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RESEARCH ARTICLE

The Protective Effect of Resveratrol in Experimentally Induced Non-Sterile Clean Wound Inflammation in Rats

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Summary

The effects of resveratrol on haematological and biochemical parameters were investigated in rats that had inflammation induced by experimental non-sterile clean wound technique. Twenty four male Wistar-Albino rats weighing 250-300 g were placed into three groups of eight after matching for age, sex and weight. In the sham-operated and resveratrol-treated groups, a 4-cm incision was made on the median line of the rats in order to create a wound. Resveratrol group was administrated resveratrol daily at a dose of 0.5 mg/kg/day for seven days. At the end of seven days, blood samples were obtained prior to sacrification and then analysed for hematological and biochemical parameters. The mean concentration of the inflammatory markers as CRP, WBC and LDH were significantly lower in resveratrol-treated group compared to the shame-operated group ($P<0.05$). Similarly, the mean values for WBC and LDH activity were significantly decreased in resveratrol-treated group compared to the control group ($P<0.05$). Resveratrol-treated group had significantly lower triglyceride concentration whereas sham-operated group had significantly higher glucose concentration when compared with the control values ($P<0.001$ and $P<0.05$, respectively). The results of the present study indicate that resveratrol attenuates inflammation and restores the alterations in blood chemistry induced by experimental clean wound inflammation in rats.

Keywords: *Resveratrol, Inflammation, Biochemical parameters, Haematological parameters, Wound*

Sıçanlarda Deneysel Oluşturulan Steril Olmayan Temiz Yara İnflamasyonunda Resveratrolün Koruyucu Etkisi

Özet

Deneysel steril olmayan temiz yara tekniği ile indüklenmiş inflamasyona sahip sıçanlarda, resveratrolün hematolojik ve biyokimyasal parametrelere olan etkisi araştırıldı. Yirmidört adet, 250-300 g ağırlığında Wistar-Albino sıçanları; yaş, cinsiyet ve ağırlıkları eşleşecek şekilde, 3 gruba yerleştirildi. Sham-operasyonu geçirmiş ve resveratrol almış gruplarda, sıçanların median hattı boyunca 4 cm'lik bir ensizyon yapılarak yara oluşturuldu. Resveratrol, 7 gün boyunca, 0.5 mg/kg/gün dozunda verildi. Yedi günün sonunda, hayvanlar öldürülmeden önce kan numuneleri alındı ve hematolojik ile biyokimyasal parametreler yönünden analiz edildi. Resveratrol almış grupta; CRP, WBC ve LDH gibi yangı belirteçleri, sham-operasyonu geçirmiş grup ile karşılaştırıldığında belirgin olarak düşüktü ($P<0.05$). Benzer şekilde, resveratrol almış grubun, WBC ve LDH aktivitelerinin ortalama değerleri, kontrol grubu ile karşılaştırıldığında, belirgin olarak düşüktü ($P<0.05$). Kontrol değerleriyle karşılaştırıldığında, resveratrol almış grup, belirgin olarak daha düşük trigliserid konsantrasyonuna sahipken, sham-operasyonu geçirmiş grup da belirgin olarak daha yüksek glukoz konsantrasyonuna sahipti (sırasıyla, $P<0.001$ ve $P<0.05$). Bu çalışmanın sonuçları, resveratrolün sıçanlarda inflamasyonu zayıflattığını ve kan kimyasında deneysel temiz yara inflamasyonu ile indüklenme sonucu oluşan değişiklikleri onardığını göstermektedir.

Anahtar sözcükler: *Resveratrol, İnflammasyon, Biyokimyasal parametreler, Hematolojik parametreler, Yara*

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INTRODUCTION

Surgical injury produces alterations in the hemodynamic, metabolic and immune responses during the postoperative period [1]. Inflammation is a complex, multiscale biological response to stress that is also required for tissue repair and regeneration following injury [2] and occurs as part of the acute response, resulting in a coordinated influx of neutrophils at the wound site. These cells produce free radicals, which are well known to be critical for defense mechanism against bacteria and other pathogens [3]. The production of oxidant at the wound site is not just obtained by neutrophils alone but may also be produced by macrophages, which appear to sustain a "long-term" response to injured cells following an acute response [4].

A number of cytokines such as interleukin 1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNF-α) are also produced mainly by resident macrophages and endo/epithelial cells and these cytokines are known to contribute to the pathogenesis of wound healing [5-7]. The suppression of neutrophil activation and lipid peroxidation by decreasing oxidative stress appears to be an important mechanism in wound healing.

Resveratrol (RSV) is a natural polyphenolic antioxidant found in a variety of foods, especially grape skin and red wine and provides diverse health benefits including cardio-protection, inhibition of low-density lipoprotein, activation of nitric oxide (NO) production and hindering of platelet aggregation [8-10]. It exerts anti-oxidant [11] and anti-inflammatory [12] effects. Some studies have reported that RSV provides protection against liver injury produced by several well-known hepatotoxins such as acetaminophen, ethanol and carbon tetrachloride (CCl_4) [13-15]. This experiment was conducted to investigate if RSV ameliorates the bio-chemical and haematological parameters in a state of inflammation induced by the non-sterile clean wound technique.

MATERIAL and METHODS

Animals

The Animal Ethics Committee of the Balikesir University approved the experimental protocol (Protocol #: 09.11.2010-2010/6-9). All experimental manipulations and postoperative care were undertaken in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. Twenty-four male Wistar-Albino rats at the age of 5 months and weighing between 250 and 300 g were housed in an individual cages at 25°C with alternating 12-h light-dark cycles. The rats were assigned randomized into three groups: intact (control), sham-operation, and sham-operation plus RSV supplementation. All rats had free access to standart laboratory diet and water until 12 h prior to surgery. RSV (>99% pure, from Sigma, Stockholm, Sweden) was administrated orally by orogastric tube at a concentration of 0.5 mg/kg/day for 7 days. In the literature, the reported dose of resveratrol to be administered varies from 0.5 to 20 oral mg/kg per day in animal models [16,17].

Wound Induction

The rats in sham-operation and sham-operation plus RSV supplementation groups were anaesthetized with intra-muscular injection of 60 mg/kg of ketamin HCl (Ketalar, Eczacıbaşı, Warner-Lambert Laboratories, Istanbul, Turkey) and 10 mg/kg of xylazine HCl (Rompun, Bayer Laboratories, Istanbul, Turkey). All procedures were performed under clean but nonsterile conditions and the animals were allowed to breathe spontaneously during the surgery. The body temperature was maintained around 37°C by the use of a heating lamp. Firstly, the abdominal skin was shaved with a povidone-iodine scrub and then a four-cm-long midline incision was made. Immediately, the abdominal fascia and skin were closed in a continuous fashion with running 3/0 silk sutures. Prior to sacrification, cardiac blood samples were collected for laboratory analyses. These rats were sacrificed on the 7th postoperative days with an over-dose of sodium pentobarbital (300 mg/kg, intraperitoneal, I. E. Ulagay, Istanbul, Turkey).

Biochemical Analysis

Following centrifugation at 825 *xg* for 10 min, sera were analyzed for cholesterol, triglyceride and C-reactive protein (CRP) as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities using commercially available kits on a chemistry autoanalyser (Cobas Integra 800; Roche Diagnostics GmbH; Mannheim, Germany). Serum levels of tumor necrosis factor-alpha (TNF-α) was determined by enzyme-linked immuno-sorbent assay (ELISA) using commercially available kits (eBioscience, Rat TNF-α Platinum ELISA, Austria) on a diagnostic instrument (BioTek, ELx 800, U.S.A).

Haematological Analysis

Blood samples placed into tubes containing K3-EDTA were subjected to flow-cytometry for red blood cells (RBC) count, hemoglobin (Hb) concentration, hematocrit (HCT) level, and white blood cells (WBC) count using fully auto-mated Blood Cell Counter Gen-S (Beckman Coulter, Coulter Corporation, USA).

Statistical Analysis

The data were analyzed by one-way ANOVA after necessary transformation for those were not normally distributed (SPSS, version 11.0, Chicago, IL). Group mean differences were attained by the Bonferoni-post hoc test. The effect was considered significant when the *P* value was < 0.05.

RESULTS

Sham-operation increased serum glucose concentration, LDH activity and WBC count but decreased serum triglyceride

when compared with control group values ($P<0.05$). Administration of RSV significantly normalised serum CRP concentration, LDH activity, WBC count but did not alter serum glucose concentration compared to sham operation rats. Likewise, administration of RSV also decreased serum triglycerde concentration compared to control group. There were no differences in concentration of serum cholesterol and TNF-α, activities of serum ALTand AST, Hb concentration, HCT value, and RBC and PLT counts.

indicate that their synthesis were inhibited by RSV and suggest that RSV can facilitates wound healing by inhibiting inflammation. It has been reported that RSV inhibits the transcription factor nuclear factor-kappa B (NF-κB), which induce the inflammatory cascade [25]. RSV has also been reported to reduce the nuclear translocation of NF-Kb [26] in addition interfere with its transcription [27]. On the other hand, there were no significant differences among RSV-treated, sham-operated and control groups in TNF-α. These data could

Table 1. *Biochemical and haematological parameters of rats in control, sham-operated and Resveratrol (RSV)-treated groups (n=8 per group)*
Tablo 1. *Kontrol, sham-operasyonu geçirmiş ve resveratrol (RSV) almış gruplardaki sıçanların biyokimyasal ve hematolojik parametreleri (n=8, her bir grup için)*

Parameters	Control Group	Sham-operated Group	RSV-treated Group
Glucose (mg/dL)	151.85±21.88[a]	226.12±41.12[b]	178.62±68.54[ab]
Triglyceride (mg/dL)	134.28±37.09[a]	63.25±24.55[b]	53.62±21.80[b]
Cholesterol (mg/dL)	55.57±6.67	53.50±9.19	44.75±18.75
ALT (IU/dL)	70.00±16.56	65.75±16.49	60.37±15.06
AST (IU/dL)	131.14±15.98	121.37±32.19	108.25±30.01
LDH (IU/dL)	487.28±142.97[a]	845.37±366.07[b]	400.25±158.32[ac]
CRP (mg/dL)	0.011±0.001[a]	0.058±0.007[b]	0.005±0.0007[ac]
TNF-α (pg/ml)	41.15±1.47	39.83±0.63	40.23±0.93
RBC10^3/µL	6.43±0.47	6.09±0.66	6.04±0.86
Hb (g/dL)	12.75±0.79	12.13±0.98	12.13±1.55
HCT (%)	36.27±2.40	34.66±2.10	34.01±4.33
WBC10^3 /µL	9.83±2.76[a]	12.68±4.10[b]	8.63±2.57[ac]
PLT/µL	860±163.35	813.00±273.41	991.12±75.27

The data are expressed as mean ± S.D., [1] Different superscripts within the same rows differ ($P<0.05$), ***ALT:*** *alanine aminotransferase;* ***AST:*** *aspartate aminotransferase;* ***LDH:*** *lactate dehydrogenase;* ***CRP:*** *C-reactive protein;* ***TNF-α:*** *tumor necrosis factor-alpha;* ***RBC:*** *red blood cells;* ***Hb:*** *hemoglobin;* ***HCT:*** *hematocrit;* ***WBC:*** *white blood cells;* ***PLT:*** *platelet*

DISCUSSION

Resveratrol had been isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) in 1940 but has since been found in various plants, including grapes, berries, and peanuts. It has many biological effects, of which anti-oxidant [18-20] and anti-inflammatory effects [21] may be pertinent to many clinical situations in which inflammation is involved. Biological effects of RSV have been well studied in *in vivo* experiments involving laboratory animals and *in vitro* experiments as well as retrospective clinical trials involving humans [22]. However, the effects of RSV *in vivo* in humans is still controversial [23]. In this experiment, the serum bio-chemical and hematological tests were used to evaluate the effect of RSV on inflammation induced by the non-sterile clean wound technique. The changes observed in serum biochemistry and hematology may be a reflection of systemic metabolic and inflammatory response.

C-reactive protein is a well-known acute-phase indicator of inflammation and a marker of systemic inflammation in the body [24]. In the current study, lower-level of CRP, WBC and LDH that occurred in RSV-administrated group may have reached significance if the study had been conducted in 12 or 24 h. Since cytokines release in early stage of inflammation. However, RSV has been shown to reduce concentration of TNF-α and other markers [IL-α, IL-1β, intra-cellular adhesion molecule-1 (ICAM-1) and inducible nitric oxide synthase (iNOS)] in animals exposed to various stressors and inflammation [28]. A cell culture study also revealed similar effects of RSV, an inhibition of cytokine release from alveolar macrophages in patients with chronic obstructive pulmonary disease by about 51% [29]. The LDH activity abnormally increases when tissues and organs are injured [30]. Administration of RSV (0.5 mg/kg) significantly normalised the activity of LDH in serum of RSV-treated rats, which indicates the inter-ference of RSV with clean non-sterile wound-induced alterations in tissues, and that may reduce the tissue damage caused by experimental wound and leakage of LDH in blood *(Table 1)*. RSV normalised the increased serum LDH activity through attenuated formation of reactive oxygen species (ROS) and increases endogenous anti-oxidant activity by its free radical scavenging property [25].

Several *in vivo* studies have demonstrated the hepato-protective effects of RSV in preclinical animal models of

hepatic insult such as those induced by ibuprofen and ethanol [31] and fed atherogenic high fat diet [32], ischaemia-reperfusion injury [33], transplant and surgical models [34] as well as radiation [35]. Administration of RSV, immediately following acetaminophen treatment, protected liver against acetaminophen-evoked hepatotoxicity in both male and female mice [13] and it also reversed the acetaminophen-induced increase in the activity of liver enzyme ALT as well as AST and TNF-α. Masubuchi et al.[36] also found that RSV offered protection against acetaminophen-induced liver injury in mice as reflected by decreased ALT, TNF-α and IL-6 levels. In this study, however, decreases in serum ALT and AST activities were insignificant. All of the results of hepatotoxicity studies have confirmed that RSV protects the liver and regulates hepatic lipid metabolism through possessing an anti-inflammatory properties and modulating expression of genes involved in hepatic nutrient metabolism. Some studies have reported the hepatoprotective effects of RSV in the high fat diet model of liver damage. Ahn et al.[32] has shown that the treatment in mice with RSV significantly decreased lipid, triglyceride as well as cholesterol levels and also attenuated the fat diet-induced elevated expression of genes involved in hepatic lipid metabolism. In our study, RSV normalised the increased glucose level in serum by induce shame-operated. Hepatoprotective effects of RSV may cause this effect in hepatic carbonhidrat metabolism.

The data demonstrate that RSV treatment reduces the specific indicators of the inflammatory response, including CRP, WBC and LDH, during the early inflammatory stage of wound healing. Thus, it could be concluded that RSV ameliorates surgical injury, through improvement of inflammatory response by attenuating of tissue damage. This was a primarily animal based study and further human studies are required to confirm the use of RSV as a possible anti-inflammatory agent in humans.

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RESEARCH ARTICLE

The Evaluation of Pullout Tests of An Expandable Newly Designed Screw

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Summary

Biomechanical evaluation of pullout forces of newly designed cortical screws with openable tips was done in the tibia bone of the young bulls. Newly designed expandable titanium 14 cortical screws with openable tips were inserted in fresh tibia bone. Of these screws, 7 were used as controls. The bones were fixed with polymethylmethacrylate after the insertion of the screws. Screw heads were attached to a custom device and prepared for pullout tests. The elastic modulus values (Newton/mm^2), yield forces (Newton) and maximum forces (Newton) of expandable and control groups were assessed. The median of yield forces (Newton) of expandable cortical screw group was found to be statistically higher than that of normal group (P=0.025). The median of maximum forces of expandable cortical screw group was found to be significantly higher than that of normal group (P=0.003). In the comparison of paired groups, it was found that the pullout forces of expandable cortical screws were significantly superior to that of normal control group. In the light of these results, it was concluded that such kind of newly designed screws are able to contribute to fracture fixation in the future, allowing more bone contact without enlarging the diameter of the screw.

Keywords: *Expandable screw, Pullout test, Fracture, Bone*

Ekspanse Olabilen Yeni Tasarım Bir Vidanın Sıyırma (Pullout) Testlerinin Değerlendirilmesi

Özet

Ucu açılabilir yeni tasarlanmış kortikal vidaların sıyırma kuvvetlerinin biomekanik olarak değerlendirilmesi genç boğaların tibia kemiğinde yapıldı. Yeni tasarlanmış ucu açılabilir 14 kortikal titanyum vida taze tibia kemiğine yerleştirildi. Bu vidaların 7 tanesi kontrol grubu olarak kullanıldı. Vidalar yerleştirildikten sonra kemikler polimetilmetakrilat ile fikse edildi. Vida başları özel bir alete bağlandı ve sıyırma testi için hazırlandı. Elastik modulus değerleri (Newton/mm^2), akma kuvveti (Newton) ve maksimum kuvvet (Newton) değerleri ucu genişleyebilen ve kontrol gruplarında değerlendirildi. Ucu genişleyebilen kortikal vida grubunun ortalama akma değerleri istatistiksel olarak normal gruba göre daha yüksek bulundu (P=0.025). Genişleyebilen kortikal vida grubunun maksimum güçlerinin ortalaması normal gruba nazaran önemli derecede yüksek bulundu (P=0.003). Eşlendirilmiş grupların karşılaştırılmasında genişleyen kortikal vidaların pullout kuvvetlerinin normal kontrol gruplara nazaran önemli derecede yüksek olduğu bulundu. Bu çalışmalarımızın ışığı altında yeni tasarlanmış böyle vidaların vida çapını genişletmeksizin daha fazla kemik kontağına izin vererek gelecekte kırık fiksasyonuna katkıda bulunabileceği tartışıldı.

Anahtar sözcükler: *Genişleyebilen vida, Çekme testi, Kırık, Kemik*

INTRODUCTION

The bone screws are commonly preferred especially in stabilization of fractures. The maximum force that can be transferred between the screw and the bone establishes the insufficiency of osteosynthesis. That maximum load is named

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as pullout strength (PS) [1]. It is difficult to assess the pullout strength of a screw. Various factors affect it. These factors are the bone features, screw diameter, screw type, predrilling and tapping [2].

The best solution to enhance the efficacy of the screws used for osteosynthesis is to make changes in design of the screw. Especially with the screws having expandable tip a better bone fixation can be achieved by enlarging the diameter of screw tip, allowing further bone contact without enlarging the diameter of the screw itself inserted in the bone [3].

The bone-screw interface is faced as a weak link in fracture fixation. An inaccurate screw fixation causes to screw loosening, implant insufficiency, loss of reduction and nonunion. The distortion of bone-screw interface occurs especially in loading condition [4].

In our present study, it was planned to assess bio-mechanically in the tibia bone the pullout strengths of a newly designed cortical screw, which has an expandable tip after the insertion into the bone.

MATERIAL and METHODS

The designs of 14 titanium cortical screws with expandable tips were drawn using AutoCAD program. Processing details were created by master cam program *(Fig. 1)*. Then, these screws designed were processed (manufactured) on Spinner TC 600 lathe. When the anatomical structure of the screws were evaluated, the length of the screw was 60 mm. The screw was designed to have an outer diameter of 7 mm; a screw thread root of 4.5 mm; a screw thread crest of 1.3 mm and a pitch of 2.7 mm. The screw was consisted of 2 *pieces (Fig. 2A* and *2B)*. The first piece was the main screw *(Fig. 2A)*, the second one was created in an ergonomic design that provides expansion of the tip of the screw, passes throughout the hole in the middle of the main screw and is able to be fastened proximally to the main screw to maintain the expansion permanently *(Fig. 3A)*. It was determined that the outer diameter of the screw reaches to 8 mm by expansion of the tip. So, it was observed an increase in outer diameter by the rate of 14.2% *(Fig. 3B)*. A groove was made at the outer side (of the head of the screw) to connect the screw head to the device that performs the pull test in pullout test *(Fig. 3B-X)*. The tibia bones used in the study were obtained from 18-month-old Black Pied breed young bulls in the mean live weight of 500-550 kg; the fresh bone blocks of 7x4 cm in size were used *(Fig. 4)*. The 14 screws were inserted into bones according to the procedure. Seven of them were expanded. All of the prepared screw-bone systems were radiologically evaluated to eliminate an osseous pathology or an inaccurate insertion of the screw.

Biomechanical Assessment

These studies were evaluated by performing a pre-study using pulling devices located in the laboratories of the

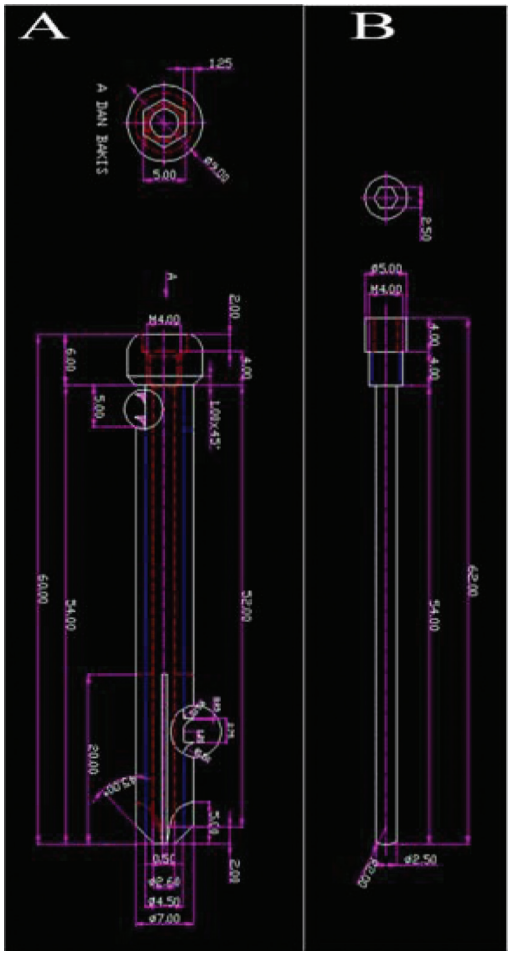

Fig 1. The AutoCAD design of the main component and the second component that allows expansion of the screw

Şekil 1. Vidanın ana komponenti ve genişlemesine izin veren ikinci komponentinin AutoCAD programı ile tasarımı

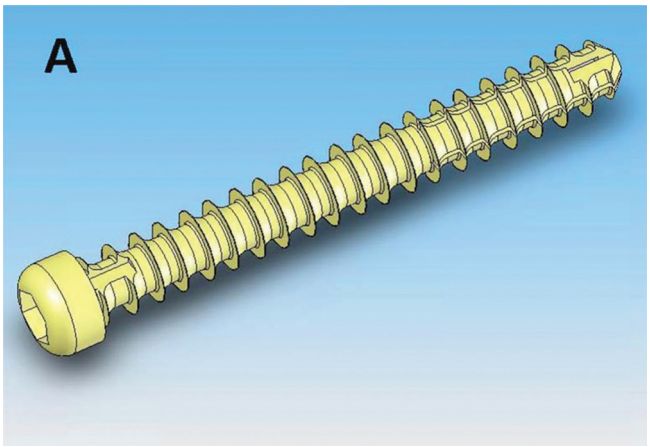

Fig 2A. The main component of the screw

Şekil 2A. Vidanın ana komponenti

Department of Mechanical Engineering of Istanbul Technical University. Screw Driven Type Testing Machine (Schimadzu brand name, autograph type AG, IS, 50 KN model) was used. This device that is able to do computer-assisted measurements with very accurate loads was

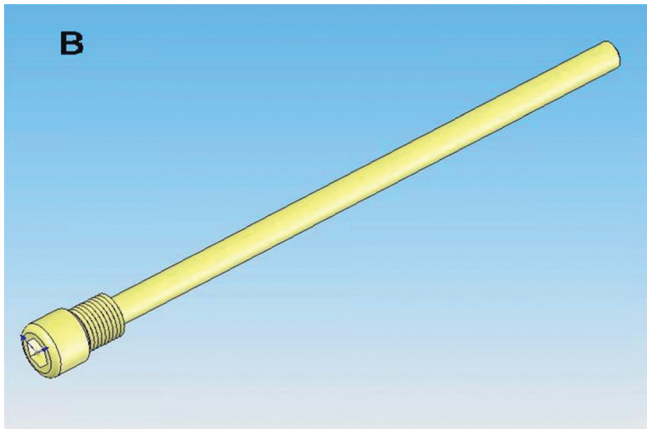

Fig 2B. The second component of the screw

Şekil 2B. Vidanın ikinci komponenti

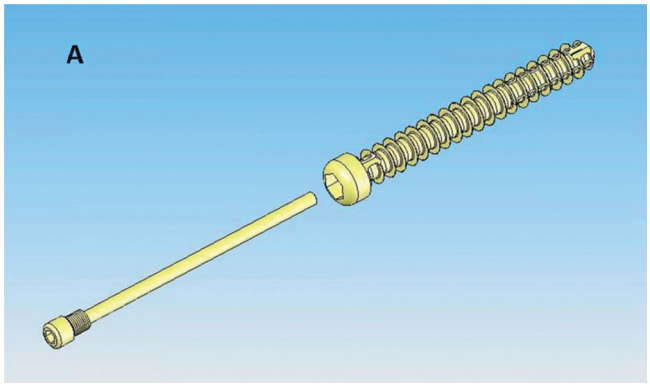

Fig 3A. The second screw component that allows expansion of the screw tip and locks of the main piece after the screw has been expanded

Şekil 3A. Vida ucunun genişlemesine izin veren ve vida ucu genişledikten sonra ana parçayı kilitleyen vidanın ikinci komponenti

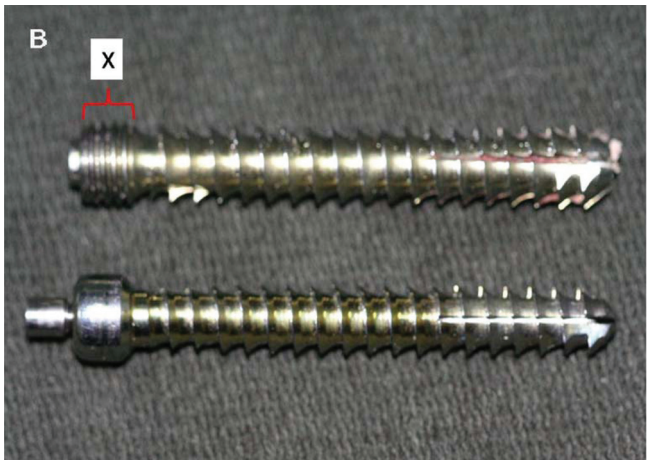

Fig 3B. X. The thread provided to connect the screw head to the pulling device for the pullout test

Şekil 3B. X. Çekme testi için çekme aletine vida başının bağlanmasını sağlayan yiv

prepared for our test. Under stroke control, the strength was measured by pulling at a constant velocity with the movement of the upper part of the device. This procedure was applied over single axis on axial plane. A test velocity

Fig 4. Fixing of the screw with methylmethacrylate after insertion into the bone and the connection to the pulling device

Şekil 4. Kemiğe yerleştirildikten sonra metilmetakrilat ile vidanın tespit edilmesi ve çekme aletine bağlanması

of 10 mm/min was chosen throughout pulling. At the end of the test, strength-elongation graphics were obtained and documented.

RESULTS

Of all of the screws exposed to pulling test, elastic modulus values (Newton/mm^2), yield forces (Newton) and maximum forces (Newton) were assessed (*Table 1, 2* and *Fig. 5, 6*). The results were compared statistically. Statistical analyses were performed by NCSS 2007 pack program. For the assessment of the data, Mann-Whitney U test for the comparison of paired groups and the definitive statistical methods (median, standard deviation) were used. The results were evaluated at the significance level of $P<0.05$.

The mean of the yield forces of the group of the expandable screws was found to be statistically higher than that of normal group ($P=0.025$). The mean of the maximum forces (Newton) of the group of the expandable screws was determined to be significantly higher than that of normal group ($P=0.003$) *(Table 3)*.

Table 1. *The elastic modulus, yield force and maximum force values of normal group*

Tablo 1. *Normal grubun elastik modulus, akma kuvveti ve maksimum kuvvet değerleri*

Normal Grup	Elastic Modulus (New/mm²)	Yield Force (Newton)	Maximum Force (Newton)
1	2381.45	1445.23	1624.14
2	2724.19	1410.12	1713.83
3	2211.39	1328.75	1345.31
4	2197.44	1647.19	2002.11
5	1969.45	1691.72	1731.95
6	2186.86	1536.88	1595.63
7	3000.23	1597.81	1722.97

Table 2. *The elastic modulus, yield force and maximum force values of expandable group*

Tablo 2. *Ekspanse olabilen grubun elastik modulus, akma kuvveti ve maksimum kuvvet değerleri*

Ekspandable Group	Elastic Modulus (New/mm²)	Yield Force (Newton)	Maximum Force (Newton)
1	2660.07	1495.94	1791.80
2	1875.77	3410.78	3555.00
3	2573.00	2662.19	2695.47
4	3428.17	2957.34	2998.59
5	2795.62	3037.66	3535.00
6	2528.35	1680.16	2328.75
7	2021.86	1598.20	2615.00

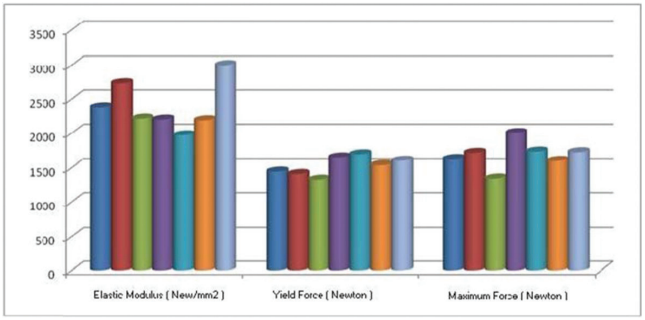

Fig 5. The elastic modulus, yield force and maximum force values of normal group

Şekil 5. Normal grubun elastik modulusu, akma kuvveti ve maksimum kuvvet değerleri

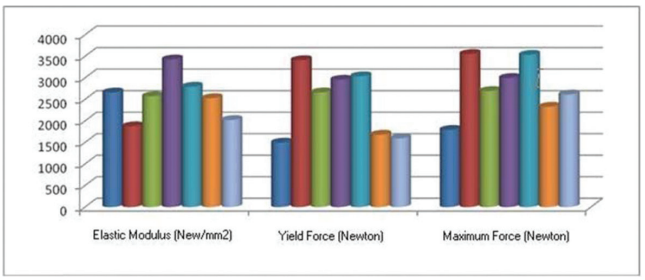

Fig 6. The elastic modulus, yield force and maximum force values of expandable group

Şekil 6. Ekspanse olabilen grubun elastik modulus, akma kuvveti ve maksimum kuvvet değerleri

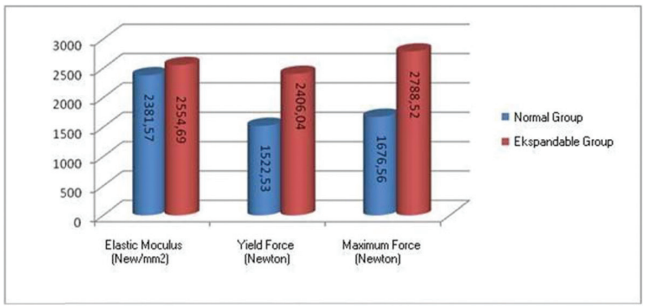

Fig 7. Comparison of the means of elastic modulus, flow force and maximum force of the normal and expandable groups

Şekil 7. Normal ve ekspanse olabilen grubun elastik modulus, akma kuvvet ortalamalarının karşılaştırılması

Table 3. *Comparison of the means of elastic modulus, yield force and maximum force of the normal and expandable groups*

Tablo 3. *Normal ve ekspanse olabilen grubun elastik modulus, akma kuvveti ve maksimum kuvvet ortalamalarının karşılaştırılması*

Comparison of Groups	Normal Group	Ekspandable Group	Median	P
Elastic Modulus (New/mm²)	2381.57±358.41	2554.69±512.83	20	0.565
Yield Force (Newton)	1522.53±133.04	2406.04±794.33	7	0.025
Maximum Force (Newton)	1676.56±196.48	2788.52±636.72	1	0.003

In the comparison of paired groups, it was observed that the pullout strengths of the expandable cortical screws were significantly superior to that of normal controls *(Fig. 7)*.

DISCUSSION

Many factors can affect the holding strength and insertional torque at the bone-screw interface. These factors are the screw thread, screw design, self-tapping/non-self-tapping screws, coating of the screw, unicortical or bicortical insertion [2,5,6].

The advantages of the screws with sharp-shaped threads should include ideally insertion of the screw, minimal soft tissue irritation and easing of the maximal screw holding power. The insertion of the screws with more than one thread is easier and they do not cause to cortical injury. In the studies carried out, it was observed that the insertion of the screws with 4 full threads was easier and had the highest level of the holding-strength [7].

The high level of screw osseointegration has being tested to ameliorate the screw fixation. For this purpose, titanium screws are recommended. Because, titanium material has a higher level of biocompatibility and better osseointegration character compared to stainless steel. The reason of why we preferred titanium material is the biocompatibility character of titanium. However, direct bone-screw contact is absent in titanium screws either [4]. Enhancing the bone-screw contact

has been achieved by either the changes in screw design or enhancing the bone-screw interface relation via coating of the screws with osteoconductive materials [4,8].

It was determined that coating the screws with osteoconductive materials provided a maximum fixation power that was significantly higher than that of standard screws [4]. Such kind of technological innovations have some risks in aspects of rigid fixation after surgery besides an additional burden of cost. Because, establishing a bone-screw interface relationship after application of such materials takes a certain period of time. But, the screws with expandable character have the opportunity to create a rigid fixation from the beginning.

The main purposes of easily applicability and effectiveness of the screw designed are very important in screw design. The thought of designing such kind of screw was arisen from the problems encountered with the traditionally applied screws. The problems especially encountered during practice with cancellous and osteoporotic bones lead us to pursuit an alternative screw design. The important steps in the practice of screws with expandable character were thought to overcome the problems particularly encountered with the pedicular screws [9]. For the problems occurred in vertebral surgery during the application of pedicular screws and in revision surgery, many authors suggested the enlargement of screw dimension, careful care and attention for the preparation of the screw hole, the use of full-threaded screw and bone cement support [3,11-17].

The usefulness of such kind of alternative screw designs and the implementations to increase the screw stability is a matter of debate. Because, we should consider the possible injury in the bone with newly designed screw application, exothermic reactions due to the biocompatible cement when used and the problems that can occur in revision surgery.

In conclusion, considering all of these studies carried out, the best solution may be the changes to do in screw design. Therefore, the use of expandable screw may provide a better bone fixation by enlarging the dimension of screw tip, allowing more bone contact without increasing the dimension of the screw itself inserted into pedicle [3].

When the screw structures were studied, it was found that a screw with wider dimension was more powerful than a screw with narrow dimension. A wide screw in dimension is stronger than a narrow screw [17]. Although the pullout strength of a screw changes with the size of the screw, it is also due to the situation whether the bone is osteoporotic or not [15]. In addition, it was determined an association between the pullout strength of the screw and the cortical thickness of the bone [18].

The studies suggesting the ability of evaluating of the presurgery screw fixation power by bone density measurement put the osteoporosis factor forward in the screw fixation [16,17,19]. Despite the problems related to the osteoporosis and the problems arising from the bone, the attempts enhancing the implant stability have always been regarded [20]. For example, it was investigated whether the therapy with the combination of alendronate and calcitriol given pre-operatively contributed to the stability of the implants coated with hydroxyapatite in osteoporotic bone, increasing the mineral density of cancellous bone [21]. It is possible to increase the screw fixation power in the osteoporotic vertebrae using pedicle screws coated with hydroxyapatite [8].

In revision patients, the increase in the dimension of the screw didn't provide the bone-screw interface power and pedicle fullness; the stability was tried to achieve with the use of expandable pedicular screw [9,22].

Although it is argued that a wide screw don't increase the fixation power in osteoporotic bone, some researchers suggested that PMMA addition or some biological treatments on the surface of the screw increased the fixation power of the screw. Nevertheless, such problems like adverse effects of PMMA exothermic reaction and foreign body effect should be considered. Calcium phosphate cement (CPC) instead of PMMA was preferred as an option [10,14,21,23-25].

The success of the osteosynthesis is established by the maximum load that can be transferred between the screw and the bone. This maximum load is called as pullout strength (PS). In the light of the knowledge mentioned, PS is faced as a very important factor in osteoporotic bone [1].

The fact that the loss of fixation is more frequent in such cases especially with rheumatoid arthritis leads the researcher to find out alternative solutions such as the use of special plate screws for these cases [26].

Only the bone density itself cannot explain the changes related to the mechanical features of the bone-screw interface. The bone structure and particularly pedicle morphology affect the pullout load in vertebrae [27].

One of the several screw designs is whether the screw is self-tapping or not. The self-tapping cortical bone screws have increasingly been begun to use in recent years. Such kind of screws is that commonly used for fracture fixation. Even this type of screws has disadvantages especially when they are used in an osteoporotic bone. One of the most important disadvantages of this type of the screws is the increase of insertion torque needed during the insertion of the screw because of the increased contact force as the bone is actively drilled. This increased friction may contribute the screw insufficiency causing cortical injury even at microscopic level [1,28].

Andrew et al. biomechanically evaluated the performance of the self-tapping cortical screws (STS) considering the deep of the insertion of the screw. They saw that the incomplete screw insertion would cause to failure in both osteoporotic and normal bones [29].

The number of the threads is very important in the screw design. It is recommended the presence of at least

three threads at the tip of the screw to avoid stripping the thread [7].

Although it has been explained many factors that may affect the holding strength of the bone-screw structure, it should be known that some malpractices in screw application may impair the results. It should be regarded that over-tightening of the screws results to future loosening and failure of the fixation in case of the excessive microinjury in the bone surrounding of the screw threads [30].

The feature of expendability of the tip part of our designed screws in a rate of 14.2% compared to the normal screw dimension after the insertion under normal conditions is very important. In the pullout tests that we performed biomechanically, suggesting a significant increase in the pullout strength of the screw after that expansion, we found that the mean of the maximum forces (Newton) of the expandable group were statistically significantly higher than that of the normal group (P=0.0003) *(Table 3)*. Moreover, it was observed that the mean of the flow forces (Newton) of the expandable group was statistically significantly higher than that of the normal group *(Fig. 7)*.

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RESEARCH ARTICLE

Clinical and Radiological Outcomes of Locking Compression Plate System in Dogs with Diaphyseal Fractures: 32 Cases [1] [2] [3]

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[2] *This study derived from the thesis of the first author*
[3] *This study was previously presented at a scientific meeting of 2nd TSAVA "Anadolum" Continuing Education Congress, İstanbul TURKEY, 2007 and the summary of the study was pressed in abstracts book*

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Summary

The aim of this study was to introduce the clinical and radiological results of long bone fractures in dogs each applied the new implant system called Locking Compression Plate (LCP). In the study 32 dogs with different breed age and sex treated using LCP system had diaphyseal zone long bone fractures. The study incorporated eight cases of each humeral, radial, femoral and tibial fractures. Analysis preoperative taking radiographs of fractures classified according to Unger system. Appropriate titanium plates and screws regarding to LCP system used for each cases surgery. Applied plate sizes, screw numbers and plate-screw density recorded immediate postoperative. Each case was evaluated clinically and radiologically on the 10th, 25th postoperative days, and the 2nd, 3rd months. By the way, postoperatively on days 10 and 25 lameness and pain on the affected limb was also scored and recorded. Most often encountered complications as osteomyelitis, delayed union, malunion and nonunion findings were not recorded. Twenty seven cases of 32 healed with primary bone healing while others healed with little callus formation.

Keywords: *Fracture healing, Dog, Long bone fractures, Locking compression plate, Radiology*

Köpeklerde Diyafizer Kırıkların Kilitli Kompresyon Plak Sistemiyle Sağaltımının Klinik ve Radyolojik Sonuçları: 32 Olgu

Özet

Bu çalışmada amaç, yeni bir implant sistemi olan Kilitli Kompresyon Plak (LCP) uygulamalarının, köpek uzun kemik kırıklarındaki klinik ve radyolojik sonuçlarının ortaya konmasıdır. Çalışmada 32 adet değişik ırk, yaş ve cinsiyetteki köpekte diyafizer bölge uzun kemik kırıkları LCP sistem kullanılarak sağaltıldı. Çalışmaya 8'er adet humerus, radius-ulna, femur ve tibia kırığı dahil edildi. Preoperatif alınan radyografiler incelenerek kırıklar Unger sistemine göre sınıflandırıldı. Operasyonlarda her bir olguya uygun boyutlarda LCP sistem setlerine ait titanyum plak ve vidalar kullanıldı. Kullanılan plak boyutu, vida sayısı ve plak-vida dansitesi kaydedildi. Her olgu klinik ve radyolojik olarak 10. ve 25. günler ile 2. ve 3. aylarda değerlendirildi. Ayrıca postoperatif 10. ve 25. günlerde ilgili ekstremitedeki topallık ve ağrı duyumu skorlanarak kaydedildi. Literatür verilere göre sık karşılaşılan kırık komplikasyonları olan osteomyelitis, gecikmeli kaynama, hatalı kaynama, kaynama yokluğu bulgularına rastlanmadı. Çalışmadaki 32 olgudan 27'sinde primer kemik kaynamasıyla geri kalanlarda ise hafif kallus oluşumuyla iyileşmenin sağlandığı görüldü.

Anahtar sözcükler: *Kırık iyileşmesi, Köpek, Uzun kemik kırığı, Kilitli kompresyon plak, Radyoloji*

INTRODUCTION

Development of the locked compression plates (LCP) was the result of a combined work of clinicians, researchers and industry. For the first time in 1998, Prof. Dr. Michael Wagner [1] tried to use threaded screws along with standard screws in combination [2]. Clinical usage of LCP began in March 2000, and it was first used in veterinary medicine in 2005. The development of a combination hole united the two techniques in a plate. The LCP allowed for the

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combination of conventional compression plating and fixation with an internal fixator [3]. The combination of two entirely different technologies in a single implant is a logical, practical and simple solution. LCP does not press to the bone unlike conventional plates and thus do not need to be contoured exactly to the bone [4]. LCP can be applied as a compression plate by using the conventional holes, as a locking internal fixator by using threaded holes, or as a combination/hybrid plate, which incorporates both types of holes [1,4]. Four different screws can be used with the LCP: the standard cortical screw, the standard cancellous screw, the self-tapping locking screw and the self-drilling locking screw [5]. The screws can be either monocortical (unicortical), so they only penetrate one cortex of the bone, or bicortical in that they penetrate both bone cortices [4]. Locked screws can be fixed when used perpendicular to the plate, however, the insertion angle could be used up to 5˚ with high stability. This construct inhibits the toggle or tilt motion between the plate and screws. This construction is four times stronger than a conventional system, where motion is possible between the screw and plate. Locking plate technology allows the surgeon to use one implant system, in different modes, for the treatment of fractures having variable fracture types, bone quality and concurrent soft tissue trauma. Since they depend on the screw purchase in bone locking internal fixators, these are more advantageous in comminuted and osteoporotic fractures [6].

Conventional plating systems provide stability due to the friction between the bone and plate as the screws are tightened [7]. The LCP can be used in a similar fashion using conventional screws. It can also be used as an internal fixator using locking screws [8]. Locked plates are also ideal for minimally-invasive fracture repair [7]. The shape and the cross-sectional geometry are similar to the limited-contact dynamic compression plate (LC-DCP), with tapered ends to allow sub-muscular insertion [8]. When using LCP like a conventional LC-DC Plate, the implant must be shaped accurately. The friction allows for load transfer from one main fragment to the other. In case a LCP is used as an internal fixator, the exact adaptation of the implant to the bony surface is not mandatory. Load transfer occurs by locking. Once the fracture fragments are properly aligned, tightening of the screws into the conical threaded plate holes does not lead to a secondary displacement and therefore no compression of the soft tissue in the interface plate-bone occurs [5]. The LCP combi hole reflects the clinical desire for integration of two treatment technologies into one implant system, without compromising the mechanical properties of the two-plate anchorage technologies [2]. Because of the specific design of the plate hole (Combi™), the locked compression plate can be used as a standard plate with standard screws and as an internal fixator using locking screws [5,8]. The internal fixator can also be utilized as a neutralizing or bridging implant [8]. These plates are designed with a threaded interface between the screw head and the screw hole to provide angular stability and to avoid bone-implant contact [1,9].

When plating with conventional techniques, the surgeon decides on the degree of tightening by the "finger tightening" technique [10]. Torque Limiting Attachment (TLA) in LCP instrumentation eliminates this situation and prevents over-tightening, especially in locked screws [11,12]. The maximum tightening limit of the screw is 1.5 Nm when using 3.5 mm, 4.5 Nm when using 4.5 mm or 5.0 mm LCP systems with TLA [11]. The LCP system is commonly used for fracture repair in human fracture patients [13]. Experience with the LCP system in veterinary fracture repair is limited [3,8,12,14].

The purpose of this study, was to investigate the healing of diaphyseal long bone fractures clinically and radiographically, in dogs operated with LCP.

MATERIAL and METHODS

This study included 32 dogs of different breeds, age, genders and body weights. The dogs were admitted to the Ankara University, Faculty of Veterinary Medicine, Department of Surgery, for long bone fractures (humerus, radius-ulna, femur, tibia-fibula), which were diagnosed clinically and radiologically. The cases were classified using the fracture classification system of Unger et al.[15]. The routine soft tissue and orthopaedic surgery instruments, 3.5 mm and 4.5 mm LCP instruments [11] were used.

All the animals received 0.2 mg/kg (0.4 mL/10 kg) subcutaneous (SC) single dose of Meloxicam (Maxicam®, 5 mg/50 ml, Sanovel) as analgesic and cefazolin sodium (Sefazol®, 500 mg IV, Mustafa Nevzat) 22 mg/kg intravenously for antibiotherapy approximately 30 min prior to surgery. 0.5 mg/kg diazepam (maximum 25 mg) (Diazem ampule® IM/IV, 10 mg/2 mL, Deva) was used to induce anaesthesia, followed by propofol (Propofol injectable emulsion® IV, 200 mg/20mL, Abbott) for endotracheal intubation. Epidural 1.5 mg/kg bupivacain (Marcain® %0.5, 20 mL, Astra Zeneca) was administered after induction for tibial and femoral fractures. The maintenance of the anesthesia was provided by 2%-3% isoflurane (Forane solution®, 100 mL, Abbott) through spontaneous ventilation. The operation site was prepared for the relevant technique approach (craniolateral approach for femoral, humeral and radial fractures, medial approach for tibial fractures). Medial approach in tibial fractures was performed avoiding any damage to the neurovascular band. After draping the surgical incision site, the plates and screws were selected using the AO/ASIF System. In addition, various strengths of TLAs (1.5 Nm for 3.5 mm LCP implants or 4.5 Nm for 4.5 mm LCP implants), designed to place LCP implants were used to fix the screws to the plate. All cases received SC amoxicillin-clavulanic acid (Synulox®, 40 mL injectable vial, Pfizer) postoperatively for five days, and SC meloxicam (Maxicam®, 5 mg/50 mL, Sanovel) for three days. No other medication other than the NSAIDs was used to control postoperative pain. Immediately after the operations cranio-caudal (CrCd) and mediolateral (ML) radiographs were

taken to control the reduction of fracture site. Following the operation, soft bandage was used to protect the operation wound and to restrict the mobility of the affected extremity. The bandage was removed on postoperative 10th day to check the operation wound. Following the first control of the patients, the bandages were not repeated and the owners were directed to limit their pets movements. The clinical healing process was followed up by radiological examination and bearing weight of limb when walking on leash. Data recorded was signalment (age, breed, gender, body weight) and cause of fracture, type of fracture, plate size, lameness and pain score in 10th and 25th days *(Table 1)*.

Table 1. *Summary of clinical data for 32 dogs with diaphyseal long bone fractures treated with locking compression plate system*
Tablo *1. Kilitli kompresyon plak sistemiyle tedavi edilen diyafizer uzun kemik kiriği bulunan 32 köpeğe ait klinik verilerin özeti*

Case No	Age (Month)	Breed	Gender	Cause of Fracture	Body Weight (kg)	Type of Fracture	Plate Size	Lameness 10th Day	Lameness 25th Day	Pain 10th Day	Pain 25th Day
1	12	Anatolian Shepherd Dog	♂	Direct trauma	45	32 A3	4.5	1	0	2	1
2	11	Cross	♂	Direct trauma	27	42 A2	3.5	1	0	2	1
3	18	English Setter	♂	Crushed by car	30	32 B2	4.5	1	0	3	2
4	12	Siberian Husky	♂	Unknown trauma	26	22 B2	3.5	1	0	3	2
5	30	Labrador Retriever	♀	Crushed by car	35	42 C1	3.5	1	0	2	1
6	72	German Shepherd Dog	♀	Crushed by car	32	42 A3	3.5	1	0	2	1
7	5	Cross	♂	Unknown trauma	15	32 A3	3.5	1	0	2	1
8	12	Labrador Retriever	♂	Crushed by car	40	32 A3	4.5	1	0	2	1
9	12	Cross	♀	Direct trauma	22	22 B1	3.5	1	0	2	1
10	10	Anatolian Shepherd Dog	♂	Crushed by car	33	22 A2	3.5	1	0	2	1
11	18	Siberian Husky	♀	Crushed by car	22	22 A2	3.5	1	0	2	1
12	12	Siberian Husky	♂	Unknown trauma	24	42 A3	3.5	1	0	3	2
13	24	Anatolian Shepherd Dog	♂	Direct trauma	35	42 C1	4.5	1	0	2	1
14	10	Cross	♂	Crushed by car	20	22 A2	3.5	1	0	2	1
15	24	Cross	♀	Crushed by car	30	32 B2	4.5	1	0	2	1
16	36	Cross	♀	Crushed by car	18	42 A2	3.5	1	0	2	1
17	9	Labrador Retriever	♂	Crushed by car	20	42 B2	3.5	1	0	2	1
18	11	Anatolian Shepherd Dog	♀	Crushed by car	32	12 A3	4.5	1	0	2	1
19	18	Anatolian Shepherd Dog	♂	Unknown trauma	41	22 A2	4.5	1	0	2	1
20	24	Anatolian Shepherd Dog	♂	Direct trauma	35	42 C1	4.5	1	0	2	1
21	12	German Shepherd Dog	♀	Crushed by car	18	12 A2	3.5	1	0	2	1
22	24	Cross	♂	High rise trauma	22	22 A2	3.5	1	0	2	1
23	48	Cross	♂	Unknown trauma	21	22 A2	3.5	2	0	3	1
24	18	Cross	♀	Crushed by car	23	12 A3	3.5	1	0	2	1
25	11	Cross	♂	Crushed by car	20	32 C1	3.5	1	0	2	1
26	9	German Shepherd Dog	♀	Crushed by car	28	32 A3	4.5	1	0	2	1
27	18	Cross	♂	Direct trauma	21	42 A3	3.5	1	0	2	1
28	36	Cross	♀	Crushed by car	23	12 A3	3.5	1	0	2	1
29	24	German Shepherd Dog	♂	Unknown trauma	25	12 A2	3.5	1	0	2	1
30	8	Cross	♂	Crushed by car	20	12 A2	3.5	1	0	2	1
31	48	Cross	♀	Crushed by car	19	12 A3	3.5	1	0	2	1
32	12	Anatolian Shepherd Dog	♀	Unknown trauma	47	12 A2	4.5	1	0	2	1

The cases were observed for lameness using a 10 m extension collar, and were graded by the "Score system for assessing the lameness in dogs undergoing fracture operation" reported by Bergmann et al.[16]. This assessment was graded as: 0 (stands and walks normally), 1 (stands normally, slight lameness when walking), 2 (stands normally, obvious lameness when walking), 3 (stands abnormally, slight to obvious lameness when walking) and 4 (non weight bearing lameness). The perception of pain in the affected extremity was assessed by palpation, using the scoring system reported by Cross et al.[17]. This evaluation was graded as: 1 (no pain response on manipulation of limb), 2 (mild-allows manipulation of limb through normal range of motion, but acknowledges pain by turning head or pulling away), 3 (moderate-will not allow manipulation through normal range of motion; acknowledges pain as score 2) and 4 (severe-will not allow manipulation of limb). The healing process was assessed radiologically using the following criteria; loss of sharp fracture edges [10], loss of fracture line [18], cortical continuity [10,19], presence of callus [19,20], presence or absence of primary and secondary reduction loss [1,5], delayed union, lack of union [21], and osteomyelitis [22].

RESULTS

Of the 32 cases included in the study, 3% were English Setter, 9% were Labrador Retriever, 9% were Siberian Husky, 13% were German Shepherd, 22% were Anatolian Sheep Dog and 44% were Cross-breed dogs. 41% of the cases were female and 59% male, their ages varied between 5 months and 6 years (mean 20.2 months). The body weights were between 15 kg and 47 kg (mean 27.2). All cases were also grouped by cause of fracture. According to the history information obtained from the patient owners, the reasons for fractures were listed as follows: 3% high rise syndrome, 22% cause unknown, 19% direct trauma and 56% traffic accident. The fractures were classified according to the location and direction of the fracture. The percentages were: 25% diaphyseal simple transversal, 6% diaphyseal simple oblique, 22% diaphyseal reducible wedge, 3% distal diaphyseal multifragmantary, 3% diaphyseal, simple, ulnar fracture, 9% diaphyseal, transversal, 19% distal diaphyseal, simple, 13% diaphyseal, oblique.

None of the cases presented neurological defects. There were no anesthetic complications. Choice of implant size was made in relation to dog's body size and anatomical region *(Table 1)*. Except for two cases axial alignment was restored successfully. These two cases (11 and 32) had an anatomic loss of reduction.

With the radiological examinations performed on the postoperative 10th day, disappearance of the sharp ends of the fracture fragments were noted *(Fig. 1)*. In addition, cases 11 *(Fig. 2)* and 32 had anatomic loss of reduction, and in cases 1, 21 *(Fig. 3)* and 22, early callus formation was observed. There were no complications with wound healing. In the postoperative 10th days, according to physical examinations except for case 23, lameness score was reported as 1, whereas case 23 presented a lameness score of 2. Pain score was 3 in four cases (3, 4, 12, 23) and 2 in others.

The 25th day radiographic examinations showed the disappearance of the fracture line in all cases and callus formation in cases 1, 11, 21, 22 and 32. Cases 11 and 32

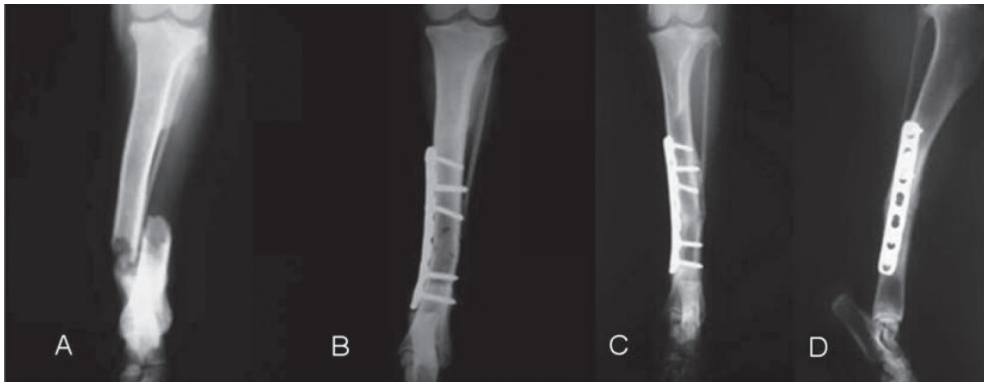

Fig 1. Preoperative CrCd radiograph (A) and postoperative 10th day CrCd radiograph (B), 60th day CrCd radiograph (C), 60th day ML radiograph (D) of the case 5. Locking screws are placed bicortically

Şekil 1. Olgu 5'in preoperatif CrCd radyografisi, postoperatif 10.gün CrCd radyografisi (B), 60.gün CrCd radyografisi (C), 60.gün ML radyografisi (D). Kilitli vidalar bikortikal yerleştirildi

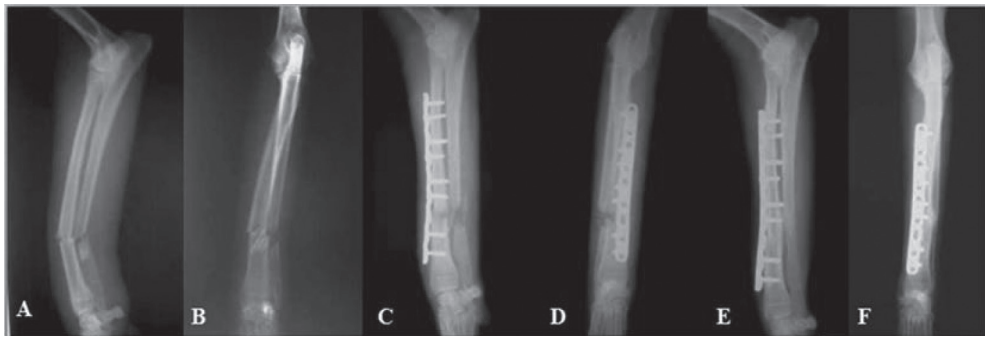

Fig 2. Failure of anatomic reduction. Preoperative ML (A), CrCd (B) radiographs and postoperative 10th day ML (C), CrCd (D) radiographs, 90th day ML radiograph (E), 90th day CrCd radiograph (F) of the case 11. The slight callus formation was seen on the 90th day radiograph

Şekil 2. Anatomik redüksiyon hatası. Olgu 11'in preoperatif ML (A), CrCd (B) radyografileri ve postoperatif 10. gün ML (C), CrCd (D) radyografileri, 90. gün ML radyografi (E), 90. gün CrCd radyografi (F). 90. gün radyografisinde hafif kallus formasyonu görülmektedir

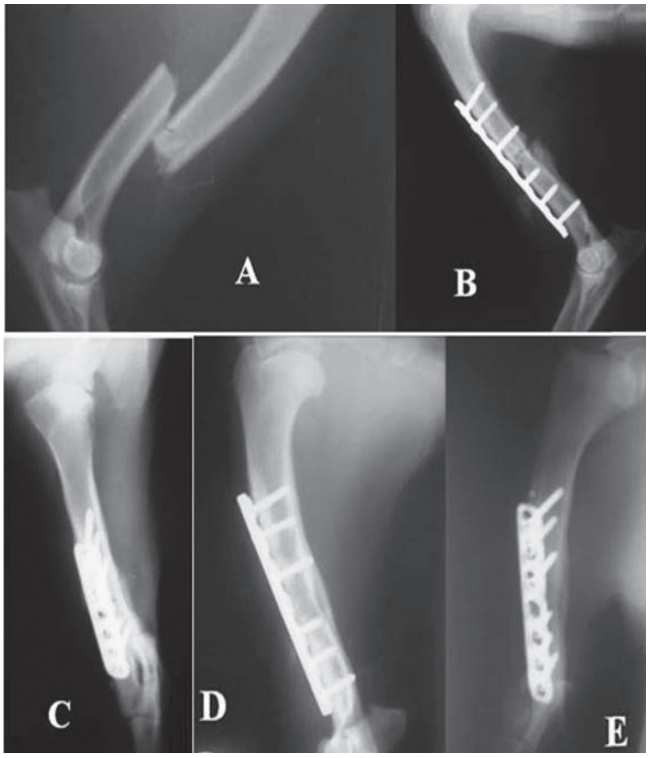

Fig 3. Radiographs are taken preoperative CrCd (A) and postoperative 10th day ML (B), 25th day CrCd (C), 90th day ML (D) and 90th day CrCd (E) of the case 21

Şekil 3. Olgu 21'in preoperative CrCd (A) ve postoperatif 10.gün ML (B), 25.gün CrCd (C), 90. gün ML (D), 90. gün CrCd (E) radyografileri alındı

showed anatomic loss of reduction, and loss of secondary reduction in the X-rays. During the physical examinations on the 25th day, the lameness score was evaluated as 0 in all cases: however, the pain score was 2 in three cases (3, 4, 12) and 1 in others.

With radiographs taken on the 60th day, it was determined that cortical integrity was sustained and reshaping had begun. In addition, cases 1, 11, 21, 22 and 32 showed reduced callus presence. After the clinical examination on the 25th day, pain and lameness scores were not reevaluated.

In the radiographic evaluation performed in the 90th day, it was recorded that all cases had cortical continuity, complete loss of fracture line and there was evidence of re-modelling. It was also found that the callus size had decreased in cases 1, 11, 21, 22 and 32. There was no evidence of osteomyelitis, delayed union, or lack of union on the follow-ups until the end of 3rd months.

Telephone interviews of owner's to assess their dog's limb function occurred between 6 and 12 months. Functional outcome was excellent in 26 dogs but the other owners could not be reached.

DISCUSSION

There is still little data on LCP systems in veterinary medicine. Osteosynthesis with plates is one of the most stable fixation techniques for veterinary orthopedic surgeons. Locked plates are designed to enable flexible internal fixation without disturbing the periosteal biology [5]. Since they do not exert pressure on the periosteal blood flow, they do not hinder fracture healing and fracture hematoma [21].

During the 3 months clinical and radiological follow up period, no fracture complications other than mild reduction loss in 2 cases (anatomic) were noticed in different control intervals. This showed that the system provides sufficient stability. Locked plating enables stable fracture fixation without the need for compressing the plate directly onto the bony surface or complete plate contouring [5,9]. Combination (Hybrid) technique was performed using both conventional and locking screws on the same plate hole. Principally the standard screws were always placed first. Since the LCP combination technique was used in this study, plates were contoured only slightly.

There are three main problems in internal fixation with plate and screws. These are primary loss of reduction, secondary loss of reduction, and compression of the periosteum leading to a disturbance of the cortical blood supply [1,5,23]. These problems were unresolved until the innovation of the LCP system [11-13,23-25].

Two cases (case 11 and 32) in the study were found to have failure of anatomic reduction intraoperatively and on radiological assessment immadiately following the operation. However, this was not found in subsequent examinations, suggesting this may have been related to both the mild loss of reduction and the stability of the implant.

Implant failure (plate failure or breakage of the screw) is a complication reported in 7% of plate fixations [26]. Sommer et al reported two plate fractures in 144 patients with LCP, despite stable screws [27]. According to a biomechanical study made in 2003, damage occurred on the DCU (Dynamic Compression Unit) when the LCP was exposed to overload [23]. Stoffel reported consistent results with Frigg. Stoffel reported that plate failure was observed on the DCU when the plates had a smaller cross sectional area [28]. In our study, no case showed complications such as plate torsion or breakage. Only for the case no, 10, where the locking screw head was broken, could be a defective screw which in controls showed no secondary reduction loss postoperatively.

No complications, such as soft tissue and/or bone infection, delayed union or non-union were observed. This was considered to be related to the right choice of antibiotic, implant, surgical technique and postoperative care.

In a study comparing vitalium, stainless steel and titanium implants on a rabbit tibia, Linder and Lundsgok concluded that titanium implants were significantly better than other implants in bone healing [29]. Millar et al.[30] compared stainless steel and titanium screws in their histological study on adult beagle dog bones, and reported that removal of the titanium which was as an expensive system, was not

required, and that it had better tissue adaptation [30]. As we also used titanium plates and screws, they were not removed.

When using the TLA, the screw is securely locked to the plate when a "click" is heard. In this study, we used TLA; thus, a standard power was applied.

It was reported that there were two main issues when evaluating the clinical outcome of the fracture: Weight-bearing on the affected limb and presence of pain when palpating the fracture line [21,31-33]. Various scores on the pain scale were recorded according to the type of surgery, age, breed and fright displayed by the case [16]. Although it does not directly evaluate pain, lameness can be used as a pain indicator [34]. In this study, the lameness scores, declined parallel with pain scores.

All the cases other than 5 showing callus formation (case 1, 11, 21, 22 and 32) healed with primary bone healing, however, the 5 cases healed secondarily as a result of lack of absolute stabilization and/or flexible fixation.

The clinical and radiological outcomes of the LCP system were found to be promising. More comprehensive detailed studies with the use of the results of this study are required, as there are little scientific data to be followed for the clinical use of LCP and the routine clinical use of this system in veterinary medicine.

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RESEARCH ARTICLE

Comparison of the Effects of Spontaneous and Mechanical Ventilation on Blood Gases During General Anaesthesia in Dogs

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Summary

Spontaneous ventilation during general anaesthesia leads to respiratory depression and atelectasis. Mechanical ventilation increases tidal volume and eliminates atelectasis. The study material consisted of a total of 20 dogs of different breed, age and gender. Dogs were divided into two groups, consisted of 10 dogs. The first group was established as the spontaneous ventilation (SV) group, while the second group was the mechanical ventilation (MV) group. For induction of anaesthesia, propofol was administered to both groups via intravenous injection at a dose of 6 mg/kg. Blood samples were collected from all dogs in 5 minutes after propofol administration. This period was determined as Minute 0 (T_0). In both groups, inhalation anaesthesia was continued with isoflurane. Venous blood samples were collected from dogs in the SV and MV groups at 15 (T_{15}), 30 (T_{30}) and 60 (T_{60}) minutes. Heart rate, respiratory rate, SpO_2, body temperature and blood gases were monitored. Statistical evaluation of the study was carried out using the Repeated Measures Analysis of Variance method. The results obtained showed that there was no statistically significant difference between the SV and MV groups regarding the examined parameters. However, in the assessments within the group, results obtained from the dogs in the MV group were more reliable from the point of view of the patients remaining stable throughout anaesthesia.

Keywords: *Spontaneous ventilation, Mechanical ventilation, Blood gases, Dog*

Köpeklerde Genel Anestezi Sürecinde Spontan ve Mekanik Ventilasyonun Kan Gazları Üzerine Etkilerinin Karşılaştırılması

Özet

Genel anestezide spontan ventilasyon, solunumun baskılanmasına ve atelektaziye yol açar. Mekanik ventilasyon tidal volümü artırır ve atelektaziyi giderir. Çalışma materyalini, farklı ırk, yaş ve cinsiyetteki toplam 20 köpek oluşturdu. Olgular, her grupta 10 köpek olacak şekilde 2'ye ayrıldı. İlk grup spontan ventilasyon (SV), ikinci grup mekanik ventilasyon (MV) grubu olarak belirlendi. Anestezi indüksiyonunda her iki gruba, propofol 6 mg/kg'dan intravenöz enjeksiyonla verildi. Tüm köpeklerden, propofol enjeksiyonunu takip eden 5. dakikada kan örnekleri alındı. Bu süre 0. dakika (T_0) olarak değerlendirildi. Her iki grubun inhalasyon anestezisi izofloranla sürdürüldü. SV ve MV grubundaki olgulardan 15. dakika (T_{15}), 30. dakika (T_{30}) ve 60. dakika (T_{60})'larda venöz kan örnekleri alındı. Kalp atım sayısı, solunum sayısı, SpO_2, vücut ısısı ve kan gazları monitörize edildi. Çalışmanın istatistiki değerlendirmesi Tekrarlı Ölçüm Varyans Analizi metodu ile yapıldı. Elde edilen bulgular, incelenen parametreler yönünden SV ve MV grupları arasında istatistiki anlamda önemli bir fark olmadığını gösterdi. Ancak grup içi değerlendirmelerde, MV grubundaki köpeklerden elde edilen sonuçların, hastaların anestezi süresince stabil kalmaları açısından daha güvenilir olduğu sonucuna ulaşıldı.

Anahtar sözcükler: *Spontan ventilasyon, Mekanik ventilasyon, Kan gazları, Köpek*

INTRODUCTION

General anaesthesia leads to respiratory depression, a decrease in the functional residual capacity and atelectasis. This situation begins with anaesthesia induction. In connection with atelectasis, clearance of airway from secretion becomes more difficult and, in turn, post-operative hypoxia and pneumonia develop [1,2].

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In spontaneous respiration, inspiration occurs via negative intra-thoracic pressure created by respiratory muscles. With positive pressure ventilation, gas flow is achieved through the airway to the lungs by creating positive pressure against atmospheric pressure in the airway. Expiration takes place passively in both respiration types [1].

In veterinary medicine, patients are mostly encouraged to breathe spontaneously during general anaesthesia. Spontaneous respiration leads to hypoventilation, which causes hypercapnia, hypoxaemia and changes in the acid-base balance [2-5].

Mechanical intermittent positive pressure ventilation (IPPV) is an invasive method providing the patient with respiratory support. It is effective in correcting hypoventilation. It supports lung function by eliminating carbondioxide and allowing oxygenisation of arterial blood. It increases tidal volume and enables atelectatic alveoli to regain their function [2,3,5-7].

The most important complication of IPPV is, the barotrauma and volutrauma occurring in connection with high inspiration pressure. Over-inflation of the alveoli leads to formation of pulmonary interstitial emphysema, pneumomediastinum and pneumothorax [6,8]. Due to increasing thoracic pressure, right ventricular dysfunction, altered left ventricular distensibility and low cardiac output, IPPV decreases venous return to the heart [2,3,5,6,9].

Another side effect of IPPV is oxygen toxication. This condition occurs in the case of the patient breathing 100% oxygen for 18-24 hours. It causes alveolar inflammation, pulmonary oedema and eventually death [5,6] .

Anaesthetic drugs, surgical interventions and body positions lead to blood gas values alteration and hypoxia and acidosis occurring. Anaesthesia affects the oxygen and fluid-electrolyte metabolism by causing hypotension and hypothermia [10].

During spontaneous ventilation, anaesthetic drugs cause a significant increase in arterial carbondioxide pressure ($PaCO_2$) and a significant decrease in pH value. In mechanical ventilation, $PaCO_2$ remains within normal limits and there is no decrease in pH value. Both in spontaneous ventilation and mechanical ventilation, no significant difference occurs in PaO_2. However, in spontaneous ventilation, arterial oxygen pressure (PaO_2) decreases with time [4]. Polis et al.[3] reported that, spontaneous ventilation in dogs increased $PaCO_2$, while mechanical ventilation significantly decreased this value and that the pH level increased. The same authors stated that neither of these two ventilation methods caused a significant difference in PaO_2 values.

Propofol is a short-acting, injectable anaesthetic with rapid effect. Used at high doses, it decreases arterial pressure and heart rate. It leads to respiratory depression and formation of apnoea. In dogs, immediately after propofol injection, PaO_2 values decrease due to hypoventilation [3,11].

All inhalation anaesthetics cause cardiopulmonary depression depending on dosage. They depress alveolar ventilation and cause to significantly decrease in respiratory rate and to increase in $PaCO_2$ values during spontaneous ventilation [3-5].

Blood gas analyses are used to determine patients' ventilation, oxygenisation and metabolic status. Arterial blood samples give important information about lung function, whereas venous blood samples give information about tissue perfusion in the whole body as well as the acid-base balance [9,12].

Attempts at collecting arterial blood may cause bleeding, temporary or permanent arterial thrombosis, haematoma or infection. While difficulties may be encountered during arterial puncture and catheter placement in small breed dogs and obese or hypotensive patients, particularly during surgery, approach to the site may prove to be a challenge. Therefore, before collecting arterial blood, especially in surgical patients, peripheral blood can be collected from the patient allowing blood gases and oxygenisation to be assessed. Venous blood can be collected from a central vein such as the jugular vein, anterior vena cava or pulmonary artery [9,13-17].

Pang et al.[14] have reported that, the values obtained from samples of lingual venous blood can be used instead of arterial blood results and that this is clinically acceptable. Malatesha et al.[18] have compared pH, bicarbonate, PO_2 and PCO_2 values in arterial and venous blood. As a result of the study, they reported that pH, bicarbonate and PCO_2 values were similar to each other, while there was a difference between PO_2 values.

Normal partial venous oxygen pressure (PvO_2) is 35-50 mmHg. Normal partial venous carbondioxide pressure ($PvCO_2$) is 3-6 mmHg higher than $PaCO_2$ values and may be used instead of $PaCO_2$ values. Low PvO_2 values indicate oxygen insufficiency in tissues, while $PvCO_2$ above 60 mmHg reveals insufficient ventilation and insufficient tissue perfusion [9,17].

Pulse oximetry is a non-invasive method used in the assessment of arterial oxyhaemoglobin saturation. Haemoglobin saturation is closely related to the PaO_2 value. Therefore, pulse oximeter data may be used in the assessment of patients' oxygenisation status. In consequence, this will decrease the need for arterial blood samples. In the case of the patient receiving 100% oxygen, SpO_2 values should be 95-100%. Saturation reducing below 95% during anaesthesia indicates hypoxia [12,19].

The aim of this study is to compare the effects of spontaneous ventilation and mechanical ventilation during general anaesthesia induced with propofol and maintained using isoflurane in dogs, on heart rate, respiratory rate, pulse oximeter data, blood gases and body temperature.

MATERIAL and METHODS

The study material comprised a total of 20 dogs of different breed, age and gender, brought to the Istanbul University Veterinary Faculty Surgery Department Clinics and operated on for various reasons.

Prior to anaesthesia, routine physical examination of the dogs was performed. Haemogram (Erythrocyte-RBC, Haemoglobin-HGB, Hematocrit-HCT, Leucocyte-WBC) and various biochemical blood analyses (AST, ALT, glucose, urea, creatinine and total protein) were evaluated.

The dogs were divided randomly into two groups, consisted of 10 dogs. The first group was established as the spontaneous ventilation (SV) group, while the second was the mechanical ventilation (MV) group. Anaesthesia was induced by intravenous injection of propofol at a dose of 6 mg/kg. Injections were administered via a 22G intravenous catheter (Vasofix; B. Braun Melsungen AG, Germany) placed into the antebrachial cephalic vein. Following relaxation of the jaw muscles, endotracheal intubation was carried out (endotracheal tube 6-10 mm internal diameter, Rüsch, Germany) in each dog.

Five minutes after propofol administration, blood samples were taken from the jugular vein from all dogs in both groups. The time was established as Minute 0 (T_0). These measurements were used as baseline values.

Inhalation anaesthesia was maintained in all dogs using 100% oxygen with isoflurane at an initial concentration of 4-5% and maintained at 2-3%. In the SV group, anaesthesia was maintained spontaneous ventilation throughout general anaesthesia, those in the MV group were attached to a mechanical ventilator (SAV 2500 Anaesthesia ventilator, Surgivet, Waukesha, WI, USA) following propofol induction and continued to breathe via controlled ventilation for the duration of inhalation anaesthesia. The mechanical ventilator was re-calibrated for each case. Calibrations were made for tidal volume to be 10 ml/kg, inspiration/expiration rate (I/E) 1:3 and respiratory rate 12 breaths/min. Accuracy of the automatic ventilator calibration was first tested on an artificial lung before being applied to the patient.

During isoflurane anaesthesia, venous blood samples were taken from the jugular vein from all dogs in both the SV and MV groups at 15 (T_{15}), 30 (T_{30}) and 60 (T_{60}) min.

Heart rate (HR), respiratory rate (RR), haemoglobin oxygen saturation (SpO_2), body temperature (BT) and blood gases [pH, pCO_2 (partial pressure of CO_2), pO_2 (partial pressure of O_2), HCO_3 (bicarbonate)] were monitored at every measurement time in all dogs.

Heart rate was determined with an ECG monitor (Advisor V9212 AR; Surgivet, Waukesha, WI, USA) using the IInd derivation.

Haemoglobin oxygen saturation (SpO_2) was taken using a pulse oximeter (Advisor V9212 AR; Surgivet, Waukesha, WI, USA)with the probe placed on the tongue.

Respiratory rates were determined in the SV group at every measurement time by observing thoracic movements during respiration. In the MV group, thoracic movement was observed at T_0, while the respiratory rate adjusted by the automatic ventilator was recorded at T_{15}, T_{30} and T_{60}.

Throughout the anaesthesia period body temperature was measured rectally using a digital thermometer (Omron, The Netherlands).

Blood samples were collected using heparinized 2 ml syringes. pH, pCO_2, pO_2 and HCO_3 were measured with a blood gas analyzer (ITC Edison, NJ 08820, USA) at 37°C. These measurements were corrected for each dog's temperature.

Statistical analysis was carried out by the Istanbul Univ. Veterinary Faculty Animal Husbandry Department.

Repeated measurements of ANOVA in SPSS 10.0 statistical package (SPSS, 1999) was used to analyse data for heart rate, respiration rate, pulse oxymetry, body temperature, pH, pCO_2, pO_2 and HCO_3. The model included measurement time (T_0, T_{15}, T_{30} and T_{60}) as a within-subject effect and group (SV and MV) as a between-subject effect, and also measurement time x group interaction. Significance control was assessed by using the least significant difference procedure. In order to determine the effect of group on investigated parameters in the specific measurement time, independent samples t-test were also performed. Furthermore, one-way repeated ANOVA included measurement time (T_0, T_{15}, T_{30} and T_{60}) as a within-subject effect was assessed in order to compare means for different sampling times for a specific group (Group SV or MV).

RESULTS

The dogs were divided into two groups, consisted of 10 dogs. Age, sex and bodyweight of the cases are shown in *Table 1*.

Table 1. *Means and (standard deviations) for age and body weights of dogs in Spontaneous Ventilation (SV) and Mechanical Ventilation (MV) groups and distribution of groups according to gender*

Tablo 1. *Spontan Ventilasyon (SV) ve Mekanik Ventilasyon (MV) gruplarındaki köpeklerin yaş ve vücut ağırlığı ortalamaları ve cinsiyete göre grup dağılımları*

Parameter	SV (n = 10)	MV (n = 10)
Age (month)[a]	43.80±16.24	41.60±13.37
Body weight (kg)[b]	20.20±5.19	23.58±4.64
Distribution of groups		
Female (number)	4	3
Male (number)	6	7

[a] Difference between SV and MV groups in terms of age was not significant (P>0.05), [b] Difference between SV and MV groups in terms of body weight was not significant (P>0.05)

The age of dogs in the SV group was determined as 43.80±16.24 months and of those in the MV group as 41.60±13.37 months. Bodyweight was recorded as 21.40±4.82 kg in the SV group and 23.58±4.64 kg in the MV group. The difference between the SV and MV groups regarding age and bodyweight was found to be statistically insignificant (P>0.05).

Following propofol injection, rapid anaesthesia induction was achieved in all cases. No apnoea or any other complication was encountered at this stage. Following relaxation of the jaw muscles and loss of the swallowing reflex, endo-tracheal intubation was performed with ease.

The means values for heart rate, respiration rate, pulse oximetry, body temperature, pO_2, pCO_2, pH and HCO_3 in SV and MV groups at different measurement times are presented in *Table 2* and *Table 3*.

The heart rate differences between the SV and MV groups in different measurement times was found to be insignificant (P>0.05). The effect of measurement time on heart rate was found to be significant in both the SV (P<0.01) and MV groups (P<0.05). In the MV group, it was observed that no statistically significant decrease occurred in the T_{15} and T_{30} measurements, only that the heart rate measured at T_{60} was lower than the heart rate at T_0. In the SV group, however, a significant decrease compared to the initial measurement was observed at T_{30} and, furthermore, an additional drop was seen to occur at T_{60}.

The effect of group on respiration rate was significant (P<0.01). When each measurement time data was examined with respect to respiratory rate, while no significant difference (P>0.05) between groups was found at T_0, at later measurements (T_{15}, T_{30} and T_{60}) the respiratory rates of dogs in the SV group were determined to be higher than the MV group (P<0.01). In this study, the respiratory rate recorded at the T_0 measurement in both groups was observed to be higher than the respiratory rates recorded at subsequent measurements. However, there was no statistically significant difference between respiratory rates obtained at T_{15}, T_{30} and T_{60} in the MV group.

The effect of the group as the main influence for pulse oximeter was determined to be insignificant. On the other hand, while the effect of measurement time on pulse oximeter was found to be insignificant in the SV group (P>0.05), a significant increase (P<0.001) was observed at T_{15} compared

Table 2. *Means and (standard deviations) for heart rate (HR), respiration rate (RR), pulse oximeter and body temperature (BT) for spontaneous ventilation (SV) and mechanical ventilation (MV) groups (G) at different measurement times*

Tablo 2. *Spontan ventilasyon (SV) ve mekanik ventilasyon (MV) grupları (G) için farklı ölçüm zamanlarında kalp atım sayısı (HR), solunum sayısı (RR), pulse oksimetre ve vücut sıcaklığı ortalamaları*

Parameter	Measurement Time (MT)	SV	MV	t-Test	Significance of Main Effects		
					G	MT	G x MT
HR (beats/minute^{-1})	0th min	155.10±6.32^{a}	144.60±8.95^{a}	NS	NS	***	NS
	15th min	147.40±7.01^{ab}	135.70±7.59^{ab}	NS			
	30th min	130.70±4.98^{b}	125.90±5.48^{ab}	NS			
	60th min	125.20±5.49^{c}	118.40±5.14^{b}	NS			
	Significance	**	*				
RR (breaths/minute^{-1})	0th min	29.90±2.85^{a}	28.20±3.85^{a}	NS	**	***	NS
	15th min	22.50±2.90^{ab}	12.00±0.00^{b}	**			
	30th min	19.30±2.52^{b}	12.00±0.00^{b}	**			
	60th min	18.20±1.85^{b}	12.00±0.00^{b}	**			
	Significance	**	***				
Pulse Oximeter (%)	0th min	94.00±0.83	87.20±2.01^{b}	**	NS	***	***
	15th min	96.10±0.74	94.70±1.34^{a}	NS			
	30th min	94.40±1.36	95.40±0.92^{a}	NS			
	60th min	92.90±1.44	95.00±0.82^{a}	NS			
	Significance	NS	***	NS			
BT (°C)	0th min	38.79±0.13^{a}	38.97±0.14^{a}	NS	NS	***	NS.
	15th min	38.18±0.19^{b}	38.32±0.30^{b}	NS			
	30th min	37.69±0.19^{c}	37.98±0.32^{c}	NS			
	60th min	37.29±0.28^{d}	37.55±0.35^{d}	NS			
	Significance	***	***				

^{a,b,c,d}***:*** *Difference between the means of measuremnet times carrying various letters in the same line are significant, * P<0.05, ** P<0.01, *** P<0.001,* ***NS:*** *Not Significant (P>0.05)*

Table 3. *Means and (standard deviations) for pH, HCO_3^-, partial pressure of CO_2 (pCO_2) and O_2 (pO_2) for spontaneous ventilation (SV) and mechanical ventilation (MV) groups (G) at different measurement times*

Tablo 3. *Spontan ventilasyon (SV) ve mekanik ventilasyon (MV) grupları (G) için farklı ölçüm zamanlarında pH, HCO_3^-, CO_2 (pCO_2) ve O_2 (pO_2) parsiyel basıncının ortalamaları*

Parameter	Measurement Time (MT)	SV	MV	t-Test	Significance of Main Effects		
					G	MT	G x MT
pH	0th min	7.31±0.02[a]	7.33±0.01	NS	NS	**	NS
	15th min	7.30±0.01[a]	7.34±0.02	NS			
	30th min	7.26±0.18[b]	7.32±0.03	NS			
	60th min	7.24±0.02[b]	7.30±0.03	NS			
	Significance	***	NS				
HCO_3 mM	0th min	23.65±0.92	24.26±0.58	NS	NS	NS	NS
	15th min	24.46±0.93	23.63±0.63	NS			
	30th min	24.42±0.93	23.38±0.71	NS			
	60th min	24.16±0.98	23.40±0.81	NS			
	Significance	NS.	NS				
pCO_2 mmHg	0th min	48.23±1.79[c]	47.38±2.02	NS	NS	*	NS
	15th min	50.38±1.36[bc]	45.58±3.00	NS			
	30th min	55.64±2.44[ab]	48.41±3.90	NS			
	60th min	56.98±2.16[a]	50.84±4.52	NS			
	Significance	***	NS				
pO_2 mmHg	0th min	124.19±22.36	64.49±2.33[c]	*	NS	NS	**
	15th min	148.53±1.36	116.02±19.53[ab]	NS			
	30th min	123.99±20.82	122.82±23.53[ab]	NS			
	60th min	95.09±11.59	165.40±44.93[ac]	NS			
	Significance	NS	*				

*[a,b,c,d]: Difference between the means of measuremnet times carrying various letters in the same line are significant, * P<0.05, ** P<0.01, *** P<0.001,* ***NS:*** *Not Significant (P>0.05)*

to the beginning, and this increased level was seen to continue until T_{60} in the MV group.

In the study, body temperatures of the SV and MV groups were found to be similar. Significant decreases in body temperature in relation to time were observed in both groups.

The mean values for blood pH difference was found to be insignificant between the groups (P>0.05). While the effect of measurement time on pH value was found to be significant in the SV group (P<0.001), similar blood pH values at various measurement times were observed among the animals in the MV group (P>0.05). Blood pH value measured at T_{30} in the SV group was seen to be lower than at the beginning, and this low level continued at T_{60}.

With respect to blood HCO_3 values, the effect of the group and measurement time was determined to be insignificant (P>0.05).

With respect to blood CO_2, the difference between the groups was found to be insignificant (P>0.05). On the other hand, in the MV group while the change in pCO_2 related to measurement time was found to be insignificant (P>0.05), in the SV group the effect of measurement time was found to be significant (P<0.001). In the SV group, an important increase was determined in the pCO_2 level compared to the beginning, and this increase continued at T_{60}.

In the study, the difference between the SV and MV groups with respect to pO_2 was found to be insignificant (P>0.05). While the difference relating to time in the SV group was found to be insignificant, a trend of pO_2 level increasing with time emerged in the MV group.

DISCUSSION

In veterinary practice, patients are enabled to breathe spontaneously during general anaesthesia. General anaesthesia creates atelectasis in the lungs and leads to respiratory depression, oxygen deficiency in tissues and disruption of the body's acid-base balance. This puts the patient's life at risk.

In this study, the effects of spontaneous and controlled ventilation during general anaesthesia on heart rate, respiratory rate, pulse oximeter data, body temperature and blood gas

values have been investigated.

While Polis et al.[3] stated that, in comparison to spontaneous respiration, mechanical ventilation increased heart rate, Cecen et al.[4] reported that neither spontaneous respiration nor mechanical ventilation had any significant effect on heart rate. In the present study, heart rate in the SV group significantly decreased at the T_{30} measurement compared to the beginning and an additional decrease occurred at the T_{60} measurement. In the MV group, however, lower values compared to the initial level were encountered only at the T_{60} measurement. Nevertheless, no significant difference was observed between the groups with regard to heart rate. The time-related decrease in heart rate in both groups suggested that this had occurred due to the cardiopulmonary depressing properties of inhalation anaesthetics [3,5].

With respect to respiratory rate, no significant difference was found between the T_0 measurements in either group. In contrast to literary sources [3,11] reporting propofol to cause respiratory depression and apnoea formation, no such complications were encountered in either group, and results were found to be similar.

Respiratory rates measured at T_{15}, T_{30} and T_{60} were found to be higher in cases in the SV group compared to the MV group. While the SV group continued spontaneous respiration throughout general anaesthesia, in order to maintain respiratory rate and the normal haemodynamic state in the MV group, the settings were adjusted to12 breaths per minute and an I/E ratio of 1:3 [5,6]. The difference between groups originated from this adjustment. The reason for the T_0 measurements in both groups being higher compared to the 3 other measurement times appeared to be due to the cardiopulmonary depressing properties and respiratory rate decreasing effects [3,6] of inhalation anaesthetics.

Cecen et al.[4] reported that spontaneous and mechanical ventilation had similar effects on SpO_2. Likewise, in the present study, the difference between groups with respect to pulse oximeter data was found to be insignificant. While the difference between measurement times for pulse oximeter data was found to be insignificant in the SV group, it was concluded that the decrease in respiratory rate after T_0 was not at a level to affect the oxygen carrying capacity of haemoglobin (SpO_2).

Pulse oximeter data began to increase starting from T_{15} in the MV group and continued to do so until the T_{60} measurement. This finding was found to support the opinion that mechanical ventilation sustained lung function and encouraged return of function of atelectatic alveoli that had occurred due to general anaesthesia [3,6,7]. The authors also thought that the ventilation provided during anaesthesia, at the tidal volume determined according to the bodyweight of patients, was effective in this increase in pulse oximeter data.

In the study, no difference was observed between the SV and MV groups regarding body temperature, however, in relation to time, decreases in body temperature were seen in both groups. This finding was similar to Simeonova [10] in which general anaesthesia has been reported to create time-related hypothermia.

With regard to blood pH, the difference between the groups was found to be insignificant. In the SV group, blood pH exhibited a decrease over time and this decrease appeared to be significant. However, the values obtained were not enough to change the patient's acid-base balance. In the MV group, the cases were seen to have similar pH values at all measurement times. The results obtained in the study were found to be compatible with the study carried out by Cecen et al.[4] On the other hand, results obtained from the MV group were different to Polis et al.[3] stating that mechanical ventilation increased blood pH.

With respect to blood HCO_3 values, the difference between groups was found to be insignificant. There was not any significant difference between measurement times within groups. This result showed that there was no pCO_2 increase enough to cause the bicarbonate increase in either group.

The difference between the groups with respect to blood pCO_2 level was found to be insignificant. In the SV group, the pCO_2 level had significantly increased at the T_{30} measurement compared to the beginning, and this increase was seen to continue at T_{60}. However, the values did not rise high enough to lead to respiratory acidosis and remained within normal limits. This finding is compatible with literatures [3,4,17].

In the MV group, the change in pCO_2 in relation to time was found to be insignificant. While being in contrast to Polis et al.[3] reporting that mechanical ventilation significantly decreased pCO_2 levels, this result is compatible with the study carried out by Cecen et al.[4]

In the present study, with respect to pO_2 values, a time-related decrease was established in the SV group, whereas there was an increase in the MV group. However, the difference between the groups was found to be insignificant. This result was thought to have occurred due to there being no respiratory complication in any of the patients and provision of 100% oxygen respiration with sufficient tissue perfusion.

During the study, no complication in relation to barotrauma or volutrauma [6,8] was encountered in patients in the MV group. This kind of complication was prevented by recalibrating the mechanical ventilator for each patient and testing on an artificial lung before use.

Oxygen toxication [5,6], which had been reported to occur in the case of patients inhaling 100% O_2 for 18-24 h, was not observed due to the duration of anaesthesia being limited to 60 min. Removal of the ventilator from patients was also carried out without any problems.

Results obtained at the end of the study showed that there was no statistically significant difference between

the SV and MV groups regarding the parameters examined. However, in the within-group evaluations, it was concluded that results obtained from cases in the MV group were more reliable for patients to remain stable during anaesthesia.

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RESEARCH ARTICLE

A Comparison of the Efficacy of Dimethyl Sulfoxide (DMSO) and Synovial Fluid in the Prevention of Peritoneal Adhesions: Experimental Rabbit Model [1]

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Summary

The purpose of this study was to compare the efficacy of dimethyl sulfoxide and synovial fluid (SF) in the prevention of peritoneal adhesions that might develop in connection with the use of mersilen mesh in a ventral hernia model created experimentally in rabbits. The forty rabbits used in the study were divided into four groups of ten. The operation was conducted under intrathecal anesthesia induced with ketamine HCl (20 mg/kg intrathecal) following xylazin HCl (5 mg/kg intramuscular) sedation. A median skin incision was made in rabbits placed on the operating table in the supine position. Then, a defect 2 cm in diameter was created on the linea alba. The defect was repaired with mersilen mesh which had been previously prepared in the shape of a disk. Until the 7th day after the operation, group I was given 10 ml saline (S) and 1.5 g/kg DMSO, group II was given 7 ml saline, 3 ml synovial fluid (SF) and 1.5 g/kg DMSO, group III was given 7 ml saline and 3 ml SF while group IV (control: C) was given only 10 ml saline. All of these were administered intraperitoneally. At the end of the 10th day, the presence and extent of peritoneal adhesion was checked using Jenkins' (1983) visual adhesion scale by performing a relaparotomy. All of the data obtained from the study was analyzed statistically using the Minitab-16 package program. Tissue samples from the region where the graft was performed were evaluated under a light microscope by staining them with the Hematoxilen-Eosin (HE) and Crossman triple stain method. The results of the adhesion scale were evaluated nonparametrically and statistical calculations were performed with the Kruskal-Wallis test. No statistical difference was found between the groups (P>0.05). The results of histopathological examination revealed that the tissue which covered the graft in all of the groups was fibrous tissue. However, it was determined that the fibrosis was weaker in the DMSO group than it was in the SF-DMSO, SF and S groups. In the end, it was concluded that synovial fluid is effective in preventing postoperative peritoneal adhesions in rabbits in light of both relaparotomic, macroscopic findings and histopathological findings.

Keywords: *Rabbit, Intraabdominal adhesion, Dimethylsulfoxide, Synovial fluid*

Peritoneal Adezyonların Önlenmesinde Dimetil Sülfoksit (DMSO) ve Synovial Sıvı Etkinliğinin Karşılaştırılması: Deneysel Tavşan Modeli

Özet

Bu çalışmada, tavşanlarda deneysel olarak oluşturulan ventral herni modelinde mersilen mesh kullanımına bağlı gelişebilecek peritoneal adezyonların önlenmesinde dimetil sülfoksit ve sinovial sıvı etkinliğinin karşılaştırılması amaçlandı. Çalışmada kullanılan 40 tavşan 4 gruba ayrıldı (n: 10). Operasyon xylazin HCl (5mg/kg im) sedasyonunu izleyerek ketamin HCl (20 mg/kg intratekal) ile elde edilen intratekal anestezi altında gerçekleştirildi. Operasyon masasına sırtüstü pozisyonda yatırılan tavşanlara median deri insizyonu yapıldıktan sonra linea alba düzeyinde 2 cm çaplı daire şeklinde bir defekt oluşturuldu. Defekt önceden disk şeklinde hazırlanan mersilen mesh ile onarıldı. Postoperatif 7. güne kadar; I. gruba 10 ml serum fizyolojik (SF) ve 1.5 g/kg DMSO, II. gruba 7 ml SF, 3 ml synovial sıvı (SS) ve 1.5 g/kg DMSO, III. Gruba 7 ml SF ve 3 ml SS, IV. gruba (kontrol: K) ise yalnızca 10 ml SF intraperitoneal olarak uygulandı. Tavşanlara 10. günün sonunda relaparotomi uygulanarak peritoneal adezyon varlığı ve derecesi Jenkins (1983)'in görsel yapışıklık skalasına göre değerlendirildi. Çalışmadan elde edilen tüm veriler Minitab-16 paket programı kullanılarak istatistiksel olarak değerlendirildi. Greft uygulanan bölgeye ait doku örnekleri Hematoksilen-Eozin (HE) ve Crossman'ın üçlü boyama yöntemi ile boyanarak ışık mikroskobunda değerlendirildi. Nonparametrik olarak değerlendirilen yapışıklık skala sonuçları Kruskal-Wallis testi ile istatistiki olarak hesaplandı ve gruplar arasında anlamlı bir fark bulunmadı (P>0.05). Histopatalojik inceleme sonuçları ise tüm gruplarda greftin üzerini örten dokunun fibröz doku karakterinde olduğunu gösterdi. Bununla birlikte fibrozisin DMSO grubunda, ES-DMSO, ES ve SF grubuna göre daha zayıf olduğu tespit edildi. Sonuçta gerek relaparotomik makroskobik bulgular gerekse histopatolojik bulgular dikkate alındığında synovial sıvının tavşanlarda postoperatif peritoneal adezyonların önlenmesinde etkili olduğu sonucuna varıldı.

Anahtar sözcükler: *Tavşan, İntraabdominal adezyon, Dimetilsülfoksit, Synovial sıvı*

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INTRODUCTION

Ventral hernias occur due to a variety of reasons either as a primary condition or as a result of complications arising during surgical procedures (incisional hernia) [1-5]. In situations where there is insufficient healthy tissue or when the hernia rupture is very large, prosthetic materials are used in treatment. These materials are applied as a patch either above or below the fascia. If the mesh comes into contact with abdominal organs, serious intraabdominal adhesions form [1-11].

The surface of the peritoneum is covered by mesothelium cells arranged in a single layer [7,8,12]. Regardless of the size of the defect created as a result of trauma, it is repaired by the mesothelium cells in the surrounding area within 3-5 days [13]. The rapid healing potential of the peritoneum and its unique physiological characteristics plays an important role both in the formation of intraabdominal adhesions and their prevention [5,7-13].

Peritoneal adhesions generally occur as fibrous bands that develop between the serosal surfaces of nearby organs, and the most important causes are previous operations, foreign objects and ischemia [1-8,11]. Trauma suppresses fibrinolytic activity and stimulates the secretion of histamine and vasoactive kinins, thereby increasing capillary permeability. As a result, a seroangiotic fluid is created which plays a role in the formation of fibrous bands between the peritoneal cavity and nearby organs [12]. Adhesions which develop due to the use of polypropylene mesh can be explained as a foreign body reaction that develops due to the mesh and as a decline in plasminogen activation due to the effect on the parietal layer [7-13]. Fibrinolytics, anticoagulants, anti-inflammatory agents, antibiotics and a number of materials that create a physical barrier are being studied for their effectiveness in preventing these adhesions which can cause intestinal obstructions, volvulus, infertility and abdominal pain [1,3-5,7,8,11,12,14-20].

Dimethyl sulfoxide (DMSO) is used for a variety of purposes in industry. It is also commonly used in medicine due to its anti-inflammatory, anti-coagulant, diuretic, analgesic and fibroblast proliferation inhibiting characteristics [7,8,21,22].

Hyaluronic acid is a polyelectrolyte with a long, linear negative electrical load. It is used in various formulations in a number of clinical fields, primarily orthopedics, ophthalmology and dermatology [23]. Due to its high viscosity, anti-inflammatory, antioxidant, and anti proliferative effects, it has found an application in surgical clinics in recent years for the purpose of preventing adhesions. One of the places in the body with the highest concentrations of HA, which is also a glycosaminoglycan, is synovial fluid [20,23-26]. HA gives synovial fluid its viscosity and its concentration in bovine synovial fluid is 2-4 mg mL [20,26].

The purpose of this study was to compare the efficacy of DMSO and bovine synovial fluid, which was used as a source of HA, in preventing the intraabdominal adhesions that might occur in connection with the mersilene mesh which is preferred for repair in experimental ventral hernia models in rabbits.

MATERIAL and METHODS

The Ethic Committee on Research Animal Care at Kafkas University of Kars, Turkey approved all procedures in this study (No: KAÜ-HADYEK-2012/52).

Animal Material

Forty adult New Zealand rabbits of the same age and gender with an average live weight of 2.5-4 kg were divided into four groups of ten. The rabbits were fed ad libitum with standard rabbit food prior to and following the surgery.

Acquisition of Synovial Fluid

Sinovial fluid obtained under sterile conditions from the tarsal joints of cattle which had undergone a multifaceted health screening was subjected to a physical examination focusing on color, appearance and viscosity. After determining the quality and quantity of the hyaluronic acid contained in the collected sinovial fluid with the Mucin-clot test, it was centrifuged to obtain the supernatant used in the study.

Stages of Implementation

Xylazin HCl (5 mg/kg IM) (Rompun-Bayer 2%) sedation and intrathechal anesthesia achieved with ketamine HCl (20 mg/kg) (Ketalar-Parkedavis) were chosen for the operation. The rabbits were laid supine on the operating table, and their abdominal areas were shaved and disinfected, after which the area was opened with a median incision approximately 4 cm long. A defect measuring 2 cm in

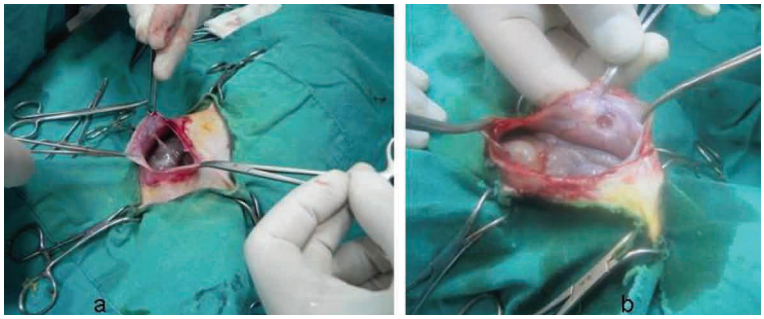

Fig 1. Macroscopic view during relaparotomy (a- Occurrence of adhesion, b- Graft completely covered with peritoneum without adhesion)

Şekil 1. Relaparatomik makroskobik görüntü (a- Adezyon oluşumu, b- Greftin adezyon olmaksızın peritonla tamamen örtülmüş hali)

diameter and including the linea alba was created to open the abdominal cavity. The defect that was created was closed up with polypropylene mesh in the shape of a disc *(Fig. 1)* as an inlay while the skin and subcutaneous connective tissue were closed routinely. 3/0 polyglactin 910 (Vicryl-Ethicon) was chosen to secure the mesh to the abdominal wall. The following treatments were administered intraperitoneally to each group in accordance with the following protocols once a day from the first day of the operation to the seventh day:

Group I: 10 ml saline and 1.5 g/kg DMSO

Group II: 7 ml saline, 3 ml SF and 1.5 g/kg DMSO

Group III: 7 ml saline and 3 ml SF

Group IV: Only 10 ml of saline (control: C)

The body temperatures, respiratory rates and pulse of the rabbits were recorded at regular intervals before, during and after the procedure. Parenteral analgesia (75 mg/kg, IM, Metamizol Sodium, Bulb, Novalgin®, Sanofi, Turkey) and antibiotics (5 mg/kg, IM, Enroflaksasin, Via, Baytril® 2.5%, Bayer, Turkey) were administered for three days following surgery.

Ten days later, the abdominal cavity was opened with a paramedian relaparotomy following the same anesthetic protocol. The existence of an intraabdominal adhesion and its extent was evaluated according to the visual adhesion scale of Jenkins et al.[13]. After taking the tissue samples required for histopathological evaluation, the laparotomy incision of the rabbits was closed with recognized methods and care was taken to ensure their survival with postoperative care and feeding.

Histopathological Examination

A circular piece was removed from the area where the graft was performed including some of the surrounding tissue and it was preserved in a 10% formaldehyde solution for histopathological examination. The tissues were blocked in paraffin after routine laboratory procedures. Cross-sections five microns thick were taken from the blocks that had been prepared and subjected to light microscopic evaluation by staining with Hematoxilen-Eosin (HE) and Crossman's triple staining technique. The cross-sections which were stained with Crossman's triple staining technique were examined for the formation and organization of fibrous tissue. The cross-sections stained with HE were evaluated for leukocyte infiltration, foreign-body giant cells, fibrosis, capillarization and necrosis.

Statistics

The Minitab-16 package program was used on all of the data obtained in the study. First of all, the data were subjected to the normality test. Then, statistical evaluation was conducted between the groups using the ANOVA method (One-way Analysis of Variance Tukey's pairwise comparisons) for parametric values while the Kruskal Wallis test was used on non-parametric values. $P<0.05$ was considered significant.

RESULTS

Pulse and respiratory rates and body temperatures were measured and recorded for all of the animals in the first three days after the operation *(Table 1)*. No statistically significant difference was found between the groups for any of these values ($P>0.05$).

The area to which the graft was applied prior to relaparotomy was evaluated with palpation and no abnormalities were found. The abdominal cavity was accessed via an approximately 5 cm incision 1-2 cm lateral from the graft, and then the peritoneal surface of the region facing the abdomen and to which the graft had been applied was examined. A single stage-1 adhesion (fibrin band) approximately 0.4 cm wide and 4 cm long was identified between the center of the graft and an organ in 2 subjects from groups I and IV and 1 subject from group II *(Fig. 2)*. In all of the cases, the graft was covered with a shiny layer consistent with peritoneum *(Fig, 3)*.

Table 1. *Statistical distribution of postoperative pulse, respiration and body temperatures*
Tablo 1. *Postoperatif nabız, solunum ve vücut ısılarının istatistiksel dağılımı*

Values		Group I	Group II	Group III	Group IV
Pulse	Day 1	146.30±12.74 [a]	161.50±5.17 [a]	168.10±25.99 [a]	157.10±18.49 [a]
	Day 2	135.00±35.91 [a]	144.40±8.49 [a]	157.80±37.69 [a]	147.20±37.43 [a]
	Day 3	153.60±23.75 [a]	146.00±19.32 [a]	149.60±36.72 [a]	149.00±31.33 [a]
Respiration	Day 1	56.90±5.78 [a]	77.40±28.03 [a]	58.00±7.06 [a]	64.50±23.52 [a]
	Day 2	64.10±15.74 [a]	55.90±10.14 [a]	70.80±19.21 [a]	55.70±9.87 [a]
	Day 3	70.40±7.53 [a]	78.70±13.90 [a]	84.80±4.13 [a]	75.40±14.82 [a]
Temperature	Day 1	37.53±0.27 [a]	37.49±9.31 [a]	38.42±0.60 [a]	37.50±0.45 [a]
	Day 2	37.79±0.71 [a]	37.75±0.45 [a]	38.14±0.61 [a]	37.83±0.57 [a]
	Day 3	37.69±0.39 [a]	37.86±0.59 [a]	37.74±0.53 [a]	37.79±0.57 [a]

a- *There was no difference between the mean values ($P>0.05$)*

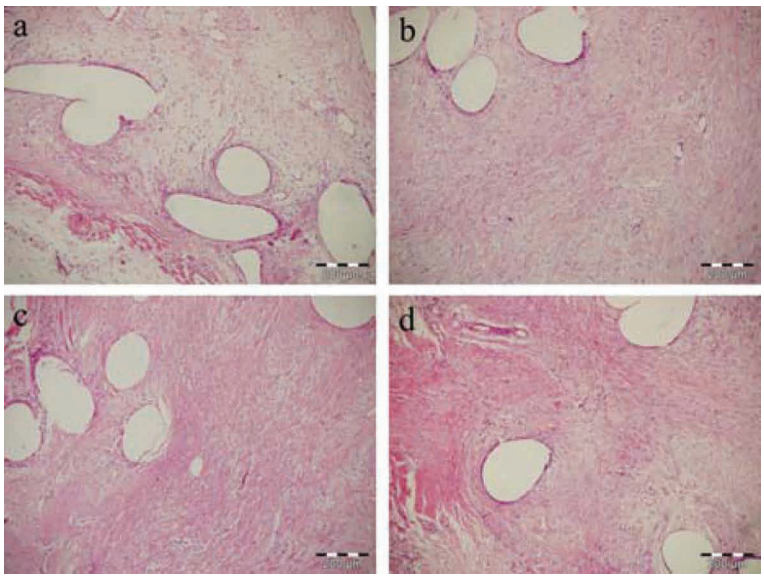

Fig 2. Fibrous tissue and healing in the DMSO (a), SF-DMSO (b), SF (c) and Control (d) groups in the areas to which the graft was applied, Hematoxilen-Eosin x 10

Şekil 2. Greft uygulanan alanlarda DMSO (a), ES-DMSO (b), ES (c) ve Kontrol (d) gruplarında fibröz doku ile iyileşme, Hematoksilen-Eosin x 10

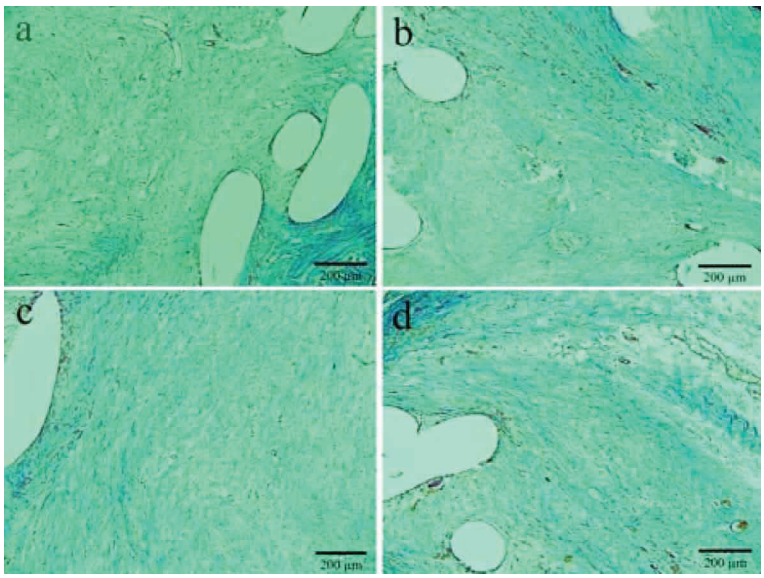

Fig 3. Fibrous tissue, collagen fiber and capillarization in the DMSO (a), SF-DMSO (b), SF (c) and Control (d) groups on the graft line. Crossman's triple stain x 10

Şekil 3. Greft hattında DMSO (a), ES-DMSO (b), ES (c) ve Kontrol (d) gruplarında kollajen lifler, kapillarizasyon, fibröz doku. Crossman'ın üçlü boyaması x 10

Table 2. *Staging according to dissection difficulty*
Tablo 2. *Diseksiyon zorluğuna göre derecelendirme*

Degree of Adhesion		**Group I**	**Group II**	**Group III**	**Group IV**
No adhesion	0	n: 8	n: 9	n: 10	n: 8
Adhesion that separated with light blunt dissection	1	n: 2	n: 1	n: -	n: 2
Adhesion that separated with aggressive blunt dissection	2	n: -	n: -	n: -	-
Adhesion that separated with sharp dissection	3	n: -	n: -	n: -	-

Healing with fibrous tissue in the graft area was observed in the DMSO group *(Fig. 1a-2a)* and fibrosis was less developed than it was in the other groups. In two of the animals from this group, it was determined that foreign body reaction and necrosis had occurred and that there was intense lymphocyte infiltration of the area to which the graft was applied. Healing with fibrous tissue was identified in the group given SF-DMSO *(Fig. 1b-2b)*. Mild mononuclear cell infiltration created primarily by lymphocytes was identified in only one animal in this group. Organization with fibrous tissue was found to be quite good in the group given SF *(Fig. 1c-2c)*. Similarly, in most of the animals in the control group, fibrosis was found to be better developed *(Fig. 1d-2d)*. In one of the animals from the control group, necrosis was observed in the grafted area and in two animals there was mild to moderate mononuclear cell infiltration.

Table 3. *Statistical results for the adhesion scale evaluation*
Tablo 3. *Yapışıklık skala değerlendirmesinin istatistiksel sonuçları*

Kruskal-Wallis Test on the Adhesion Scale	Group I	Group II	Group III	Group IV
N	10	10	10	10
Median	1.000	1.000	1.000	1.000
Av. Rank	22.0	20.0	18.0	22.0
Z	0.47	- 0.16	- 0.78	0.47

The evaluation scale results were categorized according to the visual adhesion scale reported by Jenkins et al.[13] *(Table 2)*.

As can be seen in the table, two rabbits in the first and fourth group and one rabbit in the second group had adhesion separation with light blunt dissection while there was no adhesion in the third group.

The results of the adhesion scale were evaluated non-parametrically and statistical calculations were performed with the Kruskal-Wallis test *(Table 3)*. No statistical difference was found between the groups (P=0.7337).

DISCUSSION

It has been reported that peritoneal adhesions can form due to the prosthetic mesh used to repair both ventral hernias and following any abdominal surgery including gynecological operations [1-3,7,8,11,12]. These adhesions are fibrous bands that develop between the peritoneal area where the prosthetic material is used and the serosal surface of neighboring organs or between the serosal surfaces of two different organs. The most important factors that trigger adhesion are previous operations, ischemia and foreign objects [1-8,11-13]. In this study an indirect trauma was created by forming a defect 2 cm in diameter and then creating the conditions required for adhesion by using polypropylene mesh. The formation of adhesions due to the polypropylene mesh can be explained as increased inhibition of plasminogen activators during the inflammation that occurs as a result of foreign body reaction because increased inflammation due to foreign body reaction or trauma is reported to lower fibrinolytic activity and the level of tissue plasminogen activator (tPA) even though it increases the level of type-1 and type-2 plasminogen activator inhibitor (PAI), which neutralizes the effect of tPA [7,8,12]. The fact that adhesion in all of the cases where adhesion was identified developed between the intestinal serosa and the area with the defect where polypropylene mesh was used was viewed as support of the proposed thesis.

The fact that mast cells are activated after trauma increases capillary permeability by triggering histamine and vasoactive kinins, and the result is that a seroangiotic fluid which is the basis for the formation of the fibrous bands in the peritoneal cavity begins to form. Fibrinolytic activity reportedly begins to occur three days after the trauma due to the suppressive effect of the trauma and rises above normal levels after eight days [12]. It is important to prevent adhesions during this process as they can cause intestinal obstruction, volvulus, infertility and abdominal pain. Fibrinolytics, anticoagulants, anti-inflammatory agents and a number of materials that create a physical barrier have been studied for this purpose [1-3,11,12]. Synthetic derivatives of HA esters, especially the gel form, have been used not only in a number of surgical procedures but also to prevent peritoneal adhesions in recent years [7,12,15,20,25]. Bovine synovial fluid was used for the first time in this rabbit model we created because it is easy and cheap to obtain. The concentration of HA in bovine synovial fluid is between 2-4 mg/ml and it is what gives synovial fluid its viscosity [20]. Because supernatant obtained after processing with a centrifuge was used in this study, it is very likely that this percentage was higher. As a result of the relaparotomy, a single stage-1 fibrin band approximately 0.4 cm wide and 4 cm long was identified between the center of the graft and an organ in 2 subjects from groups I and IV, and in 1 subject from group II, but no adhesions were found in any subjects from group III. Even though there was no statistical difference (P=0.7337) between the groups according to the adhesion scale results which were evaluated statistically with the Kruksal-Wallis test and viewed as nonparametric, the macroscopic findings of the relaparotomy were remarkable.

It was clear from the findings in which staging was performed according to difficulty of dissection that the fibrin band separated in all subjects with light blunt dissection. The explanation for this is probably that the fibrin band had not yet organized because of the short postoperative period. Studies have demonstrated that significant fibrous bands can be observed 10 days after peritoneal injury, reaching their highest level in 2-3 weeks and weakening as a result of remodeling in the ensuing period [5,7,8,11,12].

The results of histopathological examination revealed that the tissue which covered the graft in all of the groups was fibrous tissue. However, it was determined that the fibrosis was weaker in the DMSO group than it was in the SF-DMSO, SF and S groups. When compared in this regard, it could be said that the fibrinolytic effect of DMSO was better than SF. However, the macroscopic evaluation conducted with relaparotomy revealed no adhesions in any of the subjects given SF. Even though it was not statistically significant in preventing postoperative adhesion, SF was more effective than the other three groups from a clinical perspective. In light of these results, SF did have relative success in preventing adhesion even though its fibrinolytic effect was weak. One explanation might be that this is the result of SF serving to create a barrier and acting as a lubricant between the graft material and the serosa of internal organs due to its high viscosity.

The side of the graft facing the peritoneum in all of the subjects evaluated macroscopically with relaparotomy was covered with a shiny tissue consistent with the peritoneum. Experimental and clinical studies have demonstrated that

no matter how large a defect is it is repaired within 3-5 days by mesothelial cells from neighboring areas [2,5,7-13]. It is claimed that the rapid healing potential of the peritoneum and its unique physiological characteristics play an important role both in the formation of intraabdominal adhesions and their prevention [5-8,12]. The results of the histopathological evaluation in this study demonstrate that the surface of the graft was repaired homogenously with collagen fibers, capillarization and fibrous tissue.

Alkan et al.[7] reported that DMSO administered to rabbits in a 1 g/kg dose was effective in preventing postoperative adhesion and that immature fibrosis did develop. In the study that we did, DMSO (1.5 g/kg) was administered intraperitoneally. Histopathological examination of the tissue samples that were taken showed that fibrosis had developed to a certain degree but that it was weaker than it was in the other groups. However, foreign body reaction, lymphocyte infiltration and necrosis were found to have developed in two of the subjects. In light of the evaluation between the groups, adhesion was identified in one or two subjects in all of the groups except for the SF group. This is noteworthy from a macroscopic perspective even though it was not statistically significant as it does show that SF was effective in preventing the occurrence of adhesion without suppressing the fibrosis that is required for injuries to heal.

Although any allergic findings are not determined as clinically and histopathologically, the further detailed studies including allergic tests are needed in order to apply in clinical practice.

In light of the macroscopic and histopathological findings obtained from relaparotomy and the statistical analysis of these findings, it was concluded that SF is effective in preventing adhesions even though it does not suppress fibrosis, which is necessary for injuries to heal, as much as DMSO does Based on these results, we could say that organic formulations of HA, such as synovial fluid obtained by more modern methods, and not just the synthetic derivations can be used to prevent postoperative adhesions and that there is a need for more advanced studies to be conducted for clinical procedures.

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RESEARCH ARTICLE

Comparison of Propofol-Remifentanil and Propofol-Fentanyl Anesthesia During Ovariohysterectomy in Dogs [1] [2]

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Summary

The aim of the study was to evaluate the cardiorespiratory and clinical effects of propofol and remifentanil anesthesia compared to propofol and fentanyl anesthesia during ovariohysterectomy in dogs. Sixteen healthy dogs were randomly assigned to two groups. After premedication with atropine, anesthesia was induced with propofol and maintained with the infusion of propofol at a dose of 0.5 mg/kg/min. Once stable anesthesia was achieved, 1 µg/kg remifentanil or 2 µg/kg fentanyl was administered intravenously, and infusion was begun at a dose of 0.6 µg/kg/min and 0.5 µg/kg/min, respectively. Cardiorespiratory variables were recorded after propofol administration combined with remifentanil or fentanyl at 10-min intervals, and the quality of anesthesia, return of spontaneous ventilation, head lift and sternal position were also recorded. Apnea was observed after remifentanil and fentanyl administration in all dogs. Heart rate, systolic and mean arterial blood pressures tended to decrease rapidly after remifentanil and fentanyl administration, and during the first 20 min, in both groups. Although the difference between times was significant, the difference between groups was statistically insignificant. Recovery periods were longer in the fentanyl group than in the remifentanil group. The administration of propofol with remifentanil or fentanyl provides a stable haemodynamic state and depth of anesthesia with a constant infusion, and remifentanil could be preferred to fentanyl when aiming a rapid recovery period.

Keywords: *Propofol, Remifentanil, Fentanyl, Anesthesia, Cardiorespiratory, Recovery, Dog*

Köpeklerde Ovariohisterektomi Operasyonunda Propofol-Remifentanil ve Propofol-Fentanil Anestezisinin Karşılaştırılması

Özet

Bu çalışmanın amacı köpeklerde ovariohysterektomi operasyonunda propofol-remifentanil ile propofol-fentanil anestezisinin etkinliğini ve kardiyorespiratorik etkilerini karşılaştırmaktır. Bu amaçla 16 adet yetişkin, dişi köpek rastgele iki gruba (n=8) ayrıldı. Anesteziye atropin ile premedikasyon yapılarak başlandıktan sonra propofol ile indüksiyon yapıldı ve 0.5 mg/kg/dk dozunda propofol infüzyonuna başlandı. Stabil anesteziden sonra ilk gruba 1 µg/kg remifentanil, ikinci gruba 2 µg/kg fentanyl bolus olarak uygulandı. Remifentanil ve fentanil infüzyonu sırasıyla 0.6 µg/kg/dk ve 0.5 µg/kg/dk dozunda devam edildi. Kardiyovasküler değişiklikler propofol sonrası, remifentanil veya fentanil sonrası ve operasyon süresince 10 dakika aralıklarla kaydedildi. Anestezinin derinliği, spontan ventilasyonun başlama, kafayı kaldırma ve sternal pozisyona gelme zamanları kaydedildi. Tüm olgularda remifentanil ve fentanil uygulamasından sonra apnea oluşumu gözlendi. Her iki grupta da kalp atım hızı, sistolik (SAP) ve ortalama arteriyel basınç (MAP) değerlerinin remifentanil ve fentanil uygulanmasından sonra hızla düştüğü ve ilk 20 dakikada düşmeye devam ettiği görüldü. Bu değerlerde zaman içindeki farklılıklar istatistiksel olarak anlamlı olarak kaydedilirken, gruplar arasında istatistiksel açıdan anlamlı olmadığı saptandı. Fentanil grubundaki uyanma süresinin remifentanilden daha uzun olduğu tespit edildi. Sonuç olarak, köpeklerde ovariohisterektomi operasyonlarında sabit hızla uygulanan propofol-remifentanil veya propofol-fentanil infüzyonunun stabil hemodinamik parametreleri sağladığı, uyanma süreleri değerlendirildiğinde ise remifentanilin tercih edilebileceği kanısına varılmıştır.

Anahtar sözcükler: *Propofol, Remifentanil, Fentanil, Anestezi, Kardiopulmoner, Uyanma, Köpek*

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INTRODUCTION

Propofol is a short-acting, nonbarbiturate sedative drug, which is rapidly metabolized in dogs [1-4]. Minimal accumulation on repeated or constant administration makes propofol suitable for both the induction and maintenance of anesthesia [5-7]. Total intravenous (IV) anesthesia with propofol has been widely investigated in dogs, however, due to the lack of analgesic properties this drug is considered inadequate to provide anesthesia during surgery [5-9].

Remifentanil and fentanyl are potent synthetic μ-opioid agonists. Both drugs are administered to achieve intraoperative analgesia. Fentanyl has been widely investigated in veterinary anesthesia for many years. Fentanyl is metabolized mainly in the liver, and its half life is 2 to 3 h [10]. After prolonged infusions, its side effects continue because of its cumulative effect [2,7,11]. Its ultra-short action, rapid control of the depth of anesthesia, and lack of dependence on organ functions for breakdown and clearance make remifentanil more advantageous than fentanyl. Remifentanil does not accumulate in the body even after prolonged infusion, and its terminal half-life has been reported to be less than 6 minutes [5,12,13]. These properties make remifentanil ideal as part of a total IV anesthesia technique [8,14,15]. Although vagally mediated bradycardia often occurs, cardiovascular stability remains even when remifentanil or fentanyl are combined with propofol [7,9,16].

Remifentanil has gained popularity in human medicine in recent years. Although several pharmacokinetic and pharmacodynamic studies have demonstrated its distribution and clearance, there are only a few reports published on its clinical use in dogs [6,8,13-15,17].

Although the bolus administration of fentanyl is frequently used in veterinary practice, to the authors' knowledge, this is the first clinical study on the use of remifentanil given as a bolus administration in dogs [18].

The aims of this study were to evaluate the cardiorespiratory and clinical effects of propofol and remifentanil anesthesia compared to propofol and fentanyl anesthesia during ovariohysterectomy in dogs. The length of the recovery period was also recorded.

MATERIAL and METHODS

Sixteen client-owned, adult female dogs, aged between 8 months and 5 years (mean 1.7 years), and weighing between 14 and 36 kg (mean 23.2 kg), which were admitted for elective ovariohysterectomy, were studied. Each animal was randomly assigned to one of two groups of eight. They were considered to be healthy based on physical and haematological examination. The study was approved by the local Ethics Committee (Approvel number: 08/48). All dogs were fasted overnight and water was withheld for 2 h prior to anesthesia. All dogs were anesthetized by an anesthetist who was unaware of the treatment groups (ZP). Atropine (0.05 mg/kg, Atropin, Vetas, Turkey) was administered to all dogs subcutaneously (SC) 45 min before the induction of anesthesia. Following the placement of a catheter in both cephalic veins, propofol (Pofol, Sandoz, Turkey) was administered within 90-120 seconds as an IV bolus to induce anesthesia. Incremental doses were administered until a suitably sized, cuffed endotracheal tube could be inserted into the trachea. Postinduction apnea was defined as a period of >30 sec without spontaneous ventilation, and in such cases intermittent positive pressure ventilation (IPPV) was initiated manually until spontaneous ventilation resumed. Immediately after intubation, an IV infusion of propofol was started to maintain anesthesia. Propofol was administered using an infusion pump (Accumate 2300, Woo Young Medical, Korea), and the initial infusion rate of propofol was 0.5 mg/kg/min. The dogs were placed in dorsal recumbency and connected to a semiclosed circle rebreathing system (TMS Maxi 2200; Turkey). The fresh gas flow was 2 l/min. Lactated Ringer's solution (Ringesol, Vilsan, Turkey) was administered intravenously at a rate of 10 ml/kg/h throughout anesthesia.

A 20 G cannula was placed in the femoral artery per cutaneously to monitor arterial blood pressure and obtain samples for blood gas and acid-base analysis (Gastat Mini, Techno Medica, Germany). Heart rate (HR), systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressures, end-tidal carbon dioxide ($PE'CO_2$), oxygen saturation (SpO_2), and body temperature were recorded before the infusion of propofol and during anesthesia at 10-min intervals with a multiparameter monitor (Petas, KMA 800, Turkey). Anesthesia was considered stable in terms of no changes in the blood pressure and heart rate for 5 min, no palpebral reflexes and no tone of the jaw muscle. Once a stable plane of anesthesia was maintained, a bolus of 1 μg/kg remifentanil (Ultiva, GlaxoSmithKline, Turkey) or 2 μg/kg fentanyl (Fentanyl Citrate, Antigen Pharmaceuticals, Germany) was administered intravenously to the remifentanil and fentanyl groups, respectively, and infusion was begun at a dose of 0.6 μg/kg/min and 0.5 μg/kg/min, respectively. Following the depression of spontaneous ventilation, manual IPPV with a respiratory rate of 14 breaths/minute was initiated with 100 percent oxygen and continued to maintain $PE'CO_2$ between 35-45 mm Hg. To ensure that pH, arterial O_2 (PaO_2) and arterial bicarbonate (HCO_3) values were within the reference ranges, arterial blood gases were measured by blood gas analyzers at 15-min intervals.

The dose of propofol infused was changed according to the clinical assessment of the depth of anesthesia based on the observation of changes in blood pressure and heart rate, presence of palpebral reflex, increases in jaw and abdominal muscle tone during traction on the ovaries, as evaluated by the surgeon. Deviations of more than 20% in heart rate and blood pressure, during the incision and traction of the ovaries, without palpebral reflex or increase in the muscle tone, were

assumed to indicate inadequate analgesia, and 1 µg/kg fentanyl or 0.5 µg/kg remifentanil was administered intravenously. A heart rate less than 60 beats/min for 5 min with the presence of hypotension (MAP<60) was treated with atropine (0.02 mg/kg, IV).

Ovariohysterectomy was performed via a midline abdominal incision using a standard technique by the same surgeon.

Carprofen (2 mg/kg, IV, Rimadyl, Pfizer, Turkey) [19] and morphine (0.2 mg/kg, intramuscularly (IM), Morphine, Galen Ilac, Turkey) were administered 20 and 10 minutes before the end of the operation, respectively. Propofol, remifentanil or fentanyl infusions were stopped once surgery was completed. Manual ventilation was continued until spontaneous ventilation resumed, and the time to the first spontaneous ventilation, head lift and sternal recumbency were also recorded.

Pain scores were recorded at 0, 1, 2, 3, 4, 8, 12 and 24 h after recovery. Postoperative analgesia was provided with 0.2 mg/kg morphine administered intramuscularly every four hours for the first 24 h after surgery. Each dog received 4 mg/kg carprofen orally each day for three days after the operation. Pain was scored using a multifactorial scoring system [20]. The subjective and objective variables were recorded to assess the pain score. A score of 0 to 16 was possible, with increased scores indicative of greater pain. During the observation period, analgesia was considered inadequate if the total pain score was ≥ 8, and morphine (0.2 mg/kg, IM) was administered as a rescue analgesic postoperatively [20].

Statistical analysis: Statistical analyses were performed with commercial software (SPSS, USA). All data were reported as mean ± standard deviations (SD). The normality check of the variables was performed by the Shapiro-Wilk test, and according to these test results, between- and within-group differences in heart rate, SAP, MAP, DAP and temperature were analyzed by analysis of variance (ANOVA) for repeated measures. Also, the differences in recovery periods between groups were tested by the independent sample *t*-test. All differences were considered significant for P<0.05.

RESULT

The mean dose of propofol required to allow intubation was 5.7±0.7 mg/kg (ranged from 4.9 to 7.1) in the remifentanil group and 5.9±1.4 mg/kg (ranged from 4.5 to 7.9) in the fentanyl group. The differences between the groups for the required dose of propofol were not statistically significant. No apnea was recorded after propofol administration, and respiratory rates ranged from 10 to 16 breaths/min. Therefore, there was no need to initiate IPPV after anesthetic induction with propofol.

Mean surgical times for the remifentanil and fentanyl groups were 45.0±5.3 min and 43.75±5.2 min, respectively, and did not differ statistically between the groups.

The mean heart rate of the dogs was 143±16 beats per minute (bpm) (ranged from 111 to 170) in the remifentanil group and 140±18 bpm (ranged from 114 to 168) in the fentanyl group before remifentanil and fentanyl administration. No arrhythmias were recorded during anesthesia. Heart rate tended to decrease significantly after remifentanil and fentanyl administration (P=0.0004), and during the first 20 min in both groups but not different between groups *(Fig. 1)*. Although heart rate decreased, bradycardia was not recorded and atropine was not readministered to any of the dogs.

MAP was 85.25±20.84 mmHg in the remifentanil group and 84.86±12.95 mmHg in the fentanyl group after propofol administration, and it was decreased to 66.85±18.99 mmHg and 65.50±15.22 mmHg after remifentanil and fentanyl administration, respectively *(Fig. 2)*. In both groups, the highest values for SAP, MAP, and DAP were recorded after propofol administration alone and the lowest values were recorded after remifentanil or fentanyl administration prior to the operation. The decrease in SAP, MAP and DAP was significant between times (P<0.047) but not significant between groups. Although SAP, DAP, and MAP were approximately the same between groups for most time points, SAP and MAP were transiently higher 30 min after fentanyl administration compared to the remifentanil group (*Fig. 2, 3* and *4*).

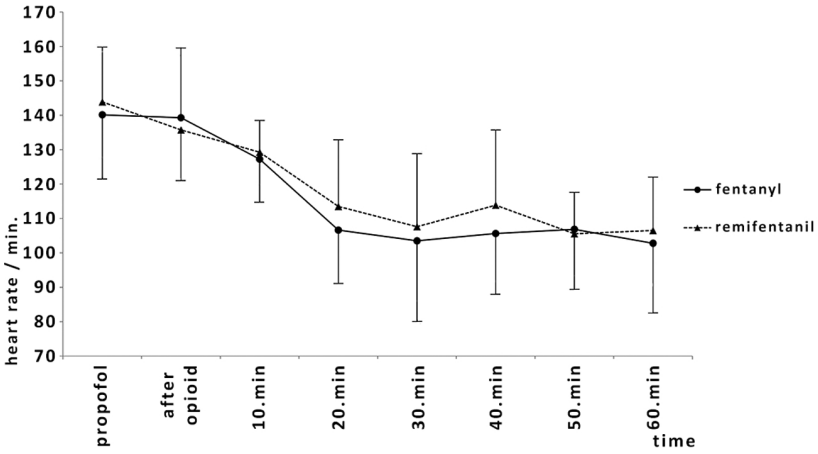

Fig 1. The heart rate of the dogs (mean±SD) during ovariohysterectomy (OH). The heart rates of the two groups did not differ significantly. The 0. minute time point shows the time at which remifentanil or fentanyl was administered

Şekil 1. Köpeklerin ovariohisterektomi (OH) sırasındaki kalp ritmleri (ortalama±SD). İki grup arasındaki kalp ritm farklılığı istatistiksel açıdan önemli bulunmamıştır. "0" zamanı fentanil veya remifentanilin uygulanmaya başlandığı zamandır

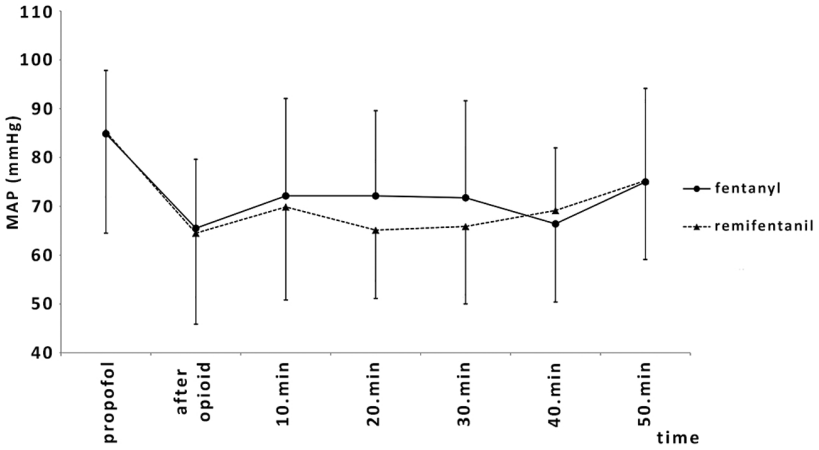

Fig 2. The mean arterial blood pressure (MAP) of the dogs (mean±SD) during ovariohysterectomy. The MAP of the two groups did not differ significantly. The 0. minute time point shows the time at which remifentanil or fentanyl was administered

Şekil 2. Köpeklerin OH sırasındaki ortalama arteriyel kan basınçları (ortalama±SD). İki grup arasındaki ortalama arteriyel kan basınç değerleri istatistiksel açıdan önemli bulunmamıştır. "0" zamanı fentanil veya remifentanilin uygulanmaya başlandığı zamandır

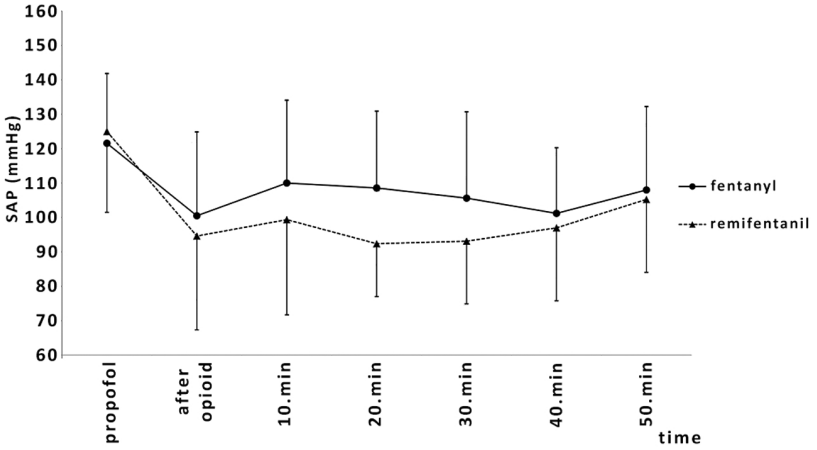

Fig 3. The systolic arterial blood pressure (SAP) of the dogs (mean±SD) during ovariohysterectomy. The SAP of the two groups did not differ significantly. The 0. minute time point shows the time at which remifentanil or fentanyl was administered

Şekil 3. Köpeklerin OH sırasındaki sistolik arteriyel kan basınçları (ortalama±SD). İki grup arasındaki sistolik arteriyel kan basınç değerleri istatistiksel açıdan önemli değildir. "0" zamanı fentanil veya remifentanilin uygulanmaya başlandığı zamandır

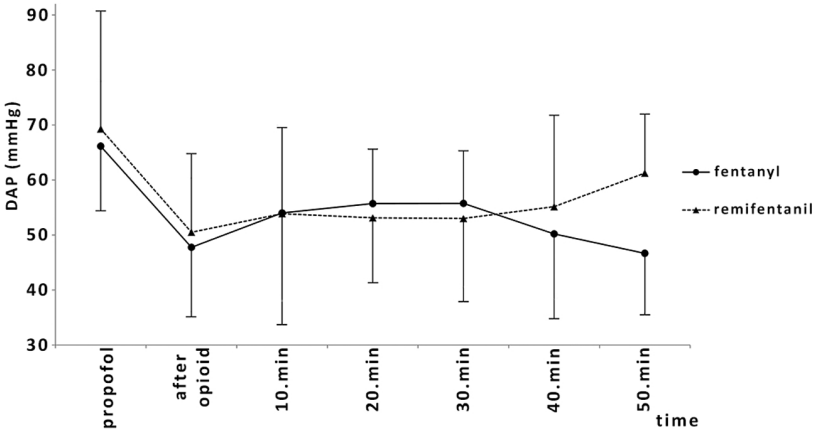

Fig 4. The diastolic arterial blood pressure (MAP) of the dogs (mean±SD) during ovariohysterectomy. The DAP of the two groups did not differ significantly. The 0. minute time point shows the time at which remifentanil or fentanyl was administered

Şekil 4. Köpeklerin OH sırasındaki diyastolik arteriyel kan basınçları (ortalama±SD). İki grup arasındaki diyastolik arteriyel kan basınç değerleri istatistiksel açıdan önemli bulunmamıştır. "0" zamanı fentanil veya remifentanilin uygulanmaya başlandığı zamandır

Apnea was recorded immediately after remifentanil and fentanyl administration in all dogs, therefore manual IPPV was continued throughout the operation. Mean times to return of spontaneous respiration, head lift and sternal position were shorter in the remifentanil group than in the fentanyl group *(Fig. 5)*. They were 17.5±6.85 min, 33.3±16.3 min and 50.8±17.1 min in the remifentanil group, and 22.7±11.04, 37.0±15.9, 66.25±35.9 min in the fentanyl group, respectively. However, the difference was statistically not significant.

There was no significant difference between the groups for mean arterial blood pH (reference range 7.35-7.45), PaO_2 (above 500 mmHg when an animal is breathing 100% O_2) and HCO_3 (reference range 18-25 mmol/l) values. The blood gas values were given in *Table 1*.

None of the dogs were given supplemental intraoperative analgesics. One dog in the remifentanil group and two dogs in the fentanyl group whined immediately after extubation, however, it only lasted 10 min. They did not appear to be in pain and supplemental analgesia was not given. The multimodal pain scale was not greater than 7/16. All dogs recovered without postoperative complications.

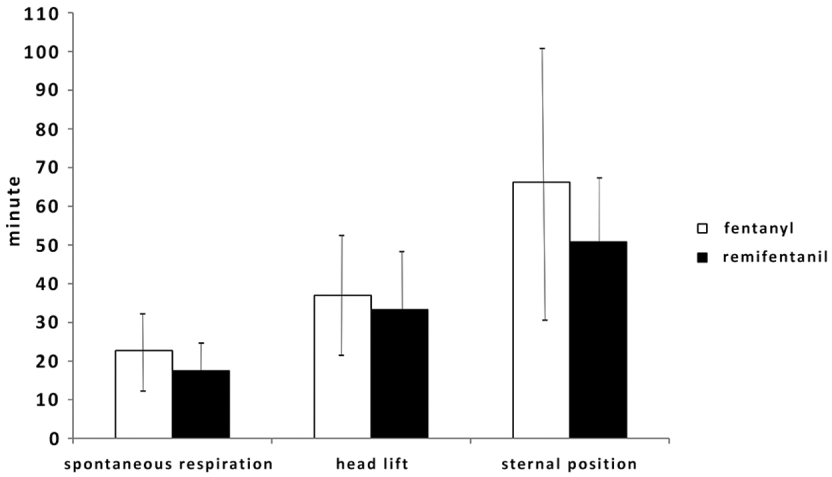

Fig 5. Mean times (mean±SD) to return of spontaneous respiration, head lift and sternal position after ovariohysterectomy in dogs. The times of the two groups did not differ significantly

Şekil 5. OH operasyonu sonlandıktan sonra köpeklerin spontan solunumlarının başladığı, kafalarını kaldırabildikleri ve sternal pozisyona geçtikleri zamanların ortalaması (mean±SD). İki grup arasındaki zamanlar açısından fark istatistiksel olarak önemli bulunmamıştır

Table 1. *Blood gas values of the dogs. The 0. min time point shows the time at which remifentanil or fentanyl was administered*
Tablo 1. *Köpeklerin kan gazı değerleri. "0" zamanı fentanil veya remifentanilin uygulanmaya başlandığı zamandır*

Parameter	Drug	0. minute	15. minute	30. minute	45. minute
pH	Remifentanil Fentanyl	7.4±0.05 7.42±0.02	7.39±0.04 7.41±0.06	7.38±0.09 7.4±0.02	7.38±0.09 7.39±0.04
PaO_2 (mmHg)	Remifentanil Fentanyl	488±20 492±60	490±15 520±74	515±45 545±32	532±20 567±38
HCO_3 (mmol/l)	Remifentanil Fentanyl	22.0±08 22.8±0.4	21.4±0.9 22.4±0.4	20.9±0.4 21.1±0.9	20.5±1.5 18.94±0.7

DISCUSSION

Propofol alone has proved to be unsatisfactory for major surgical procedures as it has no analgesic properties and the dose required to suppress responses to surgical manipulations induces severe cardiovascular and respiratory adverse effects [1,6,8,12,21]. Thus, it may be combined with some opioids like remifentanil or fentanyl. In this study, both drugs are considered as ideal analgesics for continuous infusion when combined with propofol [2,5,7,17,22,23].

The mean induction dose of propofol in this study was the same with that reported for unpremedicated dogs in several studies [24,25]. Apnea was a frequent adverse effect after rapid bolus propofol administration for induction [25-28]. Musk et al.[29] investigated 4 different doses of propofol and recorded a higher incidence of apnea with higher doses. There are also studies, in which propofol was administered slowly for induction and no adverse effects associated with respiratory depression were reported [7,8,22]. In this study, none of the animals exhibited apnea after induction with propofol, which may have resulted from slow IV administration and the discontinuation of injections after a satisfactory depth of anesthesia was achieved for intubation [5,6,9,21].

Propofol is a negative inotrope and reduces systemic vascular resistance, causing dose-dependent hypotension. Marked decreases in systemic blood pressure were reported previously [3,27]. Although the dose of propofol administered was higher than in some studies, mean SAP, MAP and DAP after propofol administration were similar to those reported in studies in which lower propofol doses were administered. It was confirmed in previous studies that hypotension is less pronounced when propofol is administered slowly [5,8,25].

The remifentanil and fentanyl infusion rates used in our study were extrapolated from published data [7,8,14]. A dose-dependent adverse effect frequently associated with the use of opioids is bradycardia [2,7,17,30]. Allweiler et al.[15] administered two different doses of remifentanil to dogs without anticholinergic injections, and reported a need for glycopyrrolate injections because of severe bradycardia related to the dose of remifentanil. In another study conducted by Murrell et al.[8], although the administered doses of remifentanil were the same as those administered in this study, atropine was re-administered during the operation because of the decrease in heart rate and blood pressure in two among 15 dogs. The bradycardia expected after the bolus administration of remifentanil or fentanyl not having been observed was attributed to the use of atropine for preanesthesia. Heart rate was not recorded below 80 beats/min during any of the procedures. It was determined that premedication with 0.05 mg/kg atropine was enough to prevent remifentanil or fentanyl-induced bradycardia.

Steagall et al.[16] administered atropine to ameliorate bradycardia, associated with a reduction in MAP, in other words, atropine was not administered as a premedicant to prevent opioid-induced cardiovascular side effects. In this study, 3 dogs in the remifentanil group exhibited low MAP (<60 mmHg) after bolus injections, but these values increased to the reference range of the anesthesia within five minutes.

During this period, atropine was not administered to any of the animals because bradycardia was not recorded and MAP increased spontaneously. It was thought that the reason for the decrease in MAP in the remifentanil group could be related to the bolus injection. In previous studies, remifentanil was not administered as a bolus for induction, and this is the first research on the use of a bolus dose of remifentanil for induction.

There are discrepancies between studies. Some researchers reported bradycardia and hypotension during remifentanil or fentanyl infusions because of the stimulation of μ-opioid receptors and the central vagotonic effect [7,17,30]. On the contrary, some other researchers observed stabilized haemodynamic variables during remifentanil or fentanyl infusions resulting from no effect on myocardial contractility, no histamine release and preserved arterial baroreflex integrity [5,9,12]. Hatschbach et al.[17] used propofol and remifentanil during ovariohysterectomy operations in bitches. These researchers reported slight decrease in blood pressure before the operation and an increase in blood pressure during the traction of the ovaries and uterus. Consequently, they reported that 0.3 μg/kg/min remifentanil was not enough to eliminate the surgical stimulus. In the same study, it was emphasized that hypotension could be observed after propofol administration. However, in our study, neither bradycardia nor hypotension was observed after propofol administration. It was considered that the hypotension recorded by Hatschbach et al.[17] prior to remifentanil administration may have arisen from the hypotensive effect of methotrimeprazine, which was used as a premedicant with propofol. The result of this study is in agreement with the report of Gimenes et al.[6], suggesting that 0.5 μg/kg/min remifentanil was enough to eliminate surgical nociception.

Grimm et al.[31] reported slight decrease in SAP, DAP and MAP after fentanyl administration alone within 60 min. Andreoni and Hughes [7] administered propofol and fentanyl with various operations in dogs. They administered atropine immediately after fentanyl to counteract anticipated bradycardia and made reductions in the rate of propofol infusions on the basis of the decrease in blood pressure Ethier et al.[23] administered fentanyl and propofol during a 24-h period without a surgical stimulation, and reported that the cardiovascular variables were slightly lower than the reference values. Furthermore, Beier et al.[9] compared propofol and propofol-remifentanil anesthesia and recorded a significant decrease in DAP and slight increase in SAP in the propofol-remifentanil group. Murrell et al.[8] administered propofol and remifentanil and reported a biphasic increase in blood pressure during surgery as the administration dose of propofol was altered according to the signs of the depth of anesthesia. When the depth of anesthesia was found to be inadequate for surgery, especially during the traction of the ovaries, an additional dose of propofol and/or remifentanil was given to the dogs. In the present study, in both groups, the lowest arterial pressures were recorded within 10 min after opioid administration, and slight increases were recorded immediately after the operation had begun in both groups. As these increases were below 20% and no muscle tone contraction was felt, they were not considered clinically important. It was suggested that the combination of propofol with remifentanil or fentanyl in these dose ranges provided good anesthesia with small individual variations in SAP, DAP and MAP.

In a recent study, a 0.3 μg/kg/min constant rate infusion of remifentanil was administered in conjunction with a target-controlled infusion of propofol, which reduced the required propofol dose by as much as 55% [9]. In the present study, the administered doses of propofol and remifentanil were approximately twice as much as that administered in the study by Beier et al.[9], and SAP, DAP and MAP were lower than those reported in the recent study. It was considered that blood pressures would have been higher if a lower dose of propofol had been administered after remifentanil or fentanyl infusion.

The arterial blood pressures were reduced after remifentanil or fentanyl administration. The decrease in SAP in the remifentanil group was clinically more pronounced than in the fentanyl group, and continued until the 40th min. The reason for the decrease in SAP in the remifentanil group could be the administration of a bolus of 1 μg/kg remifentanil, as remifentanil infusion was administered without bolus injections in previous studies [8,9,18].

Adequate anesthesia can be maintained using different doses of propofol and remifentanil [9,17]. O'Hare et al.[18] investigated the effects of different doses of propofol and remifentanil on recovery times in people. They reported shorter recovery times when the maintenance of anesthesia was achieved using a higher dose of remifentanil and lower dose of propofol instead of a lower dose of remifentanil and higher dose of propofol. It was not aimed to demonstrate a propofol-sparing effect of remifentanil or fentanyl, so the doses administered were not changed unless the depth of anesthesia was too deep or unsatisfactory. The dogs could have recovered earlier if the dose of propofol was lower and the dose of remifentanil was higher.

Mean times to return of spontaneous respiration, head lift and sternal position were similar to those reported in other studies. Hughes and Nolan [2] administered propofol and fentanyl without surgery, and recorded the first spontaneous respiration in 26±7 min and head lift in 59±12 min. In this study, although mean times to return of spontaneous respiration were similar to those reported by Hughes and Nolan [2], the mean time of head lift was shorter. The reason for a shorter period of head lift in this study could be surgery, as in the study conducted by Hughes and Nolan [2], there was no surgery or painful procedures and the dogs lay down for longer periods. Furthermore, Murrell et al.[8] administered propofol and remifentanil during ovariohysterectomy and recorded the time to return of

spontaneous respiration as 11.1 min and head lift time as 16.7 min. As higher doses of propofol and remifentanil were administered in the present study, mean times to return of spontaneous respiration and head lift were longer than those reported in the previous study. When the two groups were compared, it was observed that the mean times were longer in the fentanyl group than in the remifentanil group. The differences between the two groups throughout the recovery period can be explained by the cumulative effect of fentanyl [2], which extends the recovery period.

Due to the rapid metabolism of fentanyl and remifentanil, it is important to give an analgesic before the end of remifentanil and fentanyl administration to ensure a gradual transition of intraoperative to postoperative analgesia [8,15,20]. In the present study, carprofen and morphine were administered for postoperative analgesia prior to the end of remifentanil and fentanyl infusion. All dogs made an uneventful recovery, and none of the dogs showed signs of pain.

As the semi-conscious period during recovery is a high-risk period, it is important to select a short-acting anesthetic drug [8]. With respect to the recovery period, remifentanil could be preferred to fentanyl because of the rapid return of spontaneous respiration, consciousness and full awakening [5,8,17].

The quality of anesthesia and analgesia was judged to be satisfactory and it was concluded that a constant rate infusion of propofol combined with remifentanil or fentanyl was efficient for ovariohysterectomy in bitches. In conclusion, propofol with remifentanil or fentanyl provides haemo-dynamic stability and a stable depth of anesthesia with a constant rate of infusion during ovariohysterectomy in dogs. Remifentanil could be preferred to fentanyl when aiming a rapid recovery period. However, careful monitoring of heart rate, blood pressure and respiration is essential during remifentanil and fentanyl administration. Further studies to optimize the dose ratios are considered worthwhile.

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RESEARCH ARTICLE

Surgical Correction of Ocular Dermoids in Dogs: 22 Cases

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Summary

In this study, the treatment results of cases brought to the Istanbul University Faculty of Veterinary Medicine, Department of Surgery with a complaint of ocular discharge and diagnosed with ocular dermoids accompanying chronic epiphora, blepharospasm, keratitis, have been evaluated. The study includes 26 eyes with ocular dermoids, belonging to 22 dogs of different breed, sex and age. Due to the type and the localization of the dermoids surgical excision and superficial keratectomy of dermoids was carried out followed by pedicle conjunctival flap and/or tarsorrhaphy and medical treatment. The localization and treatment procedures for ocular dermoids and the need for further studies to determine the prevalence, species and breed distribution, as well as good clinical outcome, have been discussed.

Keywords: *Ocular dermoid, Choristoma, Superficial keratectomy, Conjunctival flap, Dog*

Köpeklerde Oküler Dermoid ve Cerrahi Sağaltımı: 22 Olgu

Özet

Bu çalışmada; İstanbul Üniversitesi Veteriner Fakültesi Cerrahi Anabilim Dalına gözyaşı akıntısı şikayetiyle getirilen ve kronik epifora, blefarospazm ya/ya da keratitisle birlikte seyreden oküler dermoid tanısı konulan olguların sağaltım sonuçları değerlendirilmiştir. Çalışmanın materyalini farklı ırk, yaş ve cinsiyette olan 22 köpeğin 26 gözü oluşturmaktadır. Sağaltımda, dermoidlerin tip ve lokalizasyonuna bağlı cerrahi eksizyon ya/ya da süperfisiyal keratektomiyi takiben pedikküllü konjunktival flap ya/ya da tarsorafi ve medikal sağaltım uygulanmıştır. Bu çalışmayla; oküler dermoidlerin lokalizasyonu, sağaltım seçenekleri, ırk, cinsiyet dağılımı ve prevalansı belirlemek ve uygulanan sağaltım yöntemlerinden elde edilen klinik sonuçların meslek pratiğine aktarılması amaçlanmıştır.

Anahtar sözcükler: *Oküler dermoid, Koristoma, Süperfisiyal keratektomi, Konjunktival flep, Köpek*

INTRODUCTION

A dermoid is a choristoma that is a histologically normal tissue in an abnormal location [1-3]. Choristomas are benign congenital over-growths of heterotopic coetaneous tissue in an inappropriate place [2,4-9]. Ocular dermoids are choristomatous malformations that involve the ocular and periocular tissues [4]. Dermoids of the orbit are usually cystic and are called ocular dermoid cysts [7,9]. Ocular dermoids are composed of dermis-like connective tissue containing skin, hair follicles, blood vessels, nerves, smooth muscle, fibrous tissue, sebaceous and sweat glands, adipose tissue, covered by keratinised stratified squamous epithelium [1,4,5,10]. Cartilage and bone are rarely seen [2,5]. However not all of the cutaneous appendages need to be present in each case [7].

Dermoids were first described in humans in 1742 [4]. Although they are well documented in humans [11], they have been reported less frequently in the animal species [9]. Dermoids are sporadic and relatively uncommon [12]. Ocular dermoids have been observed in several domestic animals, including dogs [4,6,13-15], cats [16], horses [10], cattle [17,18], sheep [8], guinea pigs [5], rabbits [19,20], birds [21] and also in wild animals like wildebeest [7].

Congenital ocular abnormalities, such as ocular dermoids, are those noted at birth or within a few weeks of life and they may occur accompanying ocular malformations [2,16]. The precise developmental mechanisms involved in the pathogenesis of ocular dermoids are not known. Numerous factors can

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influence ocular development during gestation and the early neonatal period, when portions of the eye continue to mature [7,16]. The association of a dermoid with other ocular anomalies has been reported in up to 30% of cases in humans but has not been determined in any animal species [4]. In dogs, there appears to be a breed predisposition to ocular dermoids in the German shepherd dog (GSD), Saint Bernard (SB), golden retriever and dachshund [4,22]. It is commonly believed that, this disease is generally congenital, but not hereditary [6]. They are reported to be inherited in the Burmese cat and perhaps, the GSD, SB and dachshund [4,12,23].

Ocular dermoids may affect the eyelids, conjunctiva (bulbar and palpebral), nictitating membrane or cornea [4-6,23], or may be seen as an inclusion cyst within the orbit [5]. Corneal dermoids have been classified into three broad types by Mann [11]. The first type is limbal or epibulbar dermoid, the most frequent and least severe form, which most commonly occurs at the lateral canthus. The second type is a large dermoid covering almost all of the cornea, which may extend deep into stroma but not the descement's membrane or corneal endothelium. Assessment of depth of involvement of the corneal mass can be made by ultrasound biomicroscopy. The third type of dermoid involves the entire corneal diameter [4].

The most common site of corneal dermoids in the dog is at the temporal canthus. And most of them are unilateral [22]. The tissues irritate the eye and associated structures. The hairs on the lid may point towards the eye. Therefore, patients suffer from chronic epiphora, blepharospasm and keratitis [6].

Ocular dermoids are best treated by surgical (keratectomy and/or conjunctivectomy) excision when the animal is old enough to undergo general anesthesia [22-24]. If the cornea is involved, the procedure of choice is superficial keratectomy (SK). There are two common ways to perform a superficial keratectomy. These are, Complete Incision Keratectomy (CIK) and Partial Incision Keratectomy (PIK). In the first method, an initial corneal incision is made that completely surrounds the lesion, in the second method a small corneal incision is made adjacent to the lesion to be removed [23,24]. Before performing a SK, determining the depth of the lesion using an operation microscope will help in planning the surgery [23]. For keratectomies that are extensive or reaching to 50 to 75% of the corneal thickness, the use of a conjunctival flap or other supportive surgery is additionally required to protect the cornea, prevent perforation and promote healing. The bulbar pedicle flap created from the bulbar conjunctiva is the most frequently used method by the veterinary ophthalmologist. This method has some advantages including; the graft can cover any part of the cornea, vision can usually remain while the graft is in place, the graft moves in relation to the eye and tension is created with the eye lid [23]. The conjunctival flaps will adhere to the corneal lesion and epithelialisation surrounding the flap will occur. Three to 8 weeks after placement of the flaps, the blood supply should be interrupted by cutting the base of the flap at the limbus. This can usually be done with topical anesthesia. Cutting off the blood supply will allow the conjunctival graft to recede and will lessen the resulting corneal scar. Naturally, an opaque scar will be left in the damaged cornea. Beside these techniques, after the excision of dermoids in dogs, canine amniotic membrane transplantation for corneal reconstruction is another treatment option which has been reported in literature [25].

In this study, a total of 26 cases diagnosed with ocular dermoid, a disorder rarely seen in dogs, were evaluated. The aim of the study is to assess breed and sex distribution and present to veterinary practice the clinical results obtained from the treatment methods used.

MATERIAL and METHODS

The material of the present study composed of 26 eyes with ocular dermoids from 22 dogs of different breeds that were brought to the clinics of Department of Surgery Faculty of Veterinary Medicine, Istanbul University. The breeds of the dogs were: 10 German Shepherds, 4 crossbreeds, 1 Dogo Arjentina, 1 Turkish Shepherd Dog, 1 Gordon Setter, 1 Doberman Pincher, 1 French Bulldog, 1 Golden Retriever, 1 Labrador Retriever, and 1 Rottweiler. 17 dogs were female while the remaining five dogs were male. The ages of the patients varied between 1-month and 11-months-old.

Systematic ophthalmic examinations were carried out on the dogs with complaints including chronic epiphora, ocular discharge and blepharospasm. After the examination, uni- or bilateral locations of the dermoids were determined as corneal, corneoconjunctival, and conjunctival.

Prior to surgery, routine clinical examinations were performed, followed by blood analysis. Depending on the general condition and age of the patient, anaesthesia induction was carried out using xylazine/ketamine HCl or propofol. Following endotracheal intubation, general anaesthesia was achieved with 4% isoflurane and maintained at 2%. The patients were placed on the operation table with the affected eye uppermost and their heads were positioned for the surgeon to operate with ease. In order to determine the width and particularly the depth of the lesion, an operation microscope was used.

Following asepsis and antisepsis of the ocular bulbus, the lateral canthus was opened up via canthotomy and eye retractors were put in place. Once fixation of the eye was established, the corneal, corneoconjunctival and/or conjunctival tissue was resected using a cornea knife. Lesions on the cornea were removed via lamellar superficial keratectomy (partial incision keratectomy) *(Fig.1)*. In order to encourage re-epithelization and vascularisation, fluorescein -positive corneal defects, larger than 25% of the corneal surface, were closed with a bulbar pedicle graft using simple interrupted sutures. Cases with defects less than 25% of the cornea were treated with tarsorraphy alone. Conjunctival defects were closed using simple interrupted sutures.

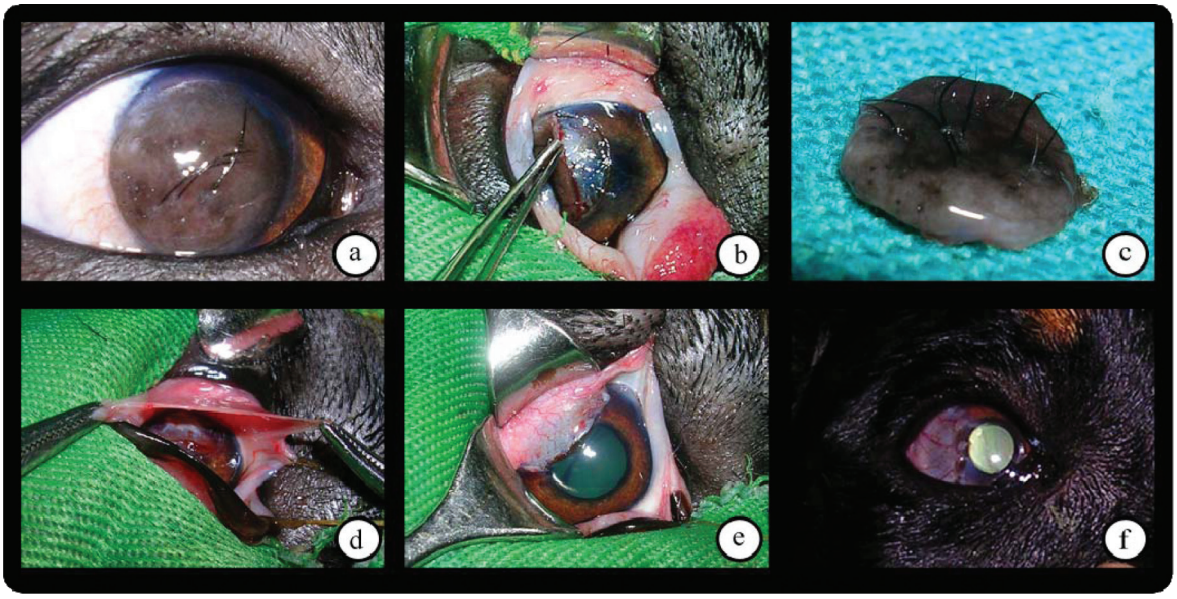

Fig 1. Case no. 6: 5-months-old Dobermann Pincher, **a-** Before excision of the corneal dermoid, **b-** Superficial keratectomy, **c-** The excised dermoid, **d,e-** Preparing the pedicle conjunctival flap, suturing to the cornea, **f-** Postoperative follow-up at 2nd week

Şekil 1. Olgu no. 6: 5 aylık Dobermann Pincher, **a-** Korneal dermoidin eksizyonundan önce, **b-** Süperfisiyel keratektomi, **c-** Eksize edilen dermoid, **d,e-**Konjunktival flebin hazırlanışı ve ve korneaya dikilmesi, **f-** 2 hafta sonraki postoperatif görünüm

Histopathologically, tissue samples were fixed in formalin-saline solution. After being routinely processed they were then embedded in paraffin. Sections of 4-5 mm in thickness were taken using a rotary microtome and then stained with hematoxylin&eosin to be evaluated by light microscopy.

In the post-operative period, ciprofloxacin ophthalmic drops (Siprogut®, Bilim, İstanbul/ Turkey) were administered for 10 days. In cases where a flap had been applied, flap connections were terminated at the end of week 2. In order to increase the resolution of the developing granulation tissue and decrease scar tissue, dexamethasone sodium phosphate (Onadron®, ophtalmic, İ.E.Ulugay, İstanbul) was also added to the treatment. The eye was protected from damage by placing an Elizabethan collar on the patients for 3-4 weeks in the post-operative period.

RESULTS

Following examination, 4 patients were diagnosed with bilateral and the other 18 with unilateral ocular dermoids. Of the cases, 16 were corneal (Mann tip II), 7 conjunctival (Mann tip I) and 3 cornea-conjunctival (Mann tip I-II). None of the cases exhibited a Type III lesion. In Case No. 20 with bilateral dermoids, one dermoid was observed in the medial angle of the left eye and 2 more in the lateral angle *(Fig. 2a)*. In 6 of the cases, in addition to ocular dermoids, entropion, follicular conjunctivitis, iris-to-iris bilateral persistent pupillary membrane (PPM) *(Fig. 2b,c)*, hyperplasia of the Harder gland and pigmentation in the cornea was also observed. All lesions except PPM were treated at the same time. Three cases were not operated on due to the owners' refusal.

Gender distribution revealed that 17 of the cases (77%) were female.

The mean age of the patients was 4.5 months.

Breed distribution showed that 11 of the cases (50%) were German Shepherd dogs *(Table 1)*. It was discovered that the sire of 4 of the patients known to be littermates had also been brought to the clinic with the same complaint two years previously.

Except one (Case No. 22) all the unilateral dermoids were located on the right eye.

Post-operative follow-up examinations of the patients were carried out on days 7, 14, 21 and at weeks 4 and 5. Patients that had undergone tarsorraphy had their sutures removed on day 7. Cases given conjunctival flaps had the flaps released on day 14.

In the follow-up examinations of the patients carried out on days 14 and 21, epithelization of the cornea was seen to be complete *(Table 2)*.

Histological examination revealed characteristics of normal epidermis with a keratinizing squamous epithelium with melanin pigmentation *(Fig. 3a-d)*. Beneath the epithelium there was dense connective tissue. The corneal mass contained numerous, well-developed hair follicles and deep and superficial adnexal structures.

Temporary opacity was observed in all of the corneal dermoid cases. The cornea started to regain its transparency between weeks 4 and 5. Case No. 18 exhibited recurrence on two occasions and was re-operated on *(Table 2)*.

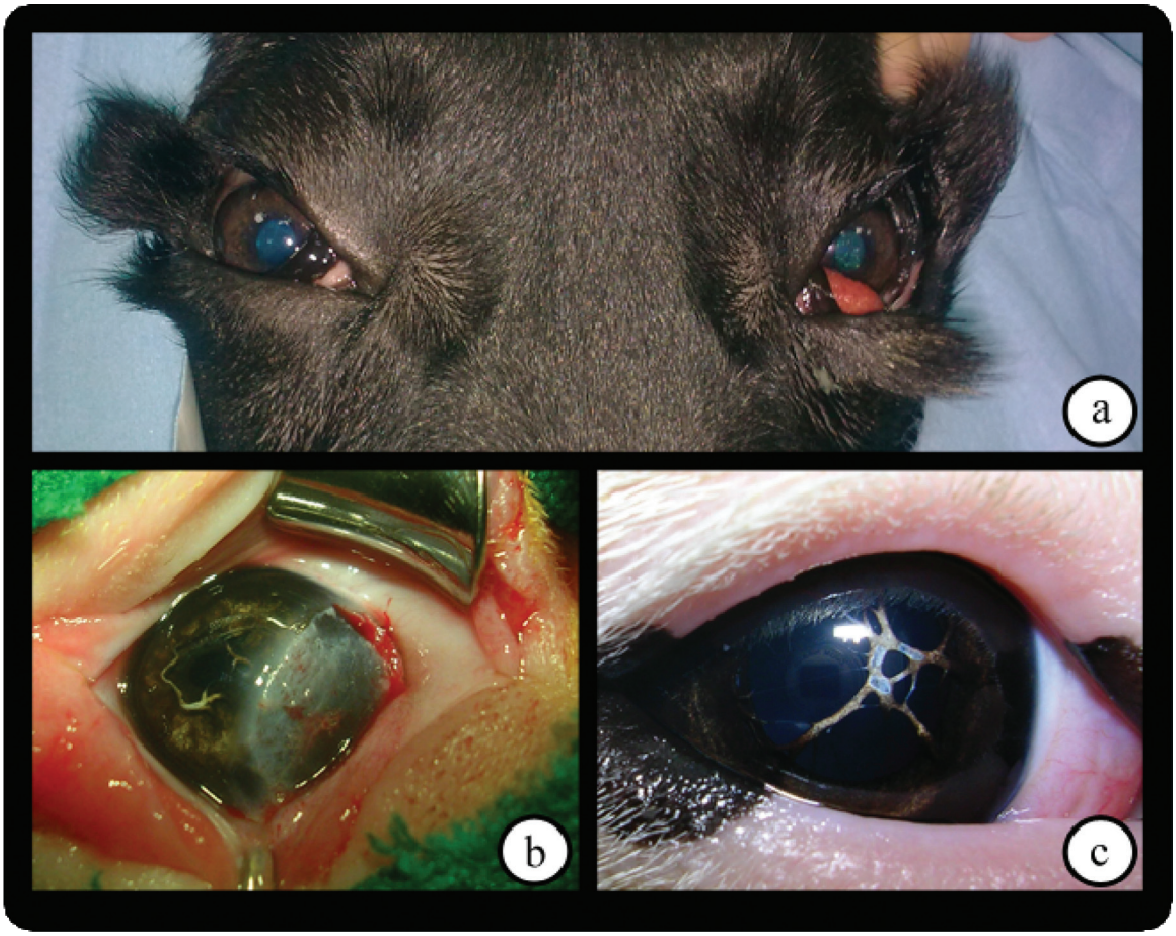

Fig 2. a- Case no. 20: 7-months-old Labrador Retriever, with bilateral dermoids, one in the medial and one in the lateral angle and hyperplasia of the Harder gland on the left eye and 2 more in the lateral angle in the right eye, **b,c-** Case no:9, 4-months-old Dogo Arjantina after superficial keratetomy on the left eye and the right eye with iris-to-iris persistent pupillary membrane (PPM)

Şekil 2. a- Olgu no. 20: 7 aylık Labrador Retriever, bilateral dermoid, sol gözün mediyal ve lateral açısında 1'er adet, sağ gözün lateral açısında 2 adet, sol gözde Harder bezi hiperplazisi, **b,c-** Olgu no: 9, 4 aylık Dogo Arjantina, sol gözde süperfisiyel keratektomiden sonraki görünüm, sağ gözde iristen irise persistent pupillar membran

Conjunctivitis that responded to medication developed in Case Nos. 1 and 20. Follow-up examination of Case No. 10 after day 14 and those of the remaining cases after week 5 could not be done.The patient owners contacted by telephone reported problem-free eyesight in their dogs.

DISCUSSION

Ocular dermoids are well-documented in humans [4,11] but they have been reported less frequently in animal species [9]. Congenital ocular abnormalities such as ocular dermoids are those noted at birth or within a few weeks of life and may occur accompanying ocular malformations [2,4,16]. The iris-to-iris persistent pupillary membrane encountered in one case (Case No. 9) confirmed this. Although no abnormalities were present in the routine pre-operative assessment of Case No. 20, which had bilateral conjunctival dermoids and various eye lesions, the patient died due to intra-operative cardiac arrest. Necropsy of the patient could not be carried, in accordance with the patient owner's wishes, and it was suspected that this patient may have had other congenital abnormalities.

In dogs, there appears to be a breed predisposition to ocular dermoids in the German shepherd dog (GSD), Saint Bernard (SB), golden retriever and dachshunds [4,22]. It is commonly believed that, this disease is generally congenital, but not hereditary [6]. Considering the breed distribution of the dogs included in this study, the facts that German Shepherd Dogs formed the majority of cases and that similar lesions were encountered in 4 puppies from the same litter, as well as in their sire, confirm the hereditary nature of the condition. For the eradication of the disorder, the authors recommend that German Shepherd Dogs with a history of congenital choristoma should not be used for breeding.

The ocular dermoid observed in the Turkish Shepherd dog in our study is the second case to be reported [14].The present case affords a second example of this condition.

In this study, lateral canthotomy was performed prior to surgical excision of conjunctival dermoids located, in particular,

Table 1. *Age, breed, sex variations, the localization of the dermoids, treatment options and additional ocular lesions of the cases*
Tablo 1. *Olguların ırk, yaş ve cinsiyet dağılımı, dermoid lokalizasyonu, sağaltım yöntemi ve diğer oküler lezyonlar*

No	Age (Month)	Breed	Sex	Side		Treatment	Note
				Right	Left		
1	5	Turkish Kangal	Female	Lateral Canthus	(-)	E	Entropium
2	1	Crossbreed	Female	Cornea + Lateral Canthus	(-)	OR	
3	3	German Shepherds	Female	Cornea	(-)	SK + PCF+T	
4	3	German Shepherds	Male	Cornea + Lateral Canthus	(-)	SK + T	
5	11	Crossbreed	Female	Lateral Canthus	(-)	OR	
6	5	Dobermann Pincher	Female	Cornea	(-)	SK + PCF	Follicular conjunctivitis
7	5	French bulldog	Male	Cornea	(-)	SK + MT	
8	6	Golden Retriever	Female	Medial Canthus	(-)	OR	
9	4	Dogo Arjantina	Female	Cornea	(-)	SK + PCF	Bilateral PPM Follicular conjunctivitis
10	10	Crossbreed	Male	Cornea	(-)	SK + PCF + T	
11	5	German Shepherds	Female	Cornea	(-)	SK + T	
12	3	German Shepherds	Female	Cornea	Cornea	SK + PCF + T (right) SK + MT (left)	
13	3	German Shepherds	Female	Cornea	Cornea	SK + MT (both)	
14	3	German Shepherds	Male	Cornea	(-)	SK + PCF + T	
15	3	German Shepherds	Female	Cornea	(-)	SK + PCF	
16	3	German Shepherds	Female	Cornea + Lateral Canthus	(-)	SK + E + T	
17	2	German Shepherds	Female	Cornea	(-)	SK + T	
18	4	German Shepherds	Male	Lateral Canthus	(-)	E	Recurred twice
19	6	German Shepherds	Female	Cornea	Cornea	SK + PCF + T (right) SK + MT (left)	
20	7	Labrador Retriever	Female	Lateral Canthus	Lateral Canthus, Medial Canthus	E (both)	Prolapse of nictitans gland (Cherry eye), Follicular conjunctivitis EX
21	7	Rottweiler	Female	Cornea	(-)	SK + PCF	Follicular conjunctivitis, Cherry eye
22	5	Gordon Setter	Female	(-)	Lateral Canthus	E	Corneal pigmentation

SK*= superficial keratectomy,* ***PCF****= pedicle conjunctival flap,* ***E****= excision,* ***T****= tarsorrhaphy,* ***MT****= medical treatment,* ***OR****= owner's refusal*

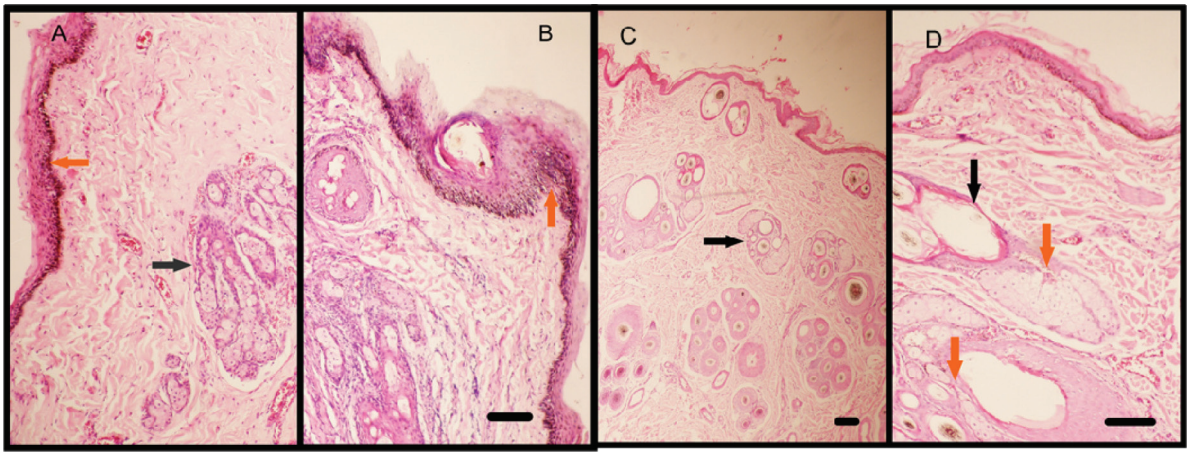

Fig 3. Histopathological view **a,b**, *Orange arrow:* dense melanin pigmentation of basal cells, *Black arrow:* sebaceous glands, *Bar:* 100 µm, **c-** *Black arrow:* hair follicle and sebaceous glands, **d-** *Black arrow:* keratinization in the hair follicle, *Orange arrow:* sebaceous glands

Şekil 3. Histopatolojik görünüm, **a,b-** *Turuncu ok:* epidermisin bazal hücrelerinde yoğun melanin pigmentasyonu, *Siyah ok:* yağ bezleri, *Bar:* 100 µm, **c-***Siyah ok:* kıl folliküleri ve yağ bezleri, **d-** *Siyah ok:* Kıl folliküllerinde keratinizasyon, *turuncu ok:* yağ bezleri, *Bar:* 100 µm

Table 2. *Age, breed, sex variations, the healing time of the corneal and conjunctival defects*
Tablo 2. *Olguların ırk, yaş ve cinsiyet dağılımı, korneal ve konjuntival defektlerin iyilişme süreleri*

No	Age (Month)	Breed	Sex	Side		Conjunctival Defects[a]	Corneal Defects[b]	
				Right (R)	Left (L)		Epithelialization[c] (Day)	NCT[d] (4.- 5. weeks)
1	5	Turkish Kangal	Female	Lateral Canthus	**(-)**	C	-	-
2	1	Crossbreed	Female	Cornea + Lateral Canthus	**(-)**	OR	-	-
3	3	German Shepherds	Female	Cornea	**(-)**	-	14	(-)
4	3	German Shepherds	Male	Cornea + Lateral Canthus	**(-)**	CH	14	(-)
5	11	Crossbreed	Female	Lateral Canthus	**(-)**	OR		-
6	5	Dobermann Pincher	Female	Cornea	**(-)**	-	14	(+) 5.week (mild cloudiness)
7	5	French Bulldog	Male	Cornea	**(-)**	-	21	(-)
8	6	Golden Retriever	Female	Medial Canthus	**(-)**	OR	-	-
9	4	Dogo Arjantina	Female	Cornea	**(-)**	-	14	(+) 5. week (mild cloudiness)
10	10	Crossbreed	Male	Cornea	**(-)**	-	14	No follow-up
11	5	German Shepherds	Female	Cornea	**(-)**	-	14	(-)
12	3	German Shepherds	Female	Cornea	Cornea	-	14	(-)
13	3	German Shepherds	Female	Cornea	Cornea	-	14 (R) 21 (L)	(-)
14	3	German Shepherds	Male	Cornea	**(-)**	-	14	(+) 5. week (mild cloudiness)
15	3	German Shepherds	Female	Cornea	**(-)**	-	14	(+) 5. week (mild cloudiness)
16	3	German Shepherds	Female	Cornea + Lateral Canthus	**(-)**	CH	21	(-)
17	2	German Shepherds	Female	Cornea	**(-)**	-	14	(-)
18	4	German Shepherds	Male	Lateral Canthus	**(-)**	2x re-operation CH	-	-
19	6	German Shepherds	Female	Cornea	Cornea	-	14	(+) 5. week (mild cloudiness)
20	7	Labrador Retriever	Female	Lateral Canthus	Lateral Kanthus Medial Kanthus	-	-	-
21	7	Rottweiler	Female	Cornea	**(-)**	-	21	(-)
22	5	Gordon Setter	Female	(-)	Lateral Kanthus	CH	-	-

*[a] - conjunctival defects, **CH**- complete healing, **C** - conjunctivitis, [b] -corneal defects, [c] - the time to complete corneal epithelialization, [d] - the time to attain normal corneal transparency*

in the lateral canthus (with the exception of Case No. 4). This technique has not been reported in literature. However, complete excision of the lesion without performing canthotomy was not possible in most cases. The cause of the double recurrence in Case No. 4 suggested that the lesion had not been completely excised due to the lack of a canthotomy. In the light of this experience, the lateral canthotomy technique was added to the surgical procedure in cases where the conjunctival dermoid was located in the lateral canthus, in particular, and no recurrence was observed thereafter.

The findings determined in the microscopical examination of the resected tissue parts were similar to those reported in literature [4,9]. Since these findings have the appearance of normal skin, in the authors' opinion, in order to prevent errors occurring with histopathological diagnosis, when material is sent for examination in cases of this nature, it is imperative to state whether the material has been taken from the cornea, conjunctiva or eyelids.

In the literature review, no information was found regarding sex predisposition in cases of ocular dermoids [22,24]. The female ratio of 77% in this study suggests a female tendency towards ocular dermoids.

Congenital ocular abnormalities such as ocular dermoids are those noted at birth or within a few weeks of life [6]. In this study, the mean age of the patients was determined as 4.5 months. The reason for Case Nos. 5 and 10 being much older than the rest of the patients can be explained by the fact

that these two patients were stray dogs with no owners.

Ocular dermoids usually appear as solitary lesions affecting only one eye [7,22]. In this study, bilaterally located dermoid cases were observed in 4 of the 22 cases this was in keeping with literature.

With the exception of one case (Case No. 10), which could not be followed-up, a link was found between the non-central localization of lesions and recovery without an eyesight-obstructing defect.

In this study, the authors did not evaluate whether any of the dogs were related. Without a history, each animal was diagnosed separately. The higher incidence of cases among certain breeds and the higher incidence in recent years suggest a hereditary predisposition to dermoid development. This study emphasizes the importance of screening for the presence of inherited ocular abnormalities, such as dermoids, especially the German Shepherds prior to breeding.

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RESEARCH ARTICLE

Evaluation of the Effects of Holes of Various Sizes on Fracture Rates in Sheep Femurs

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Summary

Defects in long bones are known to lead to increased risk of pathologic fracture. Holes weaken bones and increase the risk of fracture during bending, especially on exposure to torsional forces. Here, we investigated the effect of holes of varying numbers and sizes drilled into sheep femur bones on the resistance of the bone to torsional forces. Ninety-six fresh sheep femur bones were allocated to 8 groups, which were further subdivided into 4 groups of 3 bones each. In each group, 1 to 4 holes ranging from 2 to 5.5 mm were drilled into the femurs, and the bones were subjected to a rotation test. Forces that caused fractures and the force curves were measured and recorded. The effect of the number and size of the holes drilled in the femurs on the occurrence of fractures was compared using two-way analysis of variance, and Tukey's multiple comparison test was used for multiple comparisons. $P<0.05$ was considered statistically significant. We found that the resistance of a bone to torsional forces decreased significantly with increase in the number and size of the drilled holes ($P<0.001$). The rate of fractures increased as the number and sizes of the holes increased. The resistance of the bones to torsional forces decreased as the number of holes increased. We showed that the size of a defect in a bone is extremely important for torsional resistance and is, in fact, more important than the number of defects.

Keywords: *Fracture, Rotational forces, Bone, Femur, Sheep*

Koyun Femurlarında Çeşitli Boyutlardaki Deliklerin Kırık Oranları Üzerine Etkilerinin Değerlendirilmesi

Özet

Uzun kemiklerdeki defektlerin patololojik kırığa neden olduğu bilinmektedir. Delikler özellikle torsiyonel güçlere maruz kalınca bükülme esnasında kemiği zayıflatır ve kırık riskini artırır. Buradaki çalışmamızda biz koyun femur kemiklerinde drille delinmiş çeşitli sayı ve boyutlardaki deliklerin etkilerini araştırdık. 96 tane taze koyun kemiği her biri ilaveten 3 kemiği içeren 4'lü alt gruplara bölünerek 8 gruba ayrıldı. Her bir grubta 2 mm'den 5.5 mm'ye kadar değişen 1'den 4'e kadar delikler femurlar delinerek açıldı ve kemikler rotasyon testine tabi tutuldular. Kırığa neden olan kuvvetler ve kuvvet eğrileri ölçüldü ve kayıt edildi. Delik açılan femurlarda kırık oluşturan deliklerin boyutları ve sayısının etkisi iki yönlü varyans analizi ve Tukey'in çoklu karşılaştırma testi ile mukayese edildi. $P<0.05$ istatistiksel olarak anlamlı bulundu. Dril ile delinmiş deliklerin boyut ve sayısında artma ile birlikte kemiğin torsiyonel kuvvetlere karşı direncinin önemli derecede azaldığını bulduk ($P<0.001$). Kırık oranı deliklerin sayısı ve boyutu arttıkça artmaktadır. Torsiyonel kuvvetlere karşı kemiğin direnci deliklerin sayısı arttıkça azalmaktadır. Biz bir kemik defektinin boyutunun torsiyonel direnç için oldukça önemli olduğunu ve gerçekte de defektlerin sayısının boyutuna göre daha etkili olduğunu gösterdik.

Anahtar sözcükler: *Kırık, Rotasyonel kuvvet, Kemik, Femur, Koyun*

INTRODUCTION

Defects in long bones are known to increase the risk of pathologic fractures [1-7].When biopsy samples are taken from a bone, a weak point is formed in the biopsied region [7-9]. When osteosynthetic materials are removed fracture risk

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increases [10,11], and this risk is particularly high within the first few months after the removal of osteosynthetic materials [8]. It is well known that a hole drilled into a bone weakens the bone and increases the risk of fracture during bending, especially on exposure to torsional forces. Any hole less than 30% of the bone diameter of the bone increases the risk of fracture by weakening the bone [8].

Bones are typically exposed to several forces in vivo. Under normal conditions, a bone is subjected to cyclical loading, which is a combination of axial and torsional forces [4]. Metastatic defects appear as transcortical holes in long bones, and such defects lead to the formation of pathologic fractures, thereby decreasing the capacity of bones to carry torsional loads [12]. In this study, we investigated the effect of holes with various numbers and sizes drilled into sheep femur bones on the resistance of the bone to torsional forces.

MATERIAL and METHODS

The bones used for testing in this study were taken from the slaughterhouse, and this process did not violate animal rights in any way. In the present study, we used 96 fresh sheep femurs cut into equally sized pieces (75 mm), ensuring that the internal and external diameters of the bones were similar. The bones were divided into 8 groups, each of which was further subdivided into 4 groups (1-4 holes in the range of 2.0 to 5.5 mm. with a 0.5 mm difference between each group) were drilled in the bones. The holes were drilled in the middle of the bones at 0.5 cm intervals. The ends of the bones were fixed into a device when they were subjected to rotation tests. Torsion tests were carried out on a Tec equipment SMS21 model torsion testing machine, and the bones were securely gripped in a special grip arrangement *(Fig. 1A, 1B)*. The tests were carried out at a torsional speed of 30°/min. The torque and the corresponding torsion were continuously measured during the tests by appropriate devices attached to torsion testing machine, and the obtained values were transferred to a computer; software was used to evaluate the results. The test endpoint was partial or complete fracture of the bone identified by a drop in torque. During the application of simultaneous torsional forces, which were increased evenly in each test group, the magnitudes of the forces leading to fracture and their angles were recorded. Statistical analysis was carried out using NCSS 2007 software (NCSS).

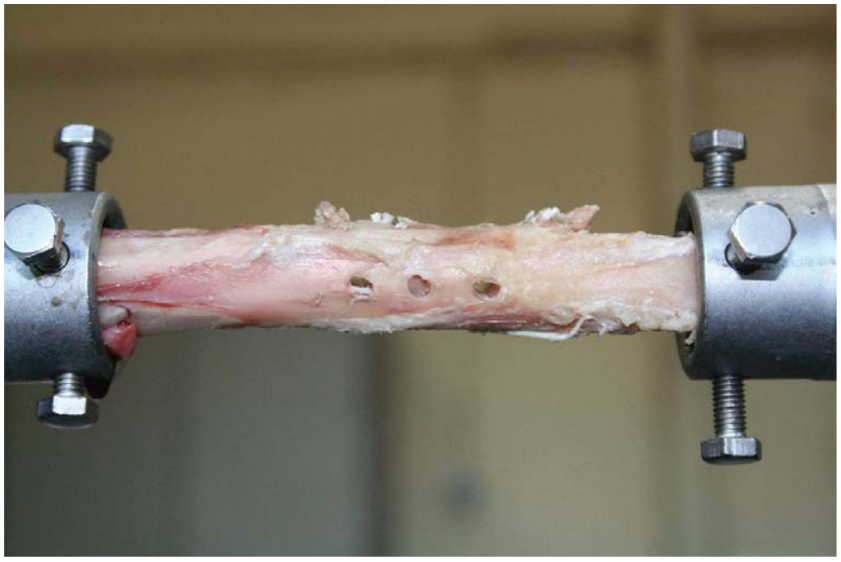

Fig 1A. The equipment used to fix the femur bones before the torsion test

Şekil 1 A. Torsiyon testinden önce femur kemiklerini tespit için kullanılan sistem

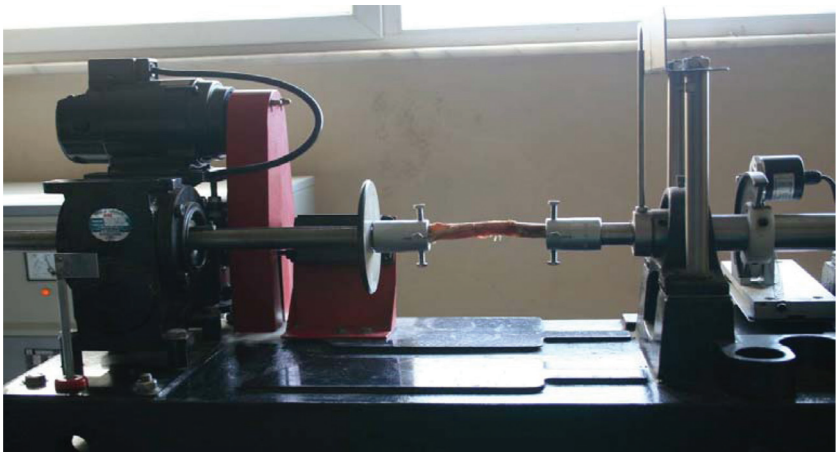

Fig 1B. The equipment used for the torsion test

Şekil 1B. Torsiyon testi için kullanılan cihaz

Descriptive statistical methods (mean±standard deviation [SD]) were used for data analysis. The effect of the number and sizes of holes drilled in the femurs on the occurrence of fractures was compared using two-way analysis of variance (ANOVA); for multiple comparisons, Tukey's multiple com-parison test was used. P<0.05 was considered statistically significant.

RESULTS

In the present study, we investigated whether the number and size of holes drilled into the bone had any effect on the development of fractures. Two-way ANOVA *(Table 1)* showed a statistically significant difference in the occurrence of fractures with different mean hole sizes (P=0.0001). Increasing the hole size resulted is progressively decreasing bone endurance for each hole size (*Table 1* and *2*). Additionally, increasing the number of holes per hole size, also decreased bone endurance *(Fig. 2)*.

Tukey's multiple comparison test was used to evaluate the differences between the groups with varying numbers and sizes of drilled holes *(Table 3)*.

Statistical evaluation for hole number related fracture rates showed significant differences between all the groups analyzed , except 3 versus 4 holes (P: 0.143) *(Table 3)*.

Table 1. *Significant differences in the mean values (± SD) of drilled hole diameters in bones*

Tablo 1. *Kemiklerde açılan deliklerin çaplarının ortalama değerlerinde (± SD) önemli farklılıklar*

Source	Type II Sum of Squares	df	Mean Square	F	*P* value
Corrected Model	517.83	31	16.70	926.94	0.0001
Intercept	1960.23	1	1960.23	108775.98	0.0001
mm	380.75	7	54.39	3018.34	0.0001
Hole	79.81	3	26.60	1476.15	0.0001
mm Hole	57.277	21	2.73	151.35	0.0001

Table 2. *The mean values (± SD) of drilled hole diameters sheep femurs*

Tablo 2. *Koyun femurlarında açılan deliklerin çaplarının ortalama değerleri (± SD)*

Diameter of Holes	1 Hole	2 Holes	3 Holes	4 Holes
2 mm	8.3±0.2	7.867±0.058	7.7±0.2	7.4±0.1
2.5 mm	7.833±0.153	7.1±0.1	5.433±0.153	5.1±0.1
3 mm	7.8±0.2	6.433±0.153	2.333±0.208	1.933±0.058
3.5 mm	6±0.1	5.767±0.058	5.4±0.2	4.9±0.2
4 mm	5.9±0.1	5.433±0.153	4.5±0.1	2.733±0.153
4.5 mm	5.6±0.1	3.133±0.153	2.6±0.1	2.267±0.153
5 mm	3±0.1	2.967±0.058	2.633±0.153	2.267±0.058
5.5 mm	1.567±0.058	1.233±0.153	0.8±0.1	0.667±0.058

Table 3. *The relationship between the number of drilled holes and fracture rate*

Tablo 3. *Açılan delik sayısı ile kırık oranı arasındaki ilişki*

Tukey's HSD Test	*P* Value for Fracture Rate (Nm)
1 Hole vs. 2 Holes	0.011
1 Hole vs. 3 Holes	0.0001
1 Hole vs. 4 Holes	0.0001
2 Holes vs. 3 Holes	0.0001
2 Holes vs. 4 Holes	0.0001
3 Holes vs. 4 Holes	0.143 (NS)

NS: *not significant*

DISCUSSION

Empty screw holes in bones are known to increase stress in the involved region [3,11,13-15]. Forming a stress riser, these regions render the bone susceptible to torsion and tension [16]. These defects may appear radiologically for 6 or more months after removal of screws from the bone, and fractures may occur along these defects [3]. It has been observed that the torsional force of bones with residual screw holes decreases by 55% in dog femurs in which 2.6 mm and 3.6 mm holes are drilled [14]. Radiologically, metastatic defects also appear as transcortical holes in long bones. These defects decrease the capacity of

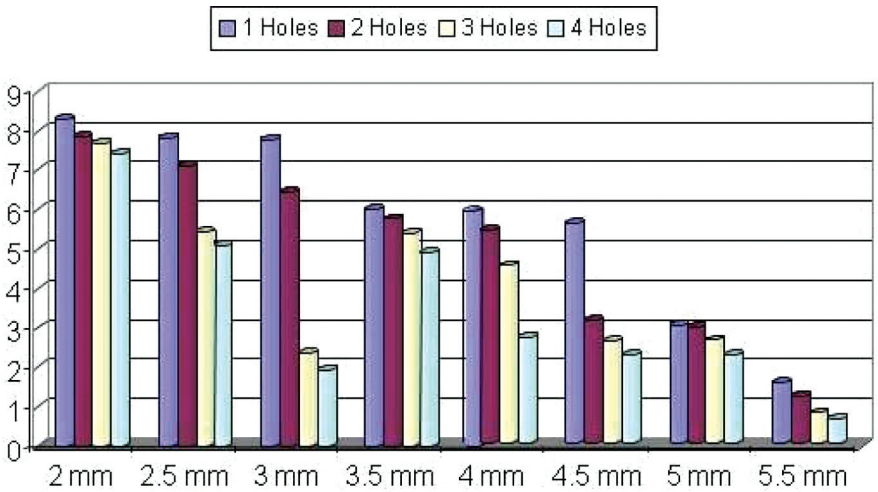

Fig 2. The relationship of the number and diameter of holes with the resistance against torsional forces

Şekil 2. Torsiyonel güçlere karşı oluşan direnç ile birlikte deliklerin çapları ve sayısının ilişkisi

bones to endure torsional force, thus producing pathological fractures [4,17].

In the present study, we demonstrated that when torsional forces were applied on bones, the rate of bone fractures was influenced by the size of the drilled holes. In addition, as the number of holes in the bone increased, even lower torsional forces could cause a fracture (*Table 1* and *2; Fig. 2*). By using Tukey's multiple comparison test, we investigated the relationship between the sizes of holes and the fracture rate. Increasing the size of the drilled hole was significantly correlated to increased rate of fracture *(Table 2)*. Therefore lower forces were sufficient to induce bone fracture. As the number of holes increasing fracture rate also increase *(Table 3)*. As the increase in the number and size of holes would increase the risk of fracture in long and weight-bearing bones, the significant increase in the occurrence of fractures observed in our study is of clinical importance. However, there was no difference in the rate of fracture between bones with 3 and 4 drilled holes. This may be explained by rotational forces leading to fractures having comparable values in the 3 and 4-hole groups. According to our study results, the presence of more than 1 drilled hole and, more importantly, a large size hole, increased the risk of fracture significantly in long bones. Hopper et al. established that 9.5 mm hole drilled in an equine radius bone had a significantly lower endurance to torsional forces as compared to 6.35 mm hole drilled. However, they found no significant relationship between endurance and the number of holes drilled [18].

Usually, redrilling the holes following screw removal eliminates fibrous tissue membranes and is an important surgical procedure for accelerated bone healing. Such procedures prevent the formation of regions of stress concentration in their in vitro study of sheep femurs demonstrated that transcortical drill holes decreased endurance against torsional forces [16,19]. It is known that in humans, the thickness of the cortical wall decreases with age [4]. Hipp et al. also emphasized the importance of the diameter of the transcortical bone and determined that a bone with a thinner cortex has lower endurance against torsional forces than the one with a thicker cortex. In long bones with transcortical defects, the importance of cortical wall thickness has been evaluated by obtaining the ratio of the endosteal diameter to the periosteal diameter; the mean value was found to be 0.653 in sheep femurs [4]. Various studies have shown that fractures do not generally occur with small holes, indicating that the effect of stress concentration increases with an increase in the size of the hole. In addition, when the geometry of round holes is compared with that of non-round holes, a decrease in stress concentration is observed [19].

Both osteolytic and osteoblastic metastatic lesions occur around areas of density change in the bone [7,12]. In our opinion, drilling is especially risky for osteolytic lesions and can lead to pathologic fractures. In tumor surgery, cortical destruction involving 50% of the cortex requires prophylactic stabilization [7,19]. Osteoporosis can significantly reduce the cortex endurance and deteriorate weight bearing capacity. Such defects, but larger than those of similar cross-section is less than the rotational forces of resistance have been reported [19,20]. However, cortical atrophy has a graeter impact on bone weakness than residual screw holes , following removal of plates and/or screws. Drilling is especially risky for osteolytic lesions (Osteoporotic patients) and can lead to pathological fractures [11,21-23].

In conclusion, the fracture risk in a bone depends not only on the geometry of the lesion but also on other factors, including the quality of bone, tumor biology (if any), and the physical activity of the patient [5]. As observed in our study, any defect in a long bone may reduce the endurance of the bone to biomechanical torsional forces. The size of the defect is extremely important in such endurance; in fact, the size is more important than the number of defects, particularly in cases with metastasis, after biopsy, and after the removal of plates and/or screws.

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RESEARCH ARTICLE

Evaluation of The Dynamic (Overground) Endoscopy Procedure in The Diagnosis of Upper Respiratory Tract Diseases Affecting Performance of Racehorses [1]

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Summary

In this study, efficiencies of dynamic (overground) and resting endoscopic examinations were compared in dynamic upper respiratory tract problems which are a cause of poor performance in race horses. Thirty actively-racing horses which were brought to Turkey Jokey Club Racehorses Hospital with poor performance and abnormal respiratory tract sounds were examined while running and rest. The diagnostic effectiveness of dynamic endoscopy and resting endoscopy procedures were compared for the diagnosis of dynamic obstructions of the upper respiratory tract. Dynamic pharyngeal collapse (DPC) in 5 race horses, third or fourth degree of left laryngeal hemiplegia (LLH) in 3 race horses, second degree of axial deviation of aryepiglottic folds (ADAF) in 3 racehorses, rostral displacement of the palatopharyngeal arch (RDPA) in 1 racehorse, and dorsal displacement of the soft palate (DDSP) along with first degree of ADAF in 1 racehorse were detected in the mobile endoscopic exams. Dynamic upper respiratory tract problem was detected totally in 13 horses. As a result, it was observed that dynamic upper respiratory tract problems could be safely detected by dynamic endoscopy and it was superior than routine endoscopic examination in racehorses.

Keywords: *Dynamic (overground) endoscopy, Poor performance, Racehorse*

Yarış Atlarında Performansı Etkileyen Üst Solunum Yolu Hastalıklarının Teşhisinde Dinamik Endoskopi Uygulamasının Değerlendirilmesi

Özet

Bu araştırmada, yarış atlarında performans kaybına neden olan dinamik üst solunum yolu problemlerinin teşhisinde dinamik (mobil) ve rutin endoskopi uygulamalarının etkinlikleri karşılaştırıldı. Performans düşüklüğü ve anormal solunum sesi şikâyetleri ile Türkiye Jokey Kulübü Yarış Atları Hastanesi'ne getirilen 30 yarış atı dinlenme halinde ve koşu sırasında değerlendirildi. Üst solunum yolunun dinamik tıkanıklarının teşhisinde, dinamik ve dinlenme halinde yapılan endoskopi uygulamalarının teşhisteki etkinlikleri karşılaştırıldı. Dinamik endoskopik muayenelerde; 5 atta dinamik farengeal kollaps (DPC), 3 atta 3. ve 4. derece sol larengeal hemipleji (LLH), 3 atta 2. derece ariepiglottik kıvrımların aksiyal deviasyonu (ADAF), 1 atta palatofarengeal kıvrımın rostral deplasmanı (RDPA), 1 atta yumuşak damağın dorsal deplasmanı (DDSP) ve 1. derece ADAF'ın birlikte bulunduğu gözlendi. Toplam 13 atta dinamik üst solunum yolu problemi belirlendi. Sonuç olarak, yarış atlarında dinamik üst solunum yolu tıkanıklıklarının, dinamik endoskopi ile güvenli ve rutin endoskopi uygulamasından daha iyi bir şekilde teşhis edilebileceği kanaatine varıldı.

Anahtar sözcükler: *Dinamik (mobil) endoskopi, Performans kaybı, Yarış atı*

INTRODUCTION

A common cause of poor performance in equine athletes occurs when there is an anatomic or functional obstruction of the upper respiratory tract. These abnormalities can be a challenge to accurately diagnose. Previous studies have

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shown that some abnormalities can be seen in the upper respiratory tract only while the horse is exercising, but the upper respiratory tract can look normal at rest [1,2]. The upper airway is most often evaluated in standing horses by resting or dynamic endoscopy. Video endoscopy is an important tool for diagnosing upper respiratory tract abnormalities [3]. High-speed treadmill endoscopy and mobile endoscopy methods are used in upper respiratory tract examination. High-speed treadmill endoscopy (HSTE) is widely accepted as the current gold standard for the diagnosis of diseases of the upper portion of the respiratory tract in horses [4,5]. The diagnosis of the HSTE upper respiratory tract disorders has been very useful in understanding its pathophysiology and its relations with dynamic upper respiratory tract abnormalities [4,6-11]. Although HSTE provides great advantages in the diagnosis of dynamic obstructions of the upper respiratory tract, it also has some disadvantages including its high initial cost, the horse being unable to get used to running on the treadmill, and the failure of the artificial environment created to fully represent the environment that the horse is introduced on the racecourse [4,12,13]. Dynamic respiratory endoscopy or mobile endoscopy is an endoscopic examination method recording the upper respiratory tract region of the horse while the horse exercises under normal exercise conditions or on the racecourse with onboard endoscopy. In mobile endoscopy, the endoscopy unit and the recording unit is attached to the horse. The endoscopy unit is placed into the nasal canal to monitor the larynx and the pharynx. Recording starts before the horse starts training. The horse is trained on the training and/or racecourse, with this equipment attached. The time and distance of the training will be determined in line with the complaints reported by a veterinary surgeon. Dynamic endoscopy is an effective method used to diagnose the obstructions of the dynamic upper respiratory tract such as axial deviation of aryepiglottic folds (ADAF) and dynamic pharyngeal collapse (DPC) that occur during high performance and cannot be detected during endoscopic examination while resting. Dynamic endoscopy allows the examination of the horse during high performance when it suffered dynamic obstructions and the accurate diagnosis of the problem. The heavy weight (14 kg) of the unit and the inability to remotely control the endoscopy probe of the endoscopy unit used in this study are its disadvantages, and these disadvantages were eliminated in different models of the same company. Upper respiratory tract diseases account for 47-49% of abnormalities causing poor performance in horses [15]. Disorders including recurrent laryngeal neuropathy (RLN), dorsal displacement of the soft palate (DDSP), epiglottic entrapment (EE), dynamic pharyngeal collapse (DPC), and rostral displacement of the palatopharyngeal arch (RDPA) are considered to be important abnormalities affecting the race performance of horses [16,17]. With regards to incidence in horses, it was reported that DDSP was seen highest (40%), followed by RLN (20%) [18]. A total of 314 abnormalities were detected by resting endoscopic and high-speed treadmill examinations in a study conducted on 291 horses with poor performance [11]. It was detected 192 abnormalities by high-speed treadmill in this study, 105 of these 192 abnormalities were ADAF (55%), 74 were DDSP (39%), and 65 were LLH (34%).

Dynamic obstructions occurring in the upper respiratory tract are the primary causes of poor performance in racehorses. Endoscopic examinations performed at rest prove insufficient in determining the source of upper respiratory tract problems. This study used the mobile endoscopy unit (Dr. Fritz® ETL-Equine Training Laryngoscope) for the first time in Turkey to investigate the presence of obstructions of the dynamic upper respiratory tract in horses where resting endoscopic examination revealed no findings despite complaints of poor performance. This study evaluates the advantages of dynamic endoscopic examination over resting endoscopic examination. The aim of the study was to investigate the suitability of dynamic endoscopy in the diagnosis of the obstructions of the upper respiratory tract in racehorses during exercise under natural racecourse conditions.

MATERIAL and METHODS

This study was conducted between August 2008 and September 2009 in Istanbul Veliefendi Hippodrome. Ethical approval for the study was obtained from the Ethical Committee, Faculty of Veterinary Medicine, University of Selcuk (September 12, 2006-2006/079).

The animal material of this study consisted of 30 racehorses of different breeds (Thoroughbred, n: 24; Arabian, n: 6) and ages (2-5 years) that were admitted to The Racecourse Hospital of the Turkey Jockey Club with complaints of abnormal respiratory sounds and poor performance. The horses included in this study were stabled within the racetrack and exercised on the polytrack course in the racetrack six days a week. The horses' histories were taken before dynamic upper respiratory tract endoscopy, their physical and laboratory analysis (haemotological, lactic acid, venous blood gas etc.) were made, other abnormalities (including the diseases of the respiratory, cardiovascular, musculoskeletal, or digestive system) that could result in poor performance were studied, and resting upper respiratory tract endoscopies were performed.

Dynamic Endoscopy

In the dynamic upper respiratory tract endoscopy procedure, the mobile endoscopy unit is mounted on to the horse together with its accessories *(Fig. 1)*. The horse was then taken to the racecourse *(Fig. 1)*. Dynamic endoscopic examination was performed while all horses were exercised on a 1.870 meter polytrack racecourse. The horses were ridden for 30 min on the racecourse before dynamic endoscopic examination. Then the horses cantered for

Fig 1. The horse in gallop on the race course (polytrack race course) with mobile endoscopy unit

Şekil 1. At mobil endoskopi cihazı ile yarış sahasında (polytrack yarış pisti) gallop yaparken

approximately 800 m, and galloped over 1.000 m the last 600 m at a fast gallop. Changes in the upper respiratory tract were recorded on a mobile endoscopy unit. Dynamic changes in the upper respiratory tract during exercise were evaluated based on these recordings. This exercise test was organized as a race experience, with the only difference being the horses running alone.

Dr. Fritz® ETL-Equine Training Laryngoscope consists of a recording unit, a 9 mm diameter 210 cm semi-rigid malleable insertion tube (Video bronco-laryngoscope), a battery, a saddle, a harness, a special snaffle and a laptop computer, with software recording and allowing the examination of patient data. An LCD screen on the recording unit enables the endoscope to be placed properly into the nasal canal and the resting upper respiratory tract examined before the workout. A full-charged system battery allows recording for about half an hour. The endoscope, recording unit, and the battery are mounted on top of a saddle specifically designed for racehorses. After the endoscope mounted on top of the saddle is connected to the recording unit, it is attached to the horse's mane and extended to the forehead section through the ears. Here, it is placed on the snaffle with the help of a cane-like apparatus. The endoscope is placed on this apparatus, aligned with the nostrils, and fixed after being positioned into the nasal canal. An endoscope mounted in this fashion will not slip out of the nasal canal during the gallop. The camera is positioned inside the nasal canal with the direction arms inside the endoscopy locked. The time from start until completion of the recording is about 5 min. The recorded images were evaluated for detection of abnormalities after the examination.

RESULTS

In this study, all horses accepted the dynamic endoscopy equipment and the mobile endoscope was well tolerated. While dynamic upper respiratory tract abnormalities (43.3%) were found in 13 horses in dynamic endoscopic examination, 17 horses (56.6%) had poor performance and/or abnormal respiratory sound complaints, no obstructive dynamic upper respiratory tract symptoms were seen. No obstructive upper respiratory tract disorders were observed in 7 (23.3%) racehorses despite complaints of poor performance and abnormal respiratory sound.

Using mobile endoscopy unit, DPC was diagnosed in 5 race horses (16.6%), third and fourth degree of LLH in 3 race horses (10%), second degree of ADAF in 3 race horses (10%) *(Fig. 2)*, RDPA in 1 race horse (3.3%), and DDSP *(Fig. 3)* along with first degree of ADAF in 1 race horse.

Resting endoscopy revealed second degree lymphoid hyperplasia in 11 race horses (36.6%), LLH in 3 race horses (10%), and partial obstruction of rima glottis by the left arytenoid cartilage from RDPA in 1 race horse (3.3%). The diagnosis rate of dynamic upper airway obstructions was only 13.3% (n: 4) in resting endoscopy.

DISCUSSION

In this study, dynamic endoscopic procedure was applied successfully under the racetrack conditions. The mobile endoscope was well tolerated in all horses. The study confirmed the efficacy of dynamic endoscopy for the diagnosis of dynamic obstructions of the upper respiratory tract (DO-URT) in racehorses.

Detection of no abnormalities in resting endoscopic examination of the upper respiratory tract does not imply that poor performance is not caused by the upper respiratory tract and/or that the upper respiratory tract is healthy. Disorders including laryngeal hemiplegia, subepiglottic cyst, and arytenoid chondropathy can be detected by endoscopic examination during resting. However, resting endoscopic examination proves insufficient for the

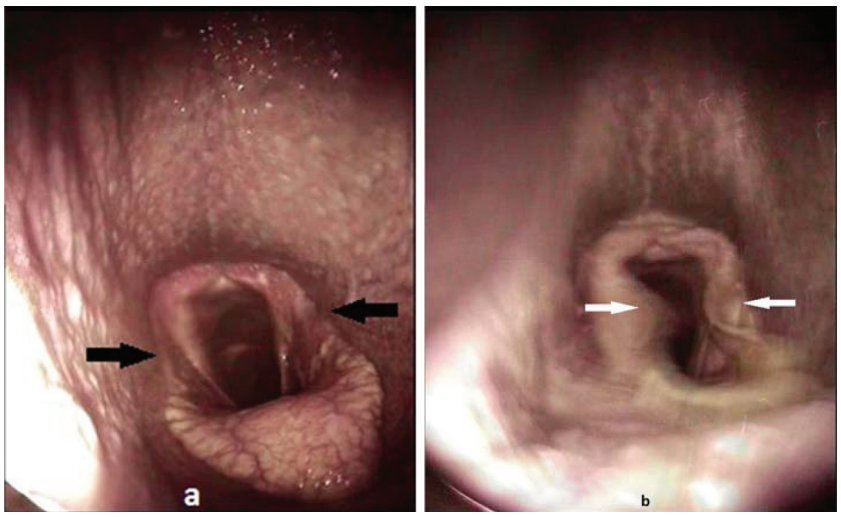

Fig 2. Normal position of the aryepiglottic folds at resting endoscopy *(a, black arrows)*. Axial deviation of aryepiglottic folds at exercise with dynamic endoscopy *(b, white arrows)*

Şekil 2. Dinlenme halinde yapılan endoskopik muayenede ariepiglottik kıvrımların normal pozisyonu *(a, siyah ok uçları)*. Dinamik endoskopide, egzersiz esnasında, ariepiglottik kıvrımların aksiyal deviasyonu *(b, beyaz ok uçları)*

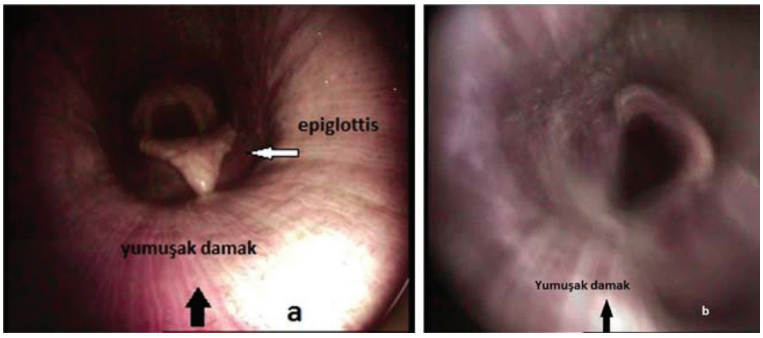

Fig 3. Normal position of the soft palate at resting endoscopy *(a, black arrow)* and epiglottis *(a, white arrow)*. Dorsal displacement of the soft palate at exercise with dynamic endoscopy *(b, black arrow)*

Şekil 3. Dinlenme halinde yapılan endoskopik muayenede yumuşak damağın normal pozisyonu *(a, siyah ok ucu)* ve epiglottis *(a, beyaz ok ucu)*. Dinamik endoskopide, egzersiz esnasında, yumuşak damağın dorsal deplasmanı *(b, siyah ok ucu)*

detection of dynamic obstructions including DPC, DDSP, ADAF, and epiglottic retroversion appearing during high performance [13,14,22]. In this case, dynamic endoscopic examination becomes compulsory. In this study, horses diagnosed with DPC and ADAF were examined many times by resting endoscopy, but a definitive diagnosis could not be made although accurate diagnoses could be made after dynamic endoscopic examination by mobile endoscopy. The cause of poor performance and abnormal respiratory sound was diagnosed by dynamic endoscopic examination.

Some researchers reported that high quality images are important in mobile endoscopic studies [13]. Because of that automatic water pump with a fixed flushing system would be useful [12,14]. During this study, images could not be collected in one case due to mucus accumulation in front of the camera, so the examination was repeated. Therefore, the addition of a fixed flushing system to the current system would be beneficial.

There are reports suggesting that dynamic upper respiratory tract abnormalities occurred during exercise (with high speed treadmill or dynamic respiratory scope (DRS)) in horses, that they should be considered at different exercise rates (during trot, gallop, jump, endurance), and that they are affected by sudden acceleration and exercising conditions [13,19]. The reported abnormal respiratory sounds and poor performance complaints were mostly experienced during the final meters of the race. So, in this study, the horses were put into a race experience/trial (trot, canter and gallop (last 600 m at a fast gallop) of 1.870 m on the racecourse to ensure that the horses were optimally stressed. Detection of dynamic upper respiratory tract abnormality in 13 of 30 horses (43.3%) in this study shows that the exercise during mobile endoscopic examination was satisfactory, and that exercises at this level were the minimum requirements for the assessment of dynamic upper respiratory tract abnormalities.

Upper airway obstructions are a significant cause of poor performance in racehorses [11,16,17,20]. In addition, abnormal respiratory sounds during exercise are frequently associated with upper airway disorders [1]. While some authors [3,20] report that there was no history of abnormal noise production in some horses with DDSP, Lumsden et al.[19] suggest that up to 30% of horses with DDSP make audible abnormal respiratory sounds during exercise. Lane et al.[4] have found a history of abnormal respiratory noise in 85% and 75% of horses with DDSP and palatal instability (PI), respectively. Kannegieter and Dore [16] reported that 89% of 75 horses with a history of abnormal respiratory noise had dynamic collapse in the upper respiratory tract (URT) during exercise whereas Tan et al.[11] reported that 82% of 146 horses had untoward respiratory noises, and only 49% of the horses produced no noises. In this study, 70% of racehorses diagnosed with DO-URT had a history of abnormal respiratory noise. Lane et al.[4] reported that there were

limitations in the evaluation of respiratory sounds by ear, and perception or interpretation of noises by the jockeys or trainers is not always reliable. In this study, too, some jockeys and trainers stated that an abnormal respiratory sound was heard from the horse controlled by the jockey until the last 600 m of the race, and that the abnormal respiratory sound disappeared when the horse stopped pulling and gained more speed during the last 600 m. This is interpreted by Strand et al.[21], who argued that the poll flexion by the jockey created pressure on the trachea and it caused mild and intermittent airway abnormalities resulting in dynamic upper respiratory tract disorders like dorsal pharyngeal collapse, bilateral aryepiglottic fold collapse, and bilateral vocal fold collapse. Strand et al.[21] also reported that such problems were not seen when the horse's head was maintained in normal position. This study demonstrates how effective the environmental factors (particularly the jockeys) are in the creation of dynamic upper respiratory tract dysfunctions that the horses are exposed to when running during exercise or on the racecourse. To this end, it is considered that dynamic endoscopy is an important tool for the accurate diagnosis of abnormal respiratory sounds and the problems during exercise and racing.

DO-URT can be diagnosed with the help of DRS and HSTE [5,11,12,14,22,23]. The primary DO-URT cases seen in horses are reported to be DDSP, Laryngeal Collapse (LC), Vocal Cord Collapse (VCC), LLH, ADAF, PI, and Epiglottic abnormalities [1,4,11,12,14,17,18]. DO-URT can also be seen alone or as multiple cases [4,11,12,14,22]. DO-URT cases identified in this study are DPC (16.6%), LLH (10%), DDSP (3.3%), ADAF (10%), DDSP along with ADAF (3.3%) and RDPA (3.3%), respectively. This data correlates with field studies and shows that the DO-URT cases observed in Thoroughbred and Arabian racehorses can be diagnosed by on-board mobile endoscopy.

This study found, by dynamic endoscopy, that upper respiratory tract hyperemia and secretion affected performance in 7 racecourses (23.3%) with no DO-URT. Desmaizieres et al.[12] reported that symptoms like abnormal mucus production or blood in the trachea in horses with negative dynamic obstructions and poor performance were indicators of the lower respiratory tract and that they could be quickly defined by DRS. In this study, symptoms described in 7 race horses with negative DO-URT are in support of this opinion.

Many researchers reported that DO-URT can be diagnosed easily by DRS during any type of performance. This technique can be used to diagnose common causes of URT associated with poor performance in horses during normal training. DRS equipment is safe and reliable. Toleration of the DRS was excellent. The results of this study verify that mobile endoscopy unit is a secure diagnostic tool and can be used in the diagnosis of dynamic upper respiratory tract abnormalities in horses in normal racecourse conditions. The horse-mounted unit and endoscopy were well-tolerated by the horse, jockey, and the horse owners, with no negative experiences. This study concludes that the mobile endoscopy unit is a practical, safe, and useful diagnostic tool that can be used to diagnose DO-URT abnormalities in horses in normal racecourse conditions. Considering that DO-URT cases are suddenly-emerging dynamic disorders [13], it is clear that examinations made by DRS or mobile endoscopic methods and in natural racecourse conditions supply useful data about DO-URT.

It is concluded that overground endoscopic examination enables safely to diagnose of DO URT at exercise in natural conditions and this technique has substantial implications for future clinical diagnosis, an enormous potential for further clinical research, and the most suitable treatment options of DO-URT pathology in racehorses.

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RESEARCH ARTICLE

Comparison of Intravenous Versus Intraperitoneal Interleukin-10 Gene Delivery in Mouse Model of Sepsis

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Summary

The most novel approach utilizing IL-10 in sepsis is IL-10 gene delivery in experimental model of sepsis. In our study, we aimed to compare kinetics of intravenous versus intraperitoneal delivery of IL-10 gene transfer in early stages of sepsis. This is a prospective controlled experimental study. Six groups were gathered with 20 Swiss-Albino female mice. Intra-abdominal sepsis was induced by cecal ligation and puncture (CLP). Animals had either intraperitoneal or intravenous IL-10 liposomal gene transfer. Animals were sacrified 24 h after injections, followed by harvest of lung,liver,spleen,vena cava tissues. Immunostaining revealed more prominent staining in liver after intraperitoneal delivery. All endothelial tissues stained with intravenous delivery. There was striking difference between tissue expressions of transgene of animals in CLP intravenous group when compared to other groups. Our results point out that pro-inflammatory action of IL-10 is prominent in intravenous gene delivery which shows itself with induction of zyon. IL-10 still may harbor therapeutical potential which still needs to be explored.

Keywords: *Interleukin-10, Interleukin-6, Tumor Necrosis Factor-alpha, Sepsis, Gene therapy*

Farelerde İntraabdominal Sepsis Modelinde İntravenöz ve İntraperitoneal İnterlökin 10 Lipozom Aracılı Gen Tedavisinin Karşılaştırılması

Özet

Sepsiste IL-10 kullanımına yönelik en yaratıcı yöntem IL-10'un deneysel modelde gen tedavisi şeklinde verilmesidir. Biz çalışmamızda IL-10'un erken sepsiste intravenöz ve intraperitoneal kinetiğini karşılaştırdık. Araştırmamız prospektif kontrollü çalışmadır. Yirmi adet Swiss-Albino dişi fare kullanılarak altı grup oluşturulmuştur. İntra-abdominal sepsis çekal ligasyon ve ponksiyon (ÇLP) yöntemiyle ortaya çıkarılmıştır. Deney hayvanlarına ya intraperitoneal veya intravenöz olarak IL-10 lipozomal gen transferi yapılmıştır. Hayvanlar enjeksiyon sonrası 24. saatte sakrifiye edilmişlerdir. Bunu akciğer, karaciğer, dalak, vena kava dokularının çıkarılması izlemiştir. İmmünhistokimyasal boyamada intraperitoneal verilişten sonra karaciğer boyanmasının daha belirgin olduğu görülmüştür. İntravenöz verilişten sonra tüm endotelyal dokuların boyandığı görülmüştür. Transgenin doku ekspresyonunun ÇLP ve intravenöz enjeksiyon yapılan grupta diğer gruplara göre çok daha belirgin olduğu görülmüştür. Sonuçlarımız aynı zamanda göstermiştir ki IL-10'un pro-inflamatuar etkisi intravenöz verilişte daha belirgindir ve kendisini IL-6 indüksiyonuyla göstermektedir. IL-10 'un halen keşfedilmeyi bekleyen tedavi edici özelliği vardır.

Anahtar sözcükler: *İnterlökin-10, İnterlökin-6, Tümör nekroz faktör alfa,Sepsis, Gen tedavisi*

INTRODUCTION

Sepsis is a systemic illness caused by microbial invasion of parts of human body during the course of bloodstream infection [1]. The classical methods of sepsis treatment include maintenance of systemic perfusion and eradication of

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infectious sources [2].

Systemic inflammatory response syndrome induced by sepsis is characterized by orchesterated release of TNF-α, interleukin (IL) 6, IL-8, and IL-1 [3].

Immunomodulatory therapeutic approaches targeting TNF-α and IL-1 to control inflammatory cytokine response were not proven to be beneficial in humans although demonstrated to be valuable in different rodent models [4,5].

IL-10 is a biphasic immunomodulator cytokine which inhibits Th1 type immune response and pro-inflammatory cytokines like TNFα and IL-1 [6]. IL-10 is first defined as T helper cell derived factor inhibiting cytokine synthesis. IL-10 inhibits T helper cytokines like interferon and IL-12 as well as pro-inflammatory cytokines like TNFα, IL-1 and nitric oxide. IL-10 is considered to have a substantial role in acute illnesses because of these antiinflammatory effects [7].

In the last decade, there has been a substantial effort to identify factors in the host response to infection that could be used for therapy. In this prospective controlled experimental study we aimed to compare kinetics of intravenous (IV) versus intraperitoneal (IP) delivery of IL-10 gene transfer in early stages of sepsis.

MATERIAL and METHODS

Animals and Surgical Procedures

The study was approved by Hacettepe University Ethics commitee with approval number of 2007/42.

There were six groups containing a total of 20 Swiss Albino female mice with weight of 22±5 g. Intraabdominal sepsis was induced by cecal ligation and puncture (CLP) with 22 G needle.

The control group consisted of three sham operated mice where only laparotomy was performed. CLP procedure was performed on the 15 mice in three treatment groups. Nothing other than cecal ligation and puncture (CLP) was performed on mice in group two (n=3). Mice in group three (n=6) had CLP and ip injection of IL-10 plasmid through right lower abdominal quadrant. Animals in group four (n=6) had CLP and iv injection via tail vein. Fifth group had one mouse which received ip injection only. Sixth group had one mouse which received iv injection only.

Injections were applied 24 h after initiation of sepsis with CLP. Animals were sacrified 24 h after injections, followed by harvest of lung, liver, spleen, vena cava tissues. Tissues were divided into 4 pieces for mRNA, protein, DNA and reverse transcriptase polymerase chain reaction (RT-PCR) analyses. National Center for Biotechnology Information numbers of primers used for TNFα and IL-6 were NM_013693 and NM_031168 respectively.

All animals were kept at room temperature and 12-h day/night cycle, having access to food and water ad libitum. All animal procedures were performed according to an institution approved protocol and under strict biological containment.

RNA Isolation and Quantitative Gene Expression Analysis

75-90 mg of sampled tissue was disrupted in a 2ml screw-cap tube containing ceramic beads and 1 ml of Trizol reagent (Invitrogen™). The tissues were rapidly disrupted using a bead-beater and kept at -80°C. The total RNA extractions from all tissues were performed simultaneously upon manufacturer's recommendations and the RNA integrity was assessed and documented via denaturing agarose gel electrophoresis. Following RNA extraction and quality assessment, reverse transcription was accomplished using Improm II reverse transcriptase (Promega™). Briefly, 0.5 µg of oligo dT primers are hybridized to 1 µg of total RNA following an initial denaturation at 70°C and reverse transcribed into cDNA following manufacturer's recommendations.

The amplification of the target genes were achieved using the quantitative PCR technique using the SYBR green dye incorporation method using the primer design and amplification strategy described before. The expression of the genes of interest were normalized to the expression of the beta actin gene and the primers and conditions regarding the technique were available upon request by e-mail. The PCR reactions were carried out on a Rotor gene 6.000 real-time PCR instrument (Corbett Life Sciences™) using Jumpstart SYBRgreen mix (Sigma™) according to manufacturer's protocols.

Plasmids and Gene Transfer

The human IL-10 cDNA (Genebank accession: NM_000572) is purchased as a GeneStorm vector (Invitrogen, Carlsbad, CA) and subcloned into pCDNA3.1 expression vector together with a C terminal fused 14 aminoacids long V5 epitope to help to discriminate from the endogeneous IL-10. The open reading frame of the expression plasmid is sequenced and amplified in to yield sufficient amount. Injection volumes were 200 µl per mice each containing IL-10 gene carrying pcDNA3.1/GS plasmid vector and antiV5-HRP antibody (hIL-10pcDNA3.1/GS transformed into GeneHogs® *E.coli*) bought from Research Genetics CO. The expression was driven via the cytomegalovirus promoter and contained a 14-amino acid V5 fusion peptide tag at the 3´ end, which assists to trace the transgene expression *(Fig. 1)*. N-(1-[2,3-dioleoyloxyl]propyl) NNNtrimethylammoniummethylsulfate: cholesterol (1:1 molar ratio) liposomal transfection reagent (Sigma Aldrich©, St. Louis, MO) was used according to manufacturer's recommendations to achieve the IP and IV gene transfer 24 h after CLP procedure.

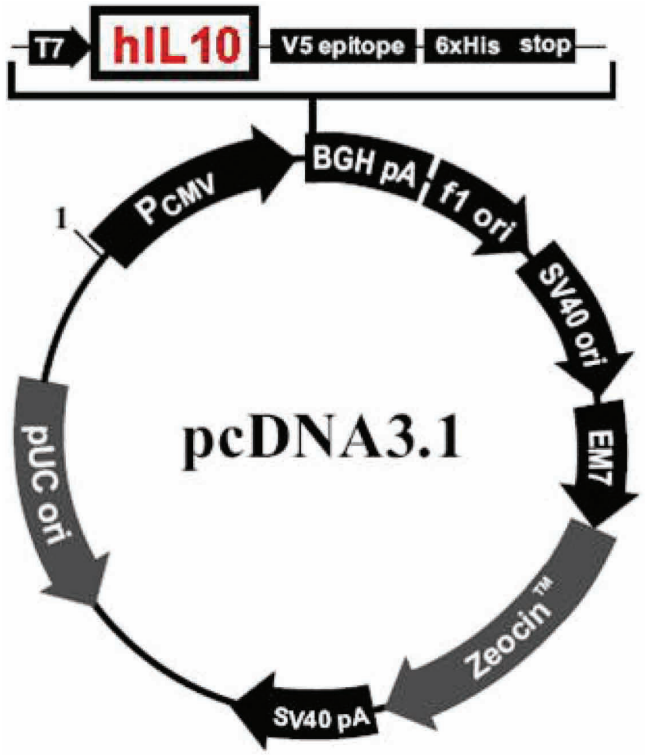


Fig 1. Schematic presentation of the gene construct delivered in injections to mice

ŞekIL-1. Hayvanlara enjekte edilen genin şematik yapısı

Immunostaining

The immunostaining was performed on the animals killed 24 h after IP and IV injections to localize and assess the expression of the IL-10 transgene in lung, liver, spleen, vena cava tissues. These tissues were fresh-frozen after the necropsy, and the standard immunostaining procedure was employed with a primary horseradish peroxidase-conjugated monoclonal anti-V5 antibody coupled to the secondary DIG-conjugated anti-horseradish peroxidase for localization. Apart from IL-10 staining, TNF-α, IL-6 gene expression levels were identified.

RESULTS

IL-10 Transgene Expression in Tissues

Immunostaining revealed more prominent staining in liver after i.p delivery while all the endothelial tissues stained with IV delivery. The signal for anti-V5 epitope antibody was used as tracer in tissues. The immunostaining images are presented in *Fig. 2* and *Fig. 3*.

IL-6 Transgene Expression in Tissues

There was a striking difference between tissue expressions of transgene of animals in CLP IV group when compared to other groups. In this group, spleen tissue showed highest expression and liver showed lowest expression *(Fig. 4)*.

CLP ip group animals had highest expression in their spleens while lowest staining was seen in lung tissues. The difference in expression when compared to CLP only group was statistically significant (P<0.05, ANOVA).

TNF-α Transgene Expression

Highest transgene expression was seen in CLP only group. In this group, spleens showed highest amount of staining whereas livers and lungs had almost equal amounts of stainings. All tissues in CLP IP group had higher expressions than CLP IV group *(Fig. 5)*. The difference was statistically significant (P<0.05, ANOVA).

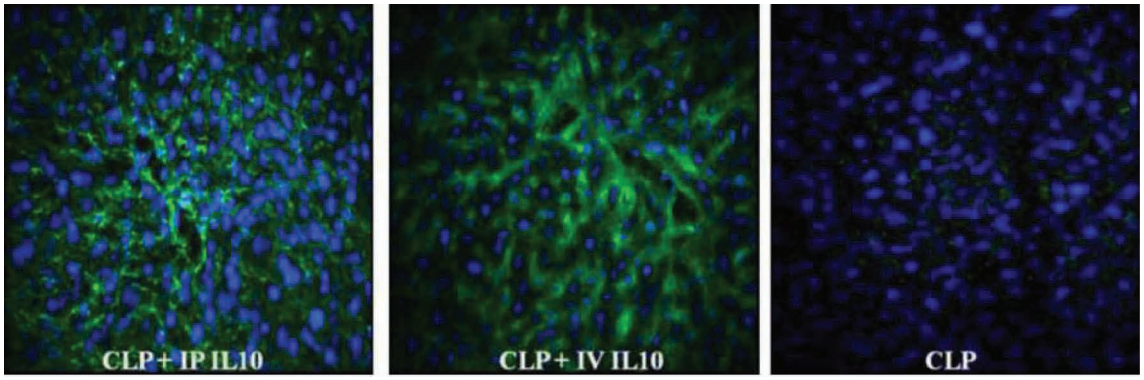


Fig 2. Liver parenchymal and sinusoidal staining after intraperitoneal and intravenous

delivery. Only CLP group staining was given for comparison (IL-10 in green, 4′,6-diamidino-2-phenylindole fluorescent stain in blue, CLP: liver specimen from cecal ligation and puncture only mouse, CLP+ IP IL10: liver specimen from mice which had intraperitoneal injection after cecal ligation and puncture, CLP+IV IL10: liver specimen from mice which had intravenous injection after cecal ligation and puncture)

Şekil 2. İntraperitoneal ve intravenöz enjeksiyon sonrası karaciğer parankimal ve sinüsoidal boyanmaları gösterilmektedir

Sadece çekal ligasyon ve delme yapılan farenin boyanması karşılaştıma amaçlı verilmiştir (IL-10 yeşil renk, 4′,6-diamidino-2-fenilindol floresan mavi renk, CLP: sadece çekal ligasyon ve delme yapılan farenin karaciğer boyanması, CLP+ IP IL10: çekal ligasyon ve delmeden sonra intraperitoneal enjeksiyon yapılan farenin boyaması, CLP+IV IL10: çekal ligasyon ve delmeden sonra intravenöz enjeksiyon yapılan farenin boyaması)

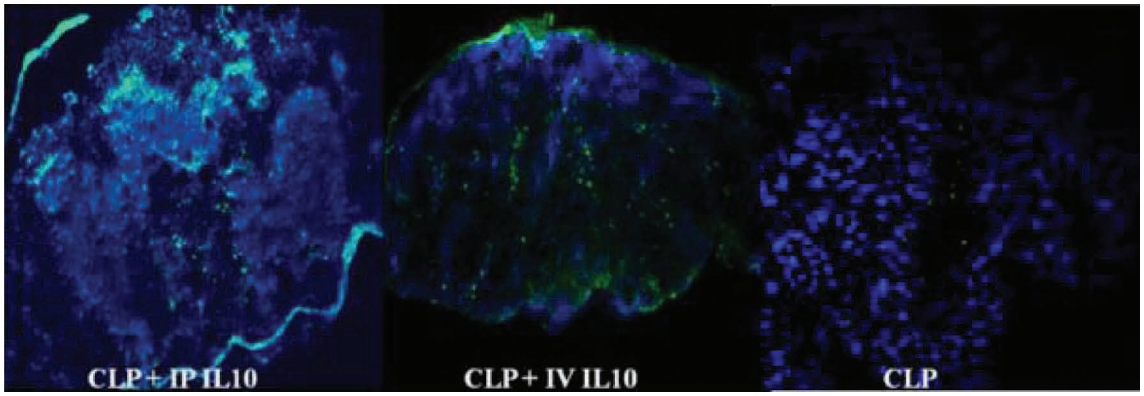


Fig 3. Spleen staining after intraperitoneal and intravenous delivery

Only CLP group staining is for comparison (IL-10 in green, 4',6-diamidino-2-phenylindole fluorescent stain in blue, CLP: spleen specimen from cecal ligation and puncture only mouse, CLP + IP IL10: spleen specimen from mice which had intraperitoneal injection after cecal ligation and puncture, CLP + IV IL10: spleen specimen from mice which had intravenous injection after cecal ligation and puncture)

Şekil 3. İntraperitoneal ve intravenöz enjeksiyon sonrası dalak boyamaları

Sadece çekal ligasyon ve delme yapılan farenin boyanması karşılaştırma amaçlı verilmiştir (IL-10 yeşil renk, 4',6-diamidino-2-phenylindol floresan mavi renk, CLP: sadece çekal ligasyon ve delme yapılan farenin dalak boyanması, CLP + IP IL10: çekal ligasyon ve delmeden sonra intraperitoneal enjeksiyon yapılan farenin dalak boyaması, CLP + IV IL10: çekal ligasyon ve delmeden sonra intravenöz enjeksiyon yapılan farenin dalak boyaması)

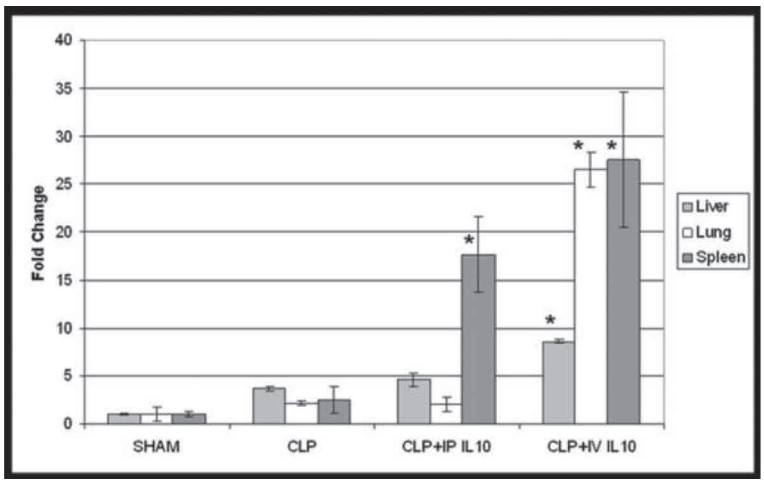
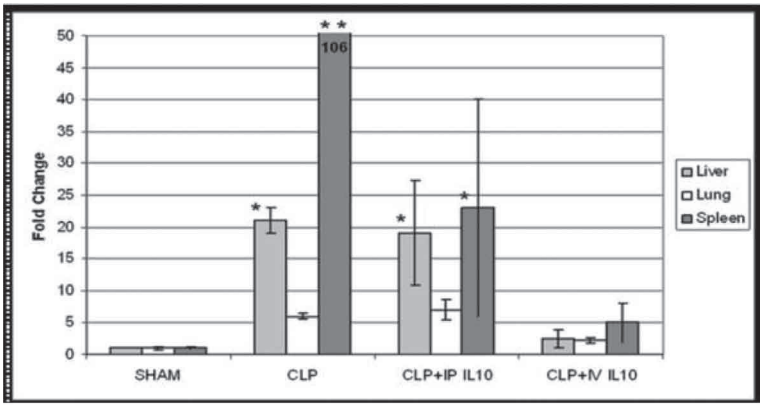


Fig 4. IL-6 transgene expression in groups (* P<0.05) (CLP: specimens from cecal ligation and puncture only mouse, CLP + IP IL10: specimens from mice which had intraperitoneal injection after cecal ligation and puncture, CLP + IV IL10: specimens from mice which had intravenous injection after cecal ligation and puncture)

Şekil 4. Gruplardaki IL-6 transgen ekspresyonu (* P<0.05 (CLP: sadece çekal ligasyon ve delme yapılan farelerden alınan dokuların analizi, CLP + IP IL10: çekal ligasyon ve delmeden sonra intraperitoneal enjeksiyon yapılan farelerden alınan dokuların analizi, CLP + IV IL10: çekal ligasyon ve delmeden sonra intravenöz enjeksiyon yapılan yapılan farelerden alınan dokuların analizi)

Fig 5. TNF-α transgene expression in groups (CLP: specimens from cecal ligation and puncture only mouse, CLP + IP IL10: specimens from mice which had intraperitoneal injection after cecal ligation and puncture, CLP + IV IL10: specimens from mice which had intravenous injection after cecal ligation and puncture)

Şekil 5. Gruplardaki TNF-α transgen ekspresyonu (CLP: sadece çekal ligasyon ve delme yapılan farelerden alınan dokuların analizi, CLP + IP IL10: çekal ligasyon ve delmeden sonra intraperitoneal enjeksiyon yapılan farelerden alınan dokuların analizi, CLP + IV IL10: çekal ligasyon ve delmeden sonra intravenöz enjeksiyon yapılan yapılan farelerden alınan dokuların analizi)



DISCUSSION

In '90s, rise of IL-10 levels has been documented in sepsis and its plasma concentration was related to mortality and the severity of multi organ dysfunction. In various studies plasma levels of IL-10 and TNF-α were found to be correlated [8,9].

In early systemic inflammatory response stage of

sepsis, IL-10 is shown to be proinflammatory whereas in multi organ dysfunction stage it is known to exert anti-inflammatory function. IL-10 was thus called as "biphasic immune modulator". Stemming from these findings it was proposed that IL-10 administration would be beneficial before inflammation is triggered.

IL-10 infusion trials in humans were proven to be untolerable causing severe fever, tremor, myalgia and hypotension while it's half life changes between 2.5 and 4 h. One alternative approach to IL-10 protein administration is to synthesize the IL-10 *de novo* in the host body. The most novel of these approaches is achieving the IL-10 gene delivery which is known to enhance survival in experimental model of sepsis [10-12].

In the second half of '90s, phase I studies in humans showed that the infusion of IL-10 was not well tolerated [13,14]. Along with TNF-α and IL-6 blockage by IL-10, other immunotherapy options were also tried without any success [15]. After these studies IL-10 was deemed unuseful. After gene transfer and gene therapy techniques were utilized in medical sciences new interventions using IL-10 became possible.

When given exogenously, IL-10 molecule has a short bioefficacy and half life. But gene transfer of IL-10 provides de novo continous expression of IL-10 inside the cell which overcomes bioefficacy and half life problems. Our previous studies have pointed out the beneficial role of IL-10 gene transfer in CLP model of sepsis [5].

In a pre-treatment model, the animals conditioned with ip IL-10 supressed TNF-α expression following CLP and exhibited better survival rate [16]. The major drawback in this was the pro-inflammatory effect exerted by the viral vectors themselves while gene transfer by cationic liposomes was free of inflammatory side effects providing safe expression. This was the main reason why we chose to use liposomal vectors.

Intravenous administration is a true systemic approach with successful gene delivery to the endothelium which is the most vulnerable target organ in sepsis. Our study proved that IL-10 liposomal gene transfer via iv or ip routes are both efficient in maintaining adequate tissue levels including endothelium 24 h after delivery.

We propose that the early expression of IL-10 in peritoneum helps to suppress and delay the overt inflammatory reaction in this irreversable model of intra-abdominal sepsis. These results also suggest that the IL-10 gene delivery via ip route helps to diminish TNF-α induction in 48th h of sepsis but the iv route is more succsessful in reducing the TNF-α levels.

Our results point out that the pro-inflammatory action of IL-10 is prominent in IV gene delivery which shows itself with induction of IL-6. IL-6 was suppressed with ip delivery in liver and lung in our study.

It is known from the kinetics of this gene transfer that the transgene expression peaks at 36 to 48th h and diminishes following the 3rd day, thus suggesting a temporary de novo source for IL-10 [17]. Further immunological and experimental data is needed to elucidate the crosstalk between IL-10 and other cytokines in sepsis.

Our study highlights the fact while preventing mortality in CLP model of sepsis, IL-10 may also harbor a therapeutical potential which still needs to be explored.

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RESEARCH ARTICLE

Structural and Histopathologic Changes of Calf Tibial Bones Subjected to Various Drilling Processes

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Summary

Bone tissue damages such as fractures, bone tissue losses, osteolysis and necrosis caused by temperature are the serious clinical concerns in orthopaedics. In order to show the effect of heat generation in bone drilling, temperatures were recorded and analysed for various drill speed, diameter, drilling force and bone densities on fresh calf tibial bones. This study revealed that high drill speeds increase the maximum temperature of the bone while high diameters and drill forces cause a decrease in the drilling temperature. SEM and histopathologic evaluations showed that high values of drill diameter and bone density caused greater damage to the bone and lowered the drill quality around the drilled site by producing rough surfaces and higher loss of osteocytes.

Keywords: *Orthopaedics, Bone drilling, Heat, SEM, Histopathology*

Çeşitli Delme İşlemlerine Tabii Tutulan Sığır Tibia Kemiklerinin Yapısal ve Histopatolojik Değişimleri

Özet

Kırık, kemik kayıpları, sıcaklığın neden olduğu osteoliz, nekroz gibi kemik dokusu hasarları ortopedide ciddi klinik sorunlardandır. Bu çalışmada, kemik delme işlemlerinde meydana gelen ısının kemiğe etkisini göstermek için taze sığır tibiaları kullanılarak farklı matkap devir sayıları, delme kuvveti ve kemik densiteleri için ortaya çıkan sıcaklıklar ölçülmüş ve analiz edilmiştir. Maksimum devir sayısı ve matkap çapında maksimum delme sıcaklığı elde edilirken artan drill kuvveti ile sıcaklığın düştüğü belirlenmiştir. SEM ve histopatolojik incelemeler; büyük matkap çapı ve yüksek kemik densitesinin kemik dokuda daha fazla osteosit kaybına yol açtığı ve kemiğe daha fazla hasar vererek delik bölgesinde kemik kalitesinin düşmesine sebep olduğunu gösterdi.

Anahtar sözcükler: *Ortopedi, Kemik delme işlemi, Isı, SEM, Histopatoloji*

INTRODUCTION

During orthopaedic operations, drilling is the common process and heat generated in bone is inevitable [1]. The heat is a serious clinical concern because of its potential to cause bone damage and bad osseointegration in the implantation processes. The bone consists of organic and inorganic structures which are affected by the temperature rise during drilling, and therefore the bone is likely to be damaged at temperatures higher than 47°C [2]. Such damages occur at the drilling site or within an explanted sample for transplantation. They may result in some complications such as the loss of an implant and aseptic necrosis [3]. Although the mechanical damage of the bone edges at the drill site alone is considered as a minor problem, the quality of bone tissue specimens is essential for the further progress of bone operations [4].

Thermal damages in drilling processes and bone implantation were studied and it was reported that the applied drill force has increased the temperature considerably [5]. The effect of the applied drill force in the generation of heat during the drilling operations of bone screw and pin assemblies was investigated [6]. In a similar

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study, it was reported that the temperature decreased with decreasing drill speed [7]. Sharpness of the dental cutter and the maximum cutting life was determined for various drill speeds [8]. Temperatures were compared at continuous drilling operations, and as expected, the tool wear was to increase the amount of heat [9].

Histology of the tissue damage for different drills used in orthopaedic surgery was examined [10] and it was found that the maximum damage to tissues was caused by the cutter that removes the largest chips from the operational site. To investigate the microstructural changes in bone, Rogers and Daniels [11] examined the bone tissue by subjecting the bones to high temperatures. Surface temperature of cutting tool was measured by the thermographic method and a numerical model was built to show the effects of heat on the bone tissue in artificial joint surgery [12]. In a parallel work, the thermal conductivity measurements were executed on calf femurs at the middle diaphysial [13]. Hamade *et al.*[14] derived various equations for the experimental cutting speed and pressure by using parameters such as drill speed, feed rate and cutting forces as the input parameters.

A number of work in the literature indicated that the drill parameters influence the heat generation in bone drilling [15-17]. If the related studies are examined, it can be seen that researchers generally focused on few drill parameters without considering structural change of bone, due to the complexity of orthogonal heat mechanism. In this study, important parameters which increased the temperature causing possible damage in bovine tibial bone were evaluated in detail. The specimens of a sacrificed tibial bone and temperature affected zones were investigated throughout the SEM and histopathologic analysis.

MATERIAL and METHODS

The use of fresh animal samples or human cadaver bones for the evaluation of the drilling process and subsequent histological examinations, are the adopted standard procedures [18,19]. The animal tibias were provided freshly and randomly choosen from a local slaughterhouse company (ELET Ltd, Elazig) as male and female calf tibias, at 2 years of age and weighing 216±30 kg for each group. Drillings have been performed in air using standard orthopaedic drills (AISI 4020) having the diameters of Æ1.5, 2.7, 3.2, 4.5 and 6 mm with an 85° tip angle. During *in vitro* experiments, temperatures were measured continuously with the use of the T-type thermocouples in the tibial bones. Drill forces (F) of 20 N and 70 N were applied throughout the drilling procedures, using a drilling rig for various speeds (n) between 230 and 1220 rpm. All drills were used no more than 15 times before being replaced with a new one [20]. The samples were divided into two groups and drilled. The standard distances between the drill sites and thermocouples sites were 0.5 mm and the depth in which thermocouples were placed in the cortical bone thickness was 5 mm, on average.

BMD Measurements: Bone mineral density (BMD) measurements were achieved by dual energy X-ray densitometry. The analysis was performed by placing the bones saparetely in the scan group by a dual energy X-ray densitometry device Discovery Wi (S/N 84440).

Temperature Measurements: The drill speed (n), applied drill force (F), drill diameter (D) and bone density (ρ) are determined as thermally effective variable parameters *(Table I)*. The temperature measurement, coupled with SEM and histomorphometry examinations at the drill site were the core of this work. A Dynamyte-2900 CNC (Numerically Controlled) machine was used to drill the bovine tibial bone samples. T-type Teflon insulated thermocouples were used for the continuous temperature measurements during the drilling operations. Fresh cadaveric calf tibias were used in the orthopaedic drilling operations (supplied by ELET Ltd. Elazig, Turkey). The tibial bone samples were separated by sawing them along the trabecular line (part) and kept in a deep freezer for 48 h. The drillings were performed at room temperature. The thermocouple slots were determined via the Master-Cam software. After the thermocouples were mounted around the drill sites, the drilling operations were performed with a precision of 10^{-3} mm by sending the values obtained from this software to CNC machine *(Fig. 1)*. The data transfer from the thermocouples was provided by the data acquisition card (Advantech).

SEM and Histopathologic Analysis: The histopathologic analyses have been executed to observe the effects of different orthopaedic drill parameters and so the temperature on the drill site of the tibial bone. Throughout these investigations some standard preparations were executed to the bone samples. The bone sections have been sawn off by a thin manual saw and then the bone sample was divided transversely into two pieces. The prepared bone samples were wrapped in a sponge with labelling and kept in a nitric acid solution (40 ml 65% vol nitric acid, 20 ml 10% vol formaldehyde and 340 ml distilled water) for ten days. The samples were checked

Table 1. *Partial correlation and standard deviations for different drill speed, force, diameter, bone sex and density*
Tablo 1. *Farklı devir sayısı, delme kuvveti, matkap çapı, kemik cinsiyeti ve kemik mineral densitesine bağlı kısmi korelasyon ve standart sapma değerleri*

Parameter	Partial Correlation (R)	P-Value
Drill Speed	0.4056	0.0320
Drill Force	0.7543	0.00004
Drill Diameter	0.4550	0.0170
Bone Sex	0.7084	0.0021
Bone Mineral Density	0.7485	0.00005
Correlation	R=0.8580	0.00000

Fig 1. Drilling at CNC machine
Şekil 1. CNC makine'sinde delme işlemi

every two days to check whether the decalcification was achieved. The decalcification solution was changed each time for a fresh one. After the decalcification process had finished, the macro sections were taken from the specimens by a microtome cutting apparatus, and then they were prosecuted with separate labelling. The prepared specimens were taken for microscopic examinations and the histograms of those sections were executed by using an optical microscope (OLYMPUS). In the histograms, the bone damage, necrotic zone of the hole-wall, volume of empty lacunas and osteocytes filled lacunas were evaluated, due to heat generation in different drilling parameters. SEM analysis took place in order to evaluate the effect of the drill temperatures on the structural changes in bone. First, the sections were taken from the drilled zone and were kept in ethyl alcohol for 30 min, for dehydration process, they were then cleaned and dried in a furnace (120°C) for 12 h.

Statistics: The Statistica 7.0 software was used for statistic analysis of the drilling parameters. The multiple regression method was used for determination of correlation between the drilling parameters via bone temperature. Partial correlation in regression analysis was used to describe the strength of the drilling parameters for bone temperature, and regression (R) and p values were determined. P=0.05 (95% probability) confidence of interval was evaluated the temperature results.

RESULTS

The prepared bone samples obtained via various drill parameters were studied via maximum temperature evaluation, SEM and histomorphometric examinations. These observations were then compared with the heat-induced alterations found from various drill parameters on the fresh tibial bones. The resistance of compact cortical bone with friction simply causes temperature increase in bone. The cellular damage and death caused by heat during drilling is reported as temperature above 50°C cause irreversible cortical bone necrosis [2].

Through this *in vitro* study, male and female tibial bones at the average age of two were drilled using different feed-rates (30, 50 and 70 mm/min) and drill speeds (200, 400 and

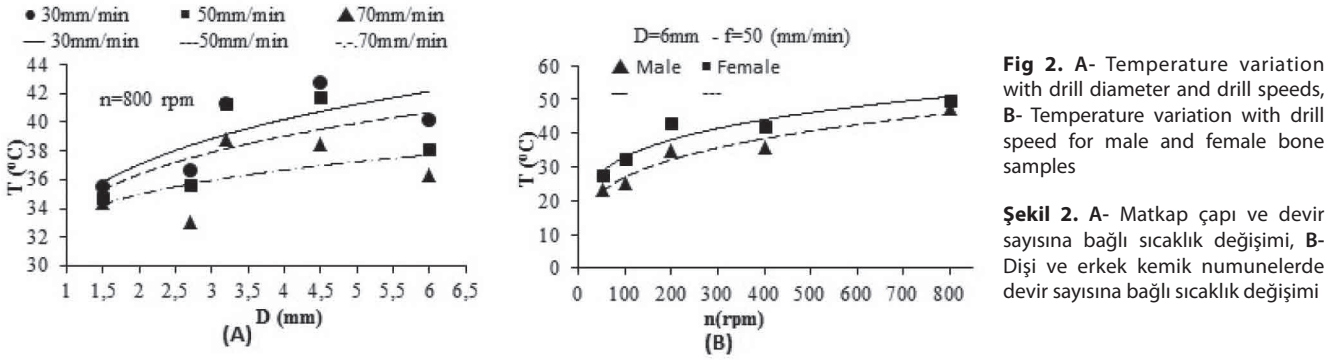

Fig 2. A- Temperature variation with drill diameter and drill speeds, **B-** Temperature variation with drill speed for male and female bone samples

Şekil 2. A- Matkap çapı ve devir sayısına bağlı sıcaklık değişimi, **B-** Dişi ve erkek kemik numunelerde devir sayısına bağlı sıcaklık değişimi

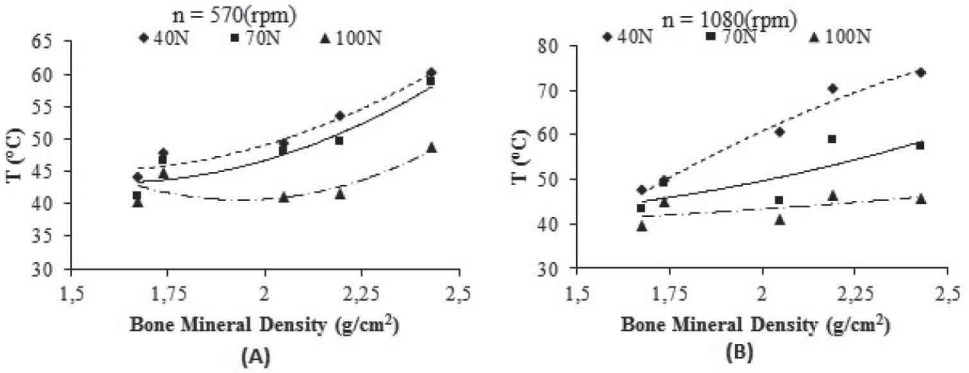

Fig 3. Temperature variations with different BMD: **A-** for n=570 rpm, **B-** for n=1.080 rpm

Şekil 3. Farklı kemik mineral yoğunluğuna bağlı sıcaklık değişimleri: **A-** n=570 d/d, **B-** n=1.080 d/d değerleri için

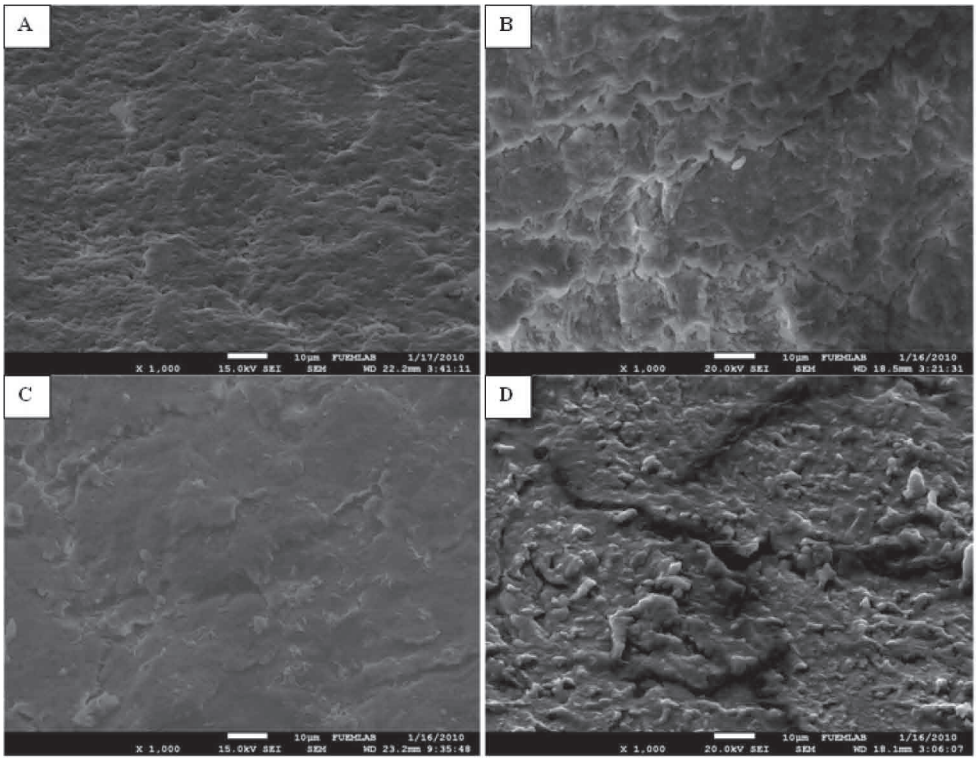

Fig 4. SEM views of drilled bone surfaces: **A-** For drill parameters D=4.5 mm, F=140 N, n=230 rpm, (T_{max}=38°C), **B-** For drill parameters D=4.5 mm, F=140 N, n=1.220 rpm, in dry condition, (T_{max}=87°C), **C-** SEM view for drill parameters of D=4.5 mm, F=40 N, n=570 rpm of the bone sample having density of 1.739 g/cm^2 (47,7°), **D-** 2.430 g/cm^2 (T_{max}=54°C)

Şekil 4. Delinen kemik yüzeylerin SEM görüntüleri: **A-** Delme parametreleri; D=4.5 mm, F=140 N, n=230 d/d (T_{max}=38°C) için, **B-** D=4.5 mm, F=140 N, n=1.220 d/d için, kuru ortamda, (T_{max}=87°C), **C-** D=4.5 mm, F=40 N, n=570 d/d ve 1.739 g/cm^2 kemik densitesi için SEM görünüşleri (47.7), **D-** 2.430 g/cm^2 (T_{max}=54°C)

800 rpm). The changes in temperature with various feed-rates and diameters are plotted in *Fig. 2A* and the influence of bone sex on temperature is given in *Fig. 2B*. In order to show the combined effects of BMD, drill force and speeds on bone temperature change, five different BMD's (1.675, 1.739, 2.051, 2.194 and 2.43 g/cm^2), three different drill forces (40, 70 and 100 N) and two different drill speeds (570 and 1.080 rpm) were used and the plotted results is given in *Fig. 3A-B*. Partial correlation in regression analysis was used for the determination of correlation between the drilling variables whilst influencing the bone temperature. To make multiple regression analysis, the Statistica 7.0 was used. Regression (R) and p values were determined and given in *Table 1*. The drill temperatures are expressed as confidence of interval, P=0.05 (95% probability) and the multiple regression analysis was used to describe the strength of the relationship between specific drilling parameters. SEM view of drilled inner surfaces of the holes by various drill parameters were shown in *Fig. 4A-B*. The effect of the drill speed, applied drill force, bone density and drill diameter and drill environment have been taken into consideration and the obtained SEM structures are shown in *Figs 4C-D*. *Fig. 4C* shows the SEM view of the bone structure obtained for the bone samples having the BMD ρ=1.739 g/cm^2, and in *Fig. 4D* the view of the samples having the BMD ρ=2.430 gr/cm^2. *Fig. 5A-B* show the histograms of the bone samples which were influenced by different applied drill forces on the tibial bone tissue e.g A) F=20 N and B) F=70 N, respectively. *Fig. 5C-D* show the histograms of the samples which were drilled at different bone mineral densities e.g C) ρ=1.739 g/cm^2 and D) ρ=*2.430* g/cm^2 at drill parameters of D=4.5 mm, F=70 N and N=570 rpm at room temperature.

DISCUSSION

Temperature Analysis: *Fig. 2A* shows the influence of drill diameter on the temperature change in bone with feed-rate, at constant drilling speed (n=800 rpm). It can be observed that the maximum reached temperature increased with increasing drill diameter. On the other hand, when the effect of feed-rate is compared, maximum temperatures tend to decrease with increasing feed-rates. As well known from the orthogonal cutting theory, some of the energy is spent for material remove from the surface and some is converted to heat [13]. Such result, therefore, is not surprising, since the length of shearing distance and friction area increase at bigger drill diameter and lowers for small diameters.

Fig. 3 shows the variation of temperature with drill speed, force and BMD. As shown in *Fig. 3A*, temperatures decrease when the drill load increases 40 to 100 N as the similar results are reported in [2]. In addition, this was confirmed in a study [5] that the drilling of bovine bone showed a temperature decrease as the feed-rate increase. The reason for this could be the increase in feed-rate causes a decrease in the drill time, and so the high temperatures in the cutting zone cannot be reached. *Fig. 3B* shows the influence of BMD on drilling temperature. The BMD was influenced by bone hardness and the temperature values were elevated with increased density. Because of this, as given in *Fig. 3A*, the temperature has increased as the bone mineral density increased. It can also be seen that the bone temperature decreased with increasing drill force, and a higher drill force maintained for a shorter drill time. This means the higher temperatures are even harder to obtain at the drill site.

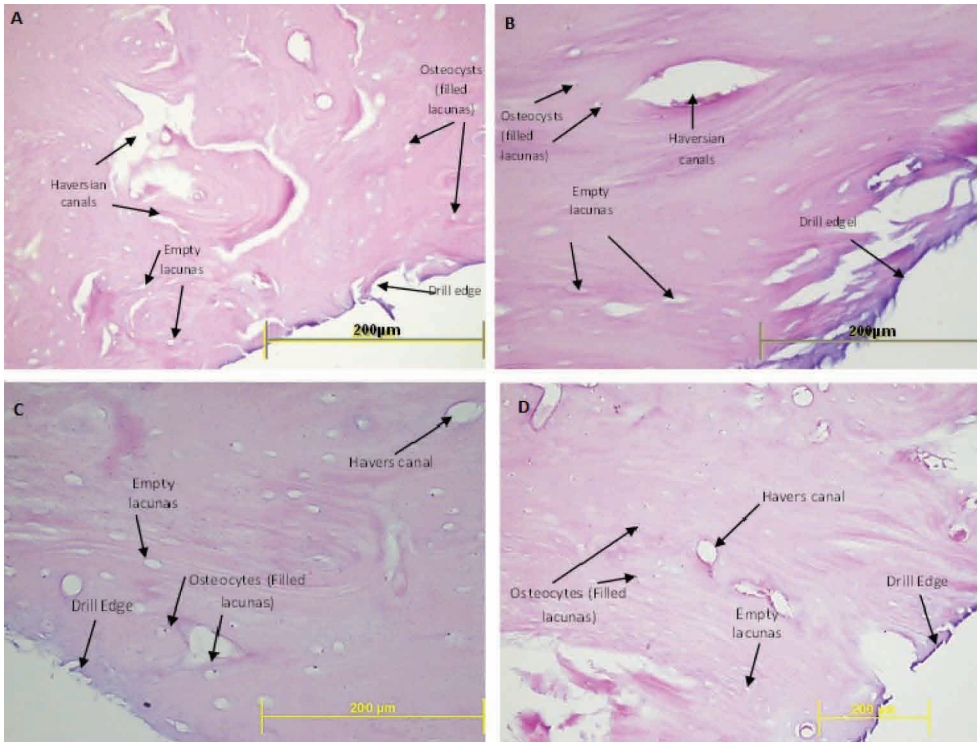

Fig 5. Histograms for drilling parameters: **A-** D=6 mm, F=20 N, n=570 rpm, (T_{max}=60°C), **B-** D=6 mm, F=70 N, n=570 rpm, (T_{max}=45°C), **C-** D=4.5 mm, F=70 N, n=570 rpm for the bone density of 1.739gr/cm^2 (T_{max}=44°C), **D-** D=4.5 mm, F=70 N, n=570 rpm for density of 2.430 g/cm^2 (T_{max}=52°C)

Şekil 5. Delme parametrelerine ait histogramlar: **A-** D=6 mm, F=20 N, n=570 d/d, (T_{max}=60°C), **B-** D=6 mm, F=70 N, n=570 d/d, (T_{max}=45°C), **C-** D=4.5 mm, F=70 N, n=570 d/d ve 1.739 g/cm^2 kemik densitesi için sıcaklık (T_{max}=44°C), **D-** D=4.5 mm, F=70 N, n=570 d/d ve 2.430 g/cm^2 kemik densitesi için sıcaklık (T_{max}=52°C)

A maximum temperature was obtained at the maximum bone density 2.43 g/cm^2 and the bone temperature was elevated with increased bone density *(Fig. 3B)*. On the other hand, increasing drill force has decreased with bone temperature. However, the drill force was reduced with the drilling time so the temperature could not rise to higher values. Because of this, the temperatures have remained at a lower degree when higher forces are used. Although the drill force and drill speed was reported to cause an increase in bone temperature [7], in other works [8,21], it was indicated that drill temperature has decreased significantly with the increasing of drill force.

Statistical Analysis: It was observed from the statistical analysis that the bone sex has a statistically significant effect on drill temperature as the correlation coefficient was found to be $R_{(sex)}$= 0.70 *(Table 1)*. But the feed-rate did not show a statistically significant effect on the bone drill temperature, as the correlation coefficient was found to be $R_{(feed\text{-}rate)}$=0.17. However, the drill speed had a higher influence on the drill temperature since P=0.032 and $R_{(speed)}$=0.77.

SEM Analysis: The effect of drill speed in the drilled hole structure at constant applied force (140 N), drill diameter (4.5 mm) was shown in *Fig. 4A*. The SEM view of the drill site shows that, low drill speed (230 rpm) produced low maximum temperature (38°C), and as a result, a good drill surface has emerged. However, as seen in *Fig. 4B*, a higher drill speed (1220 rpm) caused much higher drilling temperature (87°C) and so the surface was damaged more and some irregularities or ruptures appeared to be deeper in the drilled hole surface. *Fig. 4C-D* shows the effect of bone density on drill temperature and so microstructure of drilled bone. As the structures are shown in *Fig. 4C* and *4D*, respectively, the only slight temperature rise (5°C) was obtained, and this seems to be proportional to the densities varied between 1.739 and 2.430 g/cm^2. However, even such small temperature increase above 47°C was enough to cause bone damages as reported in [3]. *Fig. 4C* shows a diverted surface structure for a lower BMD indicating that a homogeneous and better quality drilled hole surface than that obtained for the higher BMD *(Fig. 4D)*.

Histopathologic Analysis: The effect of the drill diameter on temperature during drilling was studied elsewhere [2,5,20,21]. However, the structural changes and histopathologic evaluation were not considered in detail. In this study, the effect of temperature on bone tissue structure during drilling was evaluated. By comparing these histograms, it can be observed that the recorded maximum temperatures were found higher for lower drill forces. In addition, the osteocyte presence is far less visible in *Fig. 5A* (F=70 N), located at a distance of approximately 350 µm from the drill site, whereas they appear to be closer for a low applied drill force, e.g. F=20 N *(Fig. 5B)*. The reason for this may be due to higher forces in which need lower drilling time, hence the heat have no enough time to diffuse and penetrate into the bone. In other words, the longer drill time and applied drill force causes a higher temperature (60°C), and also damages the bone deeper and destroys the cells, thus resulting in a lower amount of filled- osteocytes. Another indication for deep penetration of heat is the appearance of osteocytes spreading far away from the drill site. The filled- osteocytes shown in *Fig. 5B* are observed to be far away (350 µm) from the drill site and the amount of filled osteocytes are found to be less than that found for

F=70 N *(Fig. 5B)*. As a result, however, although the lower temperature is reached during drilling, high drill forces should be avoided due to the occurrence of a higher bone deformity around the drilling zone.

Fig. 5C-D show the histograms for the tibias that were drilled for the BMD ρ=1.739 g/cm^2, and ρ=2.43 g/cm^2. A slightly higher temperature (52°C) was recorded for the tibial bones having the high density compared to the temperature (44°C) for bones at low density. Both histograms have similar structures in terms of damages at the drill site and the amount of filled osteocytes. When working with high BMD bones, it is necessary to use low drill speed, applied drill force and feed rates.

Higher drill speeds and diameters caused higher drill temperature and also caused more damage around the drill sites of tibial bones. As the applied drill force, and so, feed rate was increased, the temperature was also decreased. In order to avoid any undesired operational bone damages due to high drilling temperature, the applied drill force and feed rates should not be used at high levels. Although the drill temperatures were found at drill speeds of 230 rpm and at high drill forces, the SEM and histopathologic results showed that, some fractures and damages are likely to appear at the drill site. The drill speed should be kept low with a maximum 50 mm/min of feed-rate, and 70 N of drill force should be preferred when high BMD' bones are to be drilled. However, because of too many parameters and their combined effects involved in bone drilling, it is suggested that in order to minimize the bone defects and necrosis, due to high drill temperatures (T_{drill}>45-50°C). The orthopaedic surgeons should consider their specific material conditions and choose their optimum drilling parameters shown through this work.

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RESEARCH ARTICLE

Enjektabl İz Elementlerin Geçiş Dönemindeki İneklerde Metabolik Profil Üzerine Etkileri [1]

Cem AVCI * Omer KIZIL *

[1] *Bu çalışma "Enjektabl İz Elementlerin Geçiş Dönemindeki İneklerde Metabolik Profil Üzerine Etkileri" başlıklı Yüksek Lisans Tezinden özetlenmiştir*

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Özet

Bu çalışmada geçiş dönemindeki ineklerde mineral uygulamalarının metabolik profil üzerine olan etkileri araştırılmıştır. Araştırmada aynı bakım ve beslenme şartlarında tutulan 20 adet montofon ırkı inek kullanıldı. Çalışmadaki inekler her grupta 10 hayvan olacak şekilde 2 eşit gruba ayrıldı. Deney grubundaki ineklere geçiş döneminin başlangıcında, kas içi yolla (20 ml/inek) bir mineral solüsyonu uygulandı. Kontrol grubundaki ineklere geçiş döneminin başlangıcında 20 ml serum fizyolojik placebo olarak derialtı yolla uygulandı. Her iki gruptaki ineklerin v. jugularislerinden geçiş döneminin başlangıcında, doğum anı ve doğumdan 3 hafta sonra olacak şekilde kan örnekleri alınmıştır. Metabolik parametreler olarak her iki grupta da NEFA, BHBA, albumin, glukoz, T. kolesterol, VLDL-kolesterol, LDL-kolesterol, HDL-kolesterol, trigliserid, AST, ALT, GGT, T. bilirubin, kreatinin, BUN ve T. protein düzeyleri belirlenmiştir. Çalışma sonucunda, geçiş döneminin başlangıcında özellikle selenyum, bakır, çinko ve mangan içeren bir mineral solüsyon uygulanmasının metabolik profil üzerine olumlu etkilerinin olabileceği sonucuna varılmıştır.

Anahtar sözcükler: *Geçiş dönemi, İnek, Metabolik profil, Mineral madde*

The Effects of Injectable Trace Elements on Metabolic Parameters in Transition Cow

Summary

The aim of this study was to determine the effects of mineral administration on metabolic profiles in the cows during transition period. Twenty healthy pregnant cows under the same care and feeding conditions were used in the study. The animals were divided into two equal groups including 10 animals in each. The trace mineral injection was administered to the experimental group intramuscularly (20 ml/cow) on the beginning of the transition period. 20 ml isotonic sodium chloride was injected subcutaneously on beginning of the transition period in the control group as placebo. Blood samples were taken by venipuncture of the jugular vein on beginning of the transition period, parturition time and three after parturition, respectively. The NEFA, BHBA, albumin, glucose, T. cholesterol, VLDL-cholesterol, LDL- cholesterol, HDL-cholesterol, triglyceride, AST, ALT, GGT, T. bilirubin, creatinine, BUN and T. protein levels were determined in the both groups as metabolic parameters. In conclusion, the mineral solution particularly contains selenium, copper, zinc and manganese that administrated on beginning of the transition period could be effective on metabolic parameters.

Keywords: *Transition period, Cow, Metabolic profile, Trace element*

GİRİŞ

Geçiş dönemi (periparturient dönem) olarak tarif edilen dönem, gebeliğin sonları ile erken laktasyon dönemlerini kapsar. Yazarlar tarafından bu dönemin sınırları farklı tarif edilmesine rağmen genel olarak bu süre doğum öncesi ve sonrası 2-3 haftalık süreyi içine alır [1-3]. Periparturient dönem diğer dönemlerle kıyaslandığında oldukça iyi bilinmeyen bir dönemdir. Bu dönemde özellikle fizyolojik olayların çok hızlı değişim göstermesi önemli bir problemdir [4]. Gebelikten laktasyona geçiş dönemi oldukça sıkıntılı bir süreç olarak tanımlanmaktadır [2]. Özellikle bu dönemdeki birçok

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hormonal ve metabolik değişimin bu süreçte etkili olduğu düşünülmektedir [5].

Kanın çeşitli biyokimyasal parametreler yönünden incelenmesi metabolik profildeki değişimler hakkında önemli bilgiler vermektedir [6,7]. Çeşitli tipteki metabolizma hastalıkları işletmeler için önemli kayıplara neden olduğundan, özellikle belirtilerin henüz ortaya çıkmadığı subklinik seyirli olayların ortaya konulması, ayrıca teşhisi doğrulamak, prognozu tayin etmek, tedavinin etkinliğini arttırmak ve beslenme hatalarını ortaya koymada biyokimyasal olarak metabolik profil testlerinden yararlanılmaktadır [7]. Metabolik profil bölgeye, hayvanın ırkına, süt verimine, laktasyon dönemine ve beslenme şekline göre değişiklik gösterebilmektedir [6,8].

Dolaşımda esterleşmemiş yağ asitleri (NEFA) ve beta hidroksi bütirik asit (BHBA) düzeylerinin belirlenmesi geçiş dönemindeki ineklerde enerji denge durumunun ortaya konulmasında başlıca kullanılan parametrelerdir. Düşük dansiteli lipoprotein (LDL), yüksek dansiteli lipoprotein (HDL) ve çok düşük dansiteli lipoprotein (VLDL)'ler, hayvanların beslenme ve sağlık durumlarını değerlendirme yanında doğum öncesi ve sonrası dönemde şekillenebilen metabolik hastalıkların teşhisinde de kullanılmaktadır [1,9,10]. Ayrıca geçiş dönemindeki ineklerde metabolik profilin değerlendirilmesinde biyokimyasal olarak aspartat amino transferaz (AST), alanin amino transferaz (ALT), gama glutamil transferaz (GGT), glikoz, total protein, albumin, üre, kreatinin, ve total bilirubin düzeylerine bakılmaktadır [11-13].

Organizmada mikro elementlerin yeterli düzeylerde olmasının önemi, hayvanların sağlığı ile olan yakın ilişkisinden kaynaklanır. Çünkü mikro elementler organizmada çok sayıda yapısal, katalitik ve düzenleyici fonksiyona sahip olup, aynı zamanda immun sistem üzerinde çok önemli etkileri vardır [14,15]. Mikro elementlerin absorbsiyonu başlıca diyetteki düzeyleriyle alakalı olup, özellikle organik kaynaklı olanlarda daha yüksektir [16-18]. Yeterli düzeyde mikro elemente sahip anneden doğan buzağıların kanlarında da anneleriyle pozitif orantılı olarak mineral düzeylerine rastlanmaktadır. Yeni doğan bir buzağı için en iyi kaynak kolostrumdur [19]. Mikroelementler kolostrum ile sütün yapısını ve kalitesini etkileyerek meme sağlığını etkiler [17]. Yeme bakır ilavelerinin laktasyondaki ineklerde laktasyon performansını artırdığı ifade edilmektedir [20]. Bu kadar önemli fonksiyona sahip olan mikro elementlerin plasenta yoluyla fötusa, süt ve kolostrum yoluyla yeni doğan yavrulara nakledildiği düşünüldüğünde gebe hayvanlarda bu mikro elementlerin yeterli düzeylerinin sağlanması yavrularının ihtiyaçlarını karşılamada oldukça önemlidir [21-23]. Mikro elementler plasental bariyeri ve meme dokusunu geçebildiklerinden gebe hayvanlarda yeterli düzeylerin sağlanması buzağıların intrauterin ve doğum sonrası dönemde yeterli mineral desteği almasında çok önemlidir [22,24].

Bu çalışmanın amacı, geçiş dönemindeki ineklerde mineral uygulamalarının metabolik profil üzerine olan etkileri araştırmaktı.

MATERYAL ve METOT

Araştırmanın materyalini, 4-5 yaşlarında olan, aynı bakım ve beslenme şartlarında tutulan, klinik olarak sağlıklı, 20 adet montofon ırkı inek oluşturmuştur. Suni tohumlama kayıtları incelenen ineklerden, yaklaşık olarak gebeliğin son 3 haftalık döneminde oldukları belirlenenler çalışmada kullanılmıştır. Araştırmada kullanılan gebe inekler her grupta 10 hayvan olacak şekilde 2 eşit gruba ayrılarak, deney grubundaki ineklere doğuma 3 hafta kala ml'sinde 2.5 mg bakır glukonat, 1.25 mg sodyum selenit, 5 mg manganez ve 5 mg çinko glukonat içeren mineral içerikli bir solusyon (Activate, ALKE, İstanbul, 50 ml, im) hayvan başına total 20 ml dozunda tek doz kas içi yolla uygulanmış ve uygulama zamanı, doğum anı ve doğumdan 3 hafta sonra olacak şekilde analizler için kan örnekleri alınmıştır. Kontrol grubundaki ineklere ise doğuma 3 hafta kala sadece 20 ml dozunda serum fizyolojik placebo olarak derialtı yolla uygulanmış ve yine doğuma 3 hafta kala, doğum anı ve doğumdan 3 hafta sonra kan örnekleri alınmıştır.

Metabolik parametrelerden albumin, glukoz, T. kolesterol, VLDL-kolesterol, LDL-kolesterol, HDL-kolesterol, trigliserid, AST, ALT, GGT, T. bilirubin, kreatinin, BUN ve T. protein düzeyleri, Fırat Üniversitesi Tıp Fakültesi Merkez Laboratuvarı'nda ticari test kitleri yardımıyla belirlenmiştir. NEFA düzeyleri yine aynı birimde, Schimadzu GC-MS (2000 seri) cihazı yardımıyla Zivak marka ticari kitler kullanılarak, BHBA analizi ise özel bir laboratuvarda Eonia Vital Diagnostic cihazı (ABCAM/UK) kullanılarak Beta Hydroxybutyrate (beta HB) Assay Kit'leri kullanılarak ELISA yöntemiyle belirlenmiştir.

Bu çalışma için, Fırat Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Başkanlığının 09.08.2010 tarih ve 82 no'lu oturum kararıyla onay alınmıştır.

İstatistiki hesaplamalar SPSS Ms Windows Release 10.0 bilgisayar programı kullanılarak, grup içi günler arasındaki farklılıkların istatistiksel önemliliği bağımlı t-testi (paired-t test) kullanılarak yapılmıştır.

BULGULAR

Kontrol grubu ineklerde metabolik parametrelerin ortalama değerleri, standart sapmaları ve dönemler arası istatistiksel önem derecelerinin belirtildiği *Tablo 1* incelendiğinde; ALT ve albümin değerleri bakımından çalışma dönemleri arasında istatistiksel önem saptanmaz iken, NEFA ($P<0.05$), BHBA ($P<0.05$), glikoz ($P<0.05$), T. kolesterol ($P<0.01$), HDL kolesterol ($P<0.001$), LDL kolesterol ($P<0.001$), VLDL kolesterol ($P<0.001$), trigliserid ($P<0.01$), AST ($P<0.01$), GGT ($P<0.05$), BUN ($P<0.001$), kreatinin ($P<0.05$), T. bilirubin ($P<0.05$) ve T. protein ($P<0.01$) düzeyleri bakımından ise değişik derecelerde istatistiksel önem saptanmıştır.

Kontrol grubunda önemli çıkan parametreler açısından, doğuma 3 hafta kalan yapılan örneklemelerle kıyaslan-

dığında, özellikle negatif enerji dengesi ve karaciğer hasarı konusunda bilgi sunan parametrelerden NEFA, BHBA ve AST' nin doğum anında artan düzeylerinin doğumdan sonraki 3 haftalık sürede artmaya devam ettiği, buna karşın T. kolesterol, VLDL, LDL, HDL düzeylerinde doğumdan 3 hafta sonra saptanan azalmaların, doğum anındaki değerlere yakın kaldığı dikkati çekmiştir. Total protein düzeyinde ise devamlı bir azalma saptanmıştır.

Deney grubu ineklerdeki metabolik parametrelerin gösterildiği *Tablo 2* incelendiğinde ise; Glikoz, AST, ALT, GGT, kreatinin ve albümin değerleri bakımından çalışma dönemleri arasında istatistiksel önem saptanmadığı, buna karşın NEFA (P<0.01), BHBA (P<0.001), T.kolesterol (P<0.05), HDL kolesterol (P<0.01), LDL kolesterol (P<0.05), VLDL kolesterol (P<0.001), trigliserid (P<0.01), BUN (P<0.001), T. bilirubin (P<0.05) ve T. protein (P<0.01) düzeylerinde de değişik

Tablo 1. *Kontrol grubu ineklerde geçiş dönemindeki metabolik parametrelerin ortalama değerleri, standart sapmaları ve istatistiksel önem dereceleri*
Table I. *The mean values, standard deviation, and statistical differences of the metabolic parameters in the control group cows in transition period*

Parametreler	Doğuma Üç Hafta Kala	Doğum Anı	Doğumdan Üç Hafta Sonra	P
NEFA (nmol/ml)	8394.5±2937.2^{b}	9324.1±1246.7^{b}	11113.5±1577.9^{a}	*
BHBA (nmol/µl)	26.5±1.0^{b}	28.3±2.3^{a}	29.1±0.3^{a}	*
Glikoz (mg/dl)	56.7±2.7^{b}	63.8±3.6^{a}	54.8±7.8^{b}	*
T. kolesterol (mg/dl)	107.1±12.3^{a}	67.5±9.3^{b}	73.7±14.7^{b}	**
HDL-kolesterol (mg/dl)	63.5±8.4^{a}	53.6±7.6^{b}	55.2±7.3^{b}	***
LDL-kolesterol (mg/dl)	52.5±7.1^{a}	32.5±4.5^{b}	36.1±6.9^{b}	***
VLDL-kolesterol (mg/dl)	4.1±0.7^{a}	1.6±0.51^{c}	2.0±0.1^{b}	***
Trigliserid (mg/dl)	18.7±8.2^{a}	8.6±1.7^{b}	13.8±3.0^{a}	**
AST U/L	69.9±3.4^{b}	88.1±2.8^{a}	91.0±1.5^{a}	**
ALT U/L	30.4±6.0	30.5±5.6	33.9±7.2	-
GGT U/L	20.9±1.5^{c}	41.5±17.8^{a}	25.1±0.9^{b}	*
BUN (mg/dl)	19.1±2.6^{a}	13.9±4.2^{b}	9.7±1.1^{c}	***
Kreatinin (mg/dl)	1.29±0.1^{b}	1.43±0.1^{a}	1.28±0.1^{b}	*
T. bilirubin (mg/dl)	0.230±0.01^{b}	0.272±0.02^{a}	0.218±0.01^{c}	*
T. protein (g/dl)	8.32±0.6^{a}	7.48±0.6^{b}	7.25±0.1^{b}	**
Albumin (g/dl)	3.54±0.2	3.46±0.1	3.41±0.1	-

^{abc} *Farklı harflerle ifade edilen değerler arasında istatistiksel önem vardır, * P<0.05, ** P<0.01, *** P<0.001, -: önemsiz*

Tablo 2. *Deney grubu ineklerde geçiş dönemindeki metabolik parametrelerin ortalama değerleri, standart sapmaları ve istatistiksel önem dereceleri*
Table 2. *The mean values, standard deviation, and statistical differences of the metabolic parameters in the experimental group cows in transition period*

Parametreler	Doğuma Üç Hafta Kala	Doğum Anı	Doğumdan Üç Hafta Sonra	P
NEFA (nmol/ml)	6438.7±771.1^{b}	9766.4±1793.1^{a}	6924.0±2388.6^{b}	**
BHBA (nmol/µl)	20.1±1.7^{b}	25.5±2.9^{a}	21.7±1.7^{b}	***
Glikoz (mg/dl)	58.5±9.5	60.0±6.2	58.5±3.8	-
T. kolesterol (mg/dl)	82.4±7.8^{a}	75.2±35.7^{b}	81.3±34.0^{a}	*
HDL-kolesterol (mg/dl)	47.5±7.3^{a}	40.9±20.2^{b}	46.5±21.2^{a}	**
LDL-kolesterol (mg/dl)	38.5±5.7^{a}	32.1±15.4^{b}	37.1±15.8^{a}	*
VLDL-kolesterol (mg/dl)	4.1±1.4^{a}	1.8±0.4^{c}	3.4±0.8^{b}	***
Trigliserid (mg/dl)	20.7±3.3^{a}	7.1±2.6^{c}	9.6±1.2^{b}	**
AST U/L	72.4±10.3	76.0±12.5	75.9±11.2	-
ALT U/L	32.2±7.5	33.4±4.6	35.4±4.8	-
GGT U/L	25.6±8.1	28.5±8.9	24.0±4.9	-
BUN (mg/dl)	17.7±0.8^{a}	15.4±1.5^{b}	12.1±0.3^{c}	***
Kreatinin (mg/dl)	1.32±0.08	1.40±0.18	1.31±0.03	-
Total bilirubin (mg/dl)	0.224±0.03^{c}	0.322±0.02^{a}	0.285±0.01^{b}	*
Total protein (g/dl)	7.49±0.2^{a}	7.23±0.5^{b}	7.38±0.1^{a}	**
Albumin (g/dl)	3.65±0.2	3.56±0.2	3.61±0.2	-

^{abc} *Farklı harflerle ifade edilen değerler arasında istatistiksel önem vardır * P<0.05, ** P<0.01, *** P<0.001, -: önemsiz*

derecelerde istatistiksel önem saptandığı anlaşılmaktadır.

Deney grubunda önemli çıkan parametreler açısından ise doğuma 3 hafta kalan yapılan örneklemelerle kıyaslandığında, özellikle negatif enerji dengesi ve karaciğer hasarı konusunda bilgi sunan parametrelerden NEFA ve BHBA'nın doğum anında artan düzeylerinin doğumdan sonraki 3 haftalık sürede yeniden başlangıç değerlerine yaklaştığı, benzer şekilde T. kolesterol, VLDL, LDL, HDL ve T. protein düzeylerinde doğumdan 3 hafta sonra saptanan azalmaların başlangıç değerlerine yakın kaldığı dikkati çekmiştir.

TARTIŞMA ve SONUÇ

Metabolik profildeki değişimler belirlenirken bu değişimler üzerine hayvanın yaşının, ırkının, veriminin, çevresel şartların ve beslenme durumlarının etkili olduğu [8,25] dikkate alınarak, çalışmada aynı bakım ve beslenme şartlarında tutulan, aynı ırk hayvanlar kullanılmıştır.

Erken laktasyon döneminde plazma NEFA konsantrasyonlarının belirlenmesi enerji durumunun ortaya konmasında önemlidir [9,26]. Bu dönemde enerji durumunun belirlenmesinde kullanılan bir diğer önemli parametre ise BHBA'tır [1,10]. Dyk ve ark.[27] doğumdan bir hafta öncesinde, Saber [28] ile Reid ve ark.[29] da yeni doğum yapmış ineklerde NEFA konsantrasyonlarında artış olduğunu ve bu durumun ketozis, abomazum deplasmanı ve retensiyo sekundinarum için büyük risk oluşturduğunu bildirmişlerdir. Van Saun [11] doğumdan sonraki dönemde BHBA düzeylerinin önemli derecede arttığını ve bu tür hayvanların postpartum hastalıklara karşı daha duyarlı olduklarını bildirmiştir. Mevcut çalışmanın sonuçları incelendiğinde, kontrol grubu ineklerde doğum öncesi 3. haftada belirlenen NEFA düzeylerinin doğum anında artış gösterdiği ve doğum sonrası dönemde de bu artışların devam ettiği görülmektedir. Bu bulgu geçiş dönemi boyunca negatif enerji dengesinin ve yağ mobilizasyonunun devam ettiğinin göstergesi olarak kabul edilebilir. Oysa deney grubunda doğum öncesi belirlenen NEFA düzeylerinin, doğum anında artış göstermesine rağmen doğumdan sonraki 3. haftada tekrar başlangıç değerlerine yakın düzeylere gerilediği belirlenmiştir. Bu sonuçlara dayanarak, geçiş döneminde uygulanan mineral solüsyonlarının negatif enerji dengesi ve yağ mobilizasyonu üzerine pozitif etkidiğini söylemek mümkündür. Bu bulguyu destekler nitelikte, BHBA düzeyleri de kontrol grubu ineklerde doğum anında artmış ve sonrası dönemde hafif azalma göstermişken, deney grubundaki ineklerde doğum anındaki artışlar doğum sonrası dönemde yeniden başlangıç değerlerine yakın bir değere ulaşmıştır. Deney grubundaki bu etkilerin özellikle mangan gibi karbonhidrat, yağ ve protein metabolizmasıyla ilgili minerallerin [24] uygulanmasından kaynaklandığı düşünülmektedir.

Mevcut çalışmada, her iki gruptaki ineklerde geçiş dönemi boyunca saptanan kan glikoz düzeyleri, farklı araştırıcılar [6,30,31] tarafından bildirilen sınırlar içerisinde saptanmıştır. Hem kontrol hem de deney grubunda doğum öncesi ve sonrası değerlere nazaran doğum anında saptanan yüksek glikoz düzeyleri, doğum sırasında kan glikoz düzeylerinin yükseldiğini bildiren araştırıcıların [30,32,33] bulgularıyla da uyumlu bulunmuştur.

Geçiş dönemindeki karaciğer fonksiyonlarını değerlendirmede GGT, SDH, AST ve ALT gibi enzimler ve total bilirubin düzeylerine de bakılmaktadır [11,13]. Bazı yayınlarda [34] AST ve ALT düzeylerinin gebe hayvanlarda gebe olmayanlara göre daha yüksek olduğu bildirilmesine rağmen, diğer bazı çalışmalarda [13,35] kuru döneme nazaran, doğumdan sonraki dönemde AST, ALT ve T. bilirubin düzeylerinin arttığı ifade edilmiştir. Uçar ve arkadaşları ise mineral solüsyonu uyguladıkları ineklerde ALT ve LDH değerlerinin değişmeden kaldığını, AST'nin ise artma eğilimi gösterdiğini vurgulamışlardır. Bir çalışmada doğum sonu dönemde total ve direk bilirubin düzeylerinde artışlar saptanmış ve bu durumun nedeni olarak da, bu dönemde negatif enerji dengesine bağlı olarak gelişen karaciğer fonksiyon yetersizliği gösterilmiştir [36]. Elitok ve ark.[12] doğumdan sonraki ilk haftalık süreçte kan bilirubin düzeylerinde önemsiz artışlar saptamıştır. Mevcut çalışmanın sonuçlarına bakıldığında hem kontrol grubu hem de deney grubunda AST, ALT, GGT ve T. bilirubin düzeylerinin doğum öncesi döneme nazaran doğum anında artış gösterdiği anlaşılmaktadır. Bu artışların sebebinin doğuma yaklaştıkça ortaya çıkan enerji açığına bağlı olarak oluşan yağ mobilizasyonu nedeniyle karaciğerde şekillenen hücresel hasar olduğu düşünülmektedir. Özellikle kontrol grubunda NEFA ve enzim düzeylerindeki artışlar ile kolesterol düzeylerindeki azalmaları karaciğerde gelişen hasarın göstergesi olduğunu kabul etmek bu anlamda mümkündür.

Genel olarak doğum sonrası dönemde değişik derecelerde ortaya çıkan karaciğer yağlanması ile ilişkili olarak kan serumundaki lipoprotein (VLDL, LDL, HDL) düzeylerinde azalmaların olduğu yapılan araştırmalarda [13,37] ortaya konulmuştur. Mevcut çalışmada karaciğer yağlanması ile ilgili herhangi bir araştırma yapılmamasına rağmen, gerek kontrol gerekse deney gruplarında doğum anı ve sonrasında saptanan VLDL, LDL, HDL ve T. kolesterol düzeyleri doğum öncesi değerlere göre düşük saptanmıştır. Bu durumun muhtemel nedeni doğum zamanında artan NEFA düzeylerinin karaciğerde belirli derecelerde hasar oluşturması ve yetersiz VLDL üretimidir. Çünkü VLDL sentezinin azalması, bu maddeden orijin alan LDL ve HDL düzeylerinde de azalmanın oluşmasına neden olmaktadır. Çalışmada, deney grubunda doğum anında azalan lipoprotein düzeylerinin ve düzeyleri artan NEFA'ların doğumdan sonraki dönemde yeniden başlangıç değerlerine yaklaştığı saptandığından, karaciğerdeki hasarın muhtemelen geçici özellikte oluştuğunu söylemek mümkündür.

Geçiş dönemindeki ineklerde yetersiz protein alımı nedeniyle BUN'da azalmalar gözlenebileceği bir çalışmada vurgulanmıştır [38]. Başka bir araştırmada [13] ise doğum öncesi ve sonrası dönemde kan üre konsantrasyonu normal sınırlar içerisinde ölçülmüş, ancak doğum sonrası önemsiz

derecede saptanan artışların nedeni olarak doğum stresinin neden olduğu glomerular filtrasyon oranının azalması gösterilmiştir. Elitok ve ark.[12] ise yeni doğum yapmış ineklerde doğum sonrası dönemde düşük üre düzeyleri saptamışlar ve bu durumun nedeni olarak bu dönemdeki yağ infiltrasyonu nedeniyle protein anabolizmasındaki azalmayı göstermişlerdir. Mevcut çalışmada her iki grupta da doğum öncesi düzeylere göre doğum sırası ve sonrası dönemlerde belirlenen BUN düzeyleri azalma göstermiş, ancak tüm dönemlerde saptanan düzeyler normal sınırlar arasında kalmıştır. Doğum anı ve sonrası dönemdeki azalmaların nedeni olarak hem karaciğerdeki muhtemel hasar hem de bu hasara bağlı olarak protein anabolizmasındaki azalma düşünülmektedir. Bunu destekler nitelikte olarak, doğum anı ve sonrası dönemlerde albümin düzeylerinde de azalma saptanmıştır.

Geçiş döneminde özellikle albumin düzeyleri postpartum bazı hastalıklarla alakalı bulunmuş ve gerek ahırdaki gerekse meradaki sığırlar için hastalık riskini belirlemede kullanılmıştır [11]. Doğumdan sonraki süreçte serum total protein ve albumin düzeyleri doğum öncesi ve sonrası dönemlere kıyasla düşük olduğu bir çalışmada saptanmıştır [39]. Başka bir araştırmada bakır ilavelerinin laktasyondaki ineklerde albümin oranlarında bir değişime neden olmadığı saptanmıştır [32]. Ucar ve ark.[40] ise mineral solüsyonu uyguladıkları ineklerde kreatinin ve albümin düzeylerinin değişmeden kaldığını ifade etmişlerdir. Çalışmada hem kontrol hem de deney grubunda doğum öncesi değerlere nazaran doğum anındaki total protein ve albümin değerleri düşük saptanmış ve bu azalma kontrol grubunda doğum sonrası dönemde de devam etmesine rağmen deney grubunda yeniden artış göstermiştir. Gerek albümin ve gerekse de T. protein düzeylerindeki bu değişimler, deney grubunda geçiş döneminde ortaya çıkan olumsuz etkilerin geçici olarak ortaya çıktığının bir göstergesi olarak kabul edilebilir.

Çalışmada düzeyleri belirlenen metabolik parametrelerden özellikle geçiş dönemindeki olumsuz etkileri yansıtan NEFA, BHBA, ve lipid profili dikkate alındığında, geçiş döneminin başlangıcında özellikle selenyum, bakır, çinko ve mangan içeren bir mineral solüsyonu uygulamasının, metabolik profil üzerine olumlu etkilerinin olabileceği sonucuna varılmıştır.

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RESEARCH ARTICLE

Heterotopic Allogenic and Autogenic Ovarian Transplantation in Rabbits: Assessment and Comparison of the Morphological and Endocrine Characteristics [1]

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Summary

The aim of this study was to evaluate and compare the endocrine function and morphological characteristics of subcutaneous heterotopic allogenic and autogenic ovarian transplantation in immune-suppressed rabbits. Each group included seven rabbits. Group I (allogenic group) underwent freshly subcutaneous allogeneic heterotopic transplantation (n=7) (from group III to group I). Group II (autogenic group) underwent freshly subcutaneous autogenic heterotopic transplantation (n7).Group III was the donor group (n=7). The levels of serum follicle-stimulating hormone (FSH) were significantly lower during the 2nd week (P=0.017) than during the 3rd week in group I, and were significantly higher during the 4th week than at other times (P=0.001) in group II. The levels of serum 17-β estradiol (E_2) were significantly lower during the 3rd week (P=0.008) than during the 1st and 2nd weeks in group I, and were significantly lower during the 4th week than at other times (P=0.001) in group II. The levels of serum progesterone (P_4) were not vary significantly (P=0.441) in group I and were significantly higher during the 1st week than during the 3rd week (P=0.033) in group II according to the ANOVA. Histological examination was performed using light microscopy after staining with hematoxylin-eosin (HE). Our results showed that there is no remarkable difference between autogenic and allogeneic heterotopic freshly transplanted ovarian tissue, especially in terms of the FSH and P_4 levels during a four week period. We found that autogenic and allogeneic freshly transplanted ovarian tissues had similar characteristics in terms of the endocrine and histological characteristics.

Keywords: *Ovarian transplantation, Allogenic, Autogenic, FSH, E_2, P_4*

Tavşanlarda Heterotopic Allojenik ve Otojenik Yumurtalık Nakli: Morfolojik ve Endokrin Özelliklerinin Değerlendirilmesi ve Karşılaştırılması

Özet

Bu çalışmanın amacı, immün sistemi baskılanmış tavşanlara subkutan heterotopik allojenik ve otojenik yumurtalık naklinin endokrin ve morfolojik özelliklerinin değerlendirilmesi ve karşılaştırılmasıdır. Her gruba 7 tavşan dahil edildi. Grup I (allojenik: n=7) taze subkutan heterotopik allojenik transplantasyon (grup III'den, grup I'e), grup II (otojenik: n=7) taze subkutan heterotopik otojenik transplantasyon (kendi ciltaltlarına) ve grup III donor grupları olarak belirlendi. Serum follikül stimülan hormone (FSH) düzeyleri, grup I'de, 2. haftada, 3. haftadaki değerlerden (P=0.017) anlamlı derecede daha düşüktü, ve grup II'de 4. haftadaki değerler, diğer haftalardaki değerlerden anlamlı olarak daha yüksekti (P=0.001). Serum 17-β estradiol (E_2) düzeyleri, grup I'de, 3. haftada, 1. ve 2. haftalardaki değerlerden anlamlı derecede düşüktü (P=0.008) ve grup II'de 4. haftadaki değerler, diğer zamanlardaki değerlerden anlamlı derecede düşüktü (P=0.001). Serum progesterone (P_4) düzeyleri, grup I'de, anlamlı farklılık göstermedi (P=0.441) ve grup II'de, 3. haftadaki değerlerden, 1. haftadaki değerler anlamlı olarak yüksekti, ANOVA testine göre (P=0.033). Histolojik inceleme Hematoksilen-eozin (HE) ile boyanarak ışık mikroskobu ile yapıldı. Bizim sonuçlarımız özellikle dört haftalık dönemde, FSH ve P_4 düzeyleri açısından otojenik ve allojenik heterotopik taze nakledilen yumurtalık dokusu arasında anlamlı fark olmadığını göstermiştir. Sonuç olarak, otojenik ve allojenik taze nakledilen yumurtalık dokularının endokrin ve histolojik yönden benzer özelliklere sahip olduğu belirlenmiştir.

Anahtar sözcükler: *Yumurtalık nakli, Allojenik, Otojenik, FSH, E_2, P_4*

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INTRODUCTION

Currently, ovarian transplantation (OT) is a potential method for the preservation of reproductive function. The first experimental ovarian transplantation was described by Paul Bert in 1863 [1]. The first experimental study of OT using micro-vascular surgery that resulted in pregnancy following the auto-graft transplantation of fallopian tubes and ovaries in a rabbit was published by Wiston and McClure Browne in the Lancet in 1974 [1]. The first successful heterotopic ovarian auto-transplant using microsurgery in a woman was reported by Von Theobald and coworkers in 1987. The heterotopic transplanted ovary in this case had normal endocrine function and follicular development [1].

Although experimental ovarian transplantation was first performed approximately 100 years ago, during last two decades, it has gained great momentum, with many advances in assisted reproductive techniques. Recently, ovarian transplantation has been performed in many different animal models and in humans, but the optimal conditions for ovarian transplantation have not been established. Different transplantation techniques with or without microvascular anastomoses or pedicles, and various placements of the transplanted tissues, including the normal anatomic position and positions outside the normal anatomic positions (subcutaneous, intra-peritoneal, retroperitoneal, inguinal region or inside the kidney capsule) have been described in many different animal models [1-9]. The transplantation of whole ovaries and the grafting of fresh or frozen-thawed ovarian tissues have been successfully reported by many authors in human [10].

In this report, we describe and compare the morphological and endocrine characteristics of transplanted ovaries after autogenic and allogenic heterotopic ovarian transplantation without vascular anastomosis in rabbits. To the best of our knowledge, this is the first published report that compares freshly heterotopic allogenic and heterotopic autogenic ovarian transplantation without vascular anastomosis in rabbits.

MATERIAL and METHODS

This study was approved by the Ethics Committee of Animal Experiments of Kafkas University (the number: 2009-30). In this study, twenty one mature New Zealand white female rabbits were between six and eight months and weighing between 2.8 and 3.9 kg purchased from the Experimental Animal Investigation Center of Ataturk University in Erzurum, Turkey. These animals were maintained in a temperature controlled environment, illuminated for 12 h daily and fed with commercial pellets and water *ad libitum*.

During the follow-up period, before and after surgical procedure, the female rabbits received food and filtered water ad libitum in separate containers and were maintained in individual cages. All procedures were carried out under aseptic conditions in the Laboratory of Experimental Surgery, Department of Veterinary Surgery of Kafkas University. One day before surgery, and for 3 days after surgery, all animals were intramuscularly injected with 1.000 mg of cefazolin sodium (Cefamezin; Eczacıbaşı Drug Co, Turkey.).

All animals undergoing transplantation (in group I and group II) were also received daily an intramuscular injection of 25 mg/kg cyclosporine-A (Sandimmune, Novartis Drug Co., Swiss) throughout the three week period to prevent graft-versus-host rejection, which was primarily a concern for the allogenic group (group I). We also administered cyclosporine-A to the animals in group II (autogenic group) to eliminate any differences between the groups due to the effect of cyclosporine.

Anesthesia was achieved by the intramuscular injection of 25 mg/kg ketamine HCl (Ketasol 10%, Richter Pharma Drug Co., Austria) and 5 mg/kg Xylazine HCl (Rompun 2%, Bayer Drug Co. Animal Health, Germany). Each animal's abdomen was shaved and then disinfected with a Povidone-iodine (PVP-I) solution followed by a 2% alcohol solution of iodine.

The animals were randomly divided into three experimental groups. Bilateral ovariectomies were performed on group I rabbits (n=7). The ovaries retrieved from the donor group (group III) were immediately grafted to lower neck under the skin subcutaneously into group I (allogenic group). On the day before surgery and for four weeks after surgery, blood samples were taken for the analysis FSH, E_2, and P_4. One rabbits died due to immunosuppression on the twenty-ninth day. The other rabbits were euthanized, and subsequently the ovarian grafts were removed for histopathological analysis.

Bilateral ovariectomies were performed on group II rabbits (n=7) (autogenic group) and those ovaries were immediately autologously subcutaneously grafted into the lower neck of the same rabbits. On the day before surgery and for four weeks after surgery, blood samples were taken for the analysis FSH, E_2, and P_4. Blood samples were taken on days 7. 14. 21. 28. after transplantation. Unfortunately, one rabbit in group II died as a result of immunosuppression on the tenth day. The other rabbits were euthanized and subsequently, the ovarian grafts were removed for histopathological analysis.

Blood samples were collected from the marginal artery of the rabbit's ear before surgery and on days 7, 14, 21, 28 after surgery in all animals and centrifuged immediately at 2.500 rpm for 10 min. Serum samples were obtained to measure the FSH, E_2 and P_4 levels. Then, the serum was frozen at-20ºC until the hormone tests were performed using commercially available ELISA kits (Cusabio Biotech Co. Ltd. Hubei Province 430223, P.R. China).

Ovarian tissue samples were immediately fixed in 10% buffered formalin solution and were processed using conventional techniques for light microscopy analysis. Samples were stained with H.E. for histological analysis and observed by light microscopy (Olympus Optical Co., Osaka, Japan).

Statistical analyses (SPSS package version 11.5) were performed using Student's t-test for parametric data and ANOVA variance for multiple groups. Tukey's test was performed to identify the source of significant differences revealed by ANOVA.

RESULTS

We measured serum levels of FSH, E_2 and P_4 on the day before ovariectomy and on days 7. 14. 21. 28. after transplantation in all animals. We started the study with twenty-one rabbits, but one rabbit from group I and one rabbit from group II died due to immunosuppression before the end of the study.

The serum FSH and P_4 levels were not significantly different (P=0.175, P=0.147) between group I (allogenic)

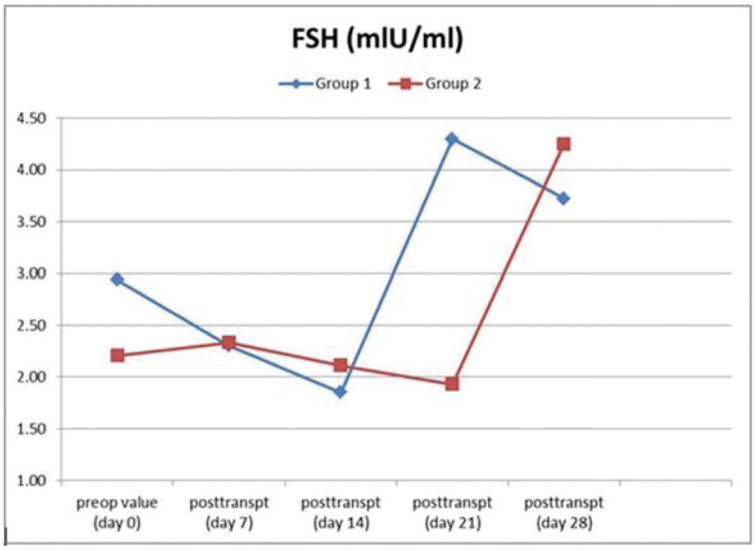

Fig 1. Variation of the serum level of FSH in allogeneic (group I) and autogenic (group II)

Şekil 1. Allojenik (grup I) ve otojenik (grup II) gruplarda serum FSH seviyelerindeki değişim

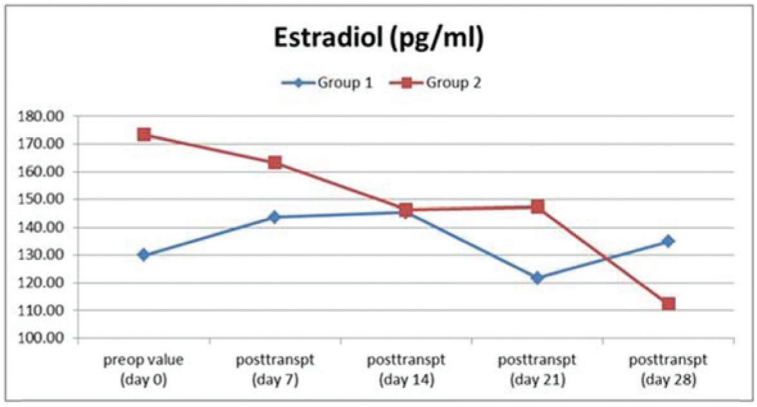

Fig 2. Variation of the serum level of E_2 allogenic (group I) and autogenic (group II)

Şekil 2. Allojenik (grup I) ve otojenik (grup II) gruplarda serum E_2 seviyelerindeki değişim

Table 1. *Serum FSH, E_2, P4 levels of group I (allogenic) and group II (autogenic)*
Tablo 1. *Grup I (alojenik) ve grup II (otojenik) de serum FSH, E_2, P_4 degerleri*

Days	Group I (Allogenic Group)			Group II (Autogenic Group)		
	FSH m IU/ml	E_2 pg/ml	P_4 ng/ml	FSH m IU/ml	E_2 pg/ml	P_4 ng/ml
Day 0 (pre-op.)	2.9±0.3	129.9±6.0	56.6±4.8	2.2±0.2	173.4 ±7.6	69.3±4.5
Day 7 (post-transp.)	2.3±0.3	143.8±2.4	59.0±2.2	2.3±0.2	163.2±11.8	73.2±2.9
Day 14 (post-transp.)	**1.9±0.1****	145.5±4.5	59.5±4.3	2.1±0.1	146.4±4.7	58.7±3.2
Day 21 (post-transp.)	4.3±0.7	**121.8±5.0***	62.9±1.8	1.9±0.1	147.4±7.2	**55.2±5.9****
Day 28 (post-transp.)	3.7±0.8	134.9±4.7	53.6±3.5	**4.3±0.8***	**112.3±6.0***	63.7±3.7

** P<0.01, ** P<0.05*

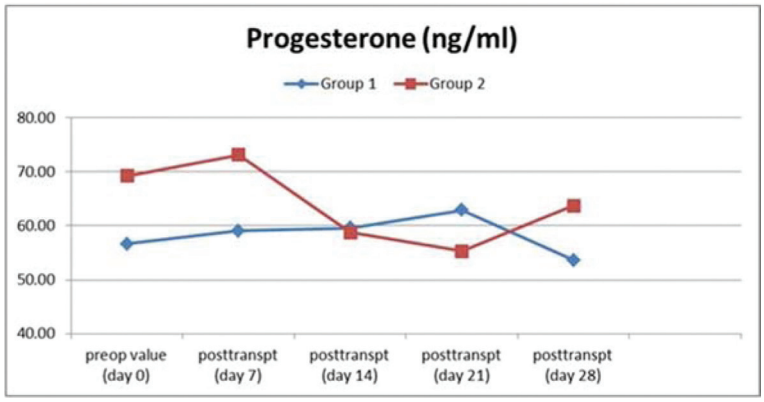

Fig 3. Variation of the serum level of P4 allogeneic (group I) and autogenic (group II)

Şekil 3. Allojenik (grup I) ve otojenik (grup II) gruplarda serum P4 seviyelerindeki değişim

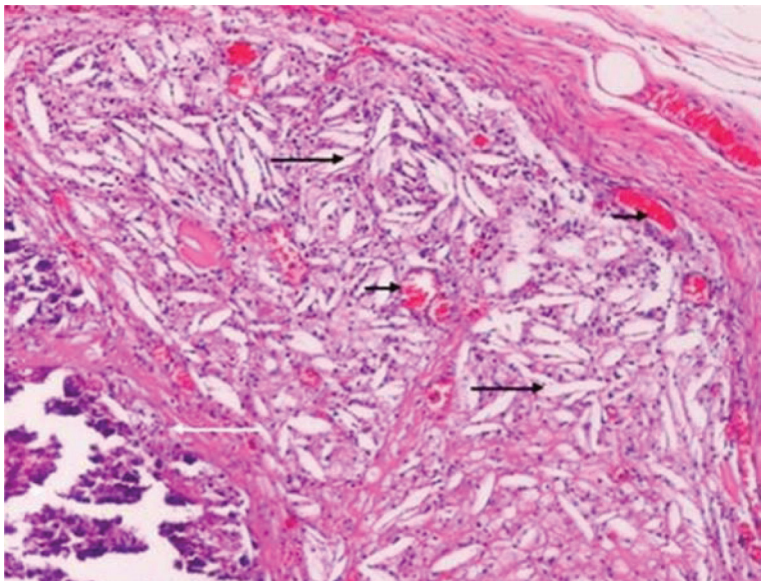

Fig 4. Rabbit ovary from group II (autogenic). No follicles were observed in the cortical regions. Common cholesterol necrosis in the cortical region, cholesterol clefts *(long black arrows)* and vascularization were presented in the necrotic tissue *(short black arrows)*. Severe necrosis of the corpus luteum *(white arrow)* was observed. HEx10

Şekil 4. Grup II (otojenik) tavşan yumurtalığı. Kortekste follikül gözlenmedi. Kortekste yaygın kolesterol nekrozu *(uzun siyah oklar)* ve vaskularizasyon *(kısa siyah oklar)*. Korpus luteumun ciddi nekrozu *(beyaz ok)*

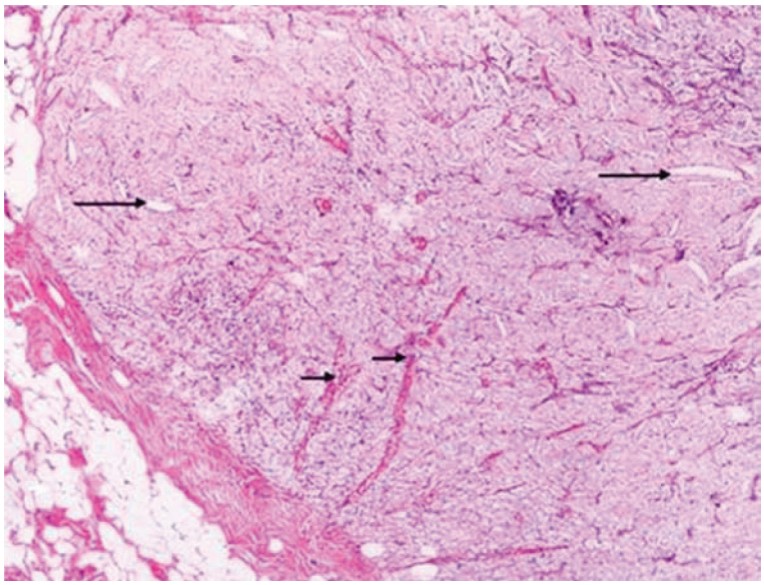

Fig 5. Rabbit ovary from group I (allogeneic). No follicles were observed in the cortical regions. Common cholesterol necrosis in the cortical region, cholesterol clefts *(long black arrows)* and vascularization were present in the necrotic tissue *(short black arrows)*. HE x 4

Şekil 5. Grup I (allojenik) tavşan yumurtalığı. Kortekste follikul gözlenmedi. Kortekste yaygın kolesterol nekrozu *(uzun siyah oklar)* ve vaskularizasyon *(kısa siyah oklar)*

and group II (autogenic), but the serum E_2 levels were significantly different between these two groups (P=0.012) (135.17±2.54 versus 148.53±5.04) *(Table. 1)*.

The difference in the FSH level between the two groups was not significant according to a Student's t-test used (P=0.198). The levels of serum follicle-stimulating hormone (FSH) were significantly lower during the 2nd week (P=0.017) than during the 3rd week in group I, and were significantly higher during the 4th week than at other times (P=0.001)

in group II according to the results of the ANOVA *(Fig. 1)*.

The difference in the E_2 level between the two groups was statistically significant according to a Student's t-test (P=0.021, P=0.023). The levels of serum 17-β estradiol (E2) were significantly lower during the 3rd week (P=0.008) than during the 1st and 2nd weeks in group I, and were significantly lower during the 4th week than at other times (P=0.001) in group II according to the ANOVA *(Fig. 2)*.

The difference in the P_4 levels between two groups was statistically significant according to the Student's t-test (P=0.036 in both groups). The levels of serum progesterone (P_4) were not vary significantly (P=0.441) in group I and were significantly higher during the 1st week than during the 3rd week (P=0.033) in group II according to the ANOVA *(Fig. 3)*.

Histological examinations were performed for the allogeneic and autogenic groups and the features of the ovarian tissues are presented in *Fig. 4* (group II-autogenic) and *Fig. 5* (group I-allogenic).

DISCUSSION

Experimental ovarian transplantation (OT) in animals was first performed in the late 18th century. In the past 20 years, great progress has been made in ovarian transplantation, and these procedures, both in animals and in humans, have become more reliable in recent years. However, there are still few reports on the restoration of ovarian function that show a satisfactory reproductive rate.

The autogenic and allogenic heterotopic ovarian transplantation with micro vascular technique in rabbits were reported by Meraz et al.[1]. The authors, in this study, also in both groups, after hCG administration, values of E2 (1.75 pg/ml versus 148.5 p/ml in autogenic, 0.25 pg/ml versus 135.1 pg/ml in allogenic) and P4 (2.15 ng/ml verus 64.0 ng/ml in autogenic, 0.20 ng/ml versus 58.3 ng/ml in allogenic) were reported remarkably lower than our values [1].

The allogeneic orthotropic transplantation of intact and sliced ovarian tissue without the vascular pedicle were reported by Petroianu et al.[2,3]. These authors analyzed the differences among the groups according to the hormone levels and histological features. The groups of this study were included animals that received intact ovaries (G2A) (n=8), animals that received sliced ovaries (G2B) (n=8), animals that received an intact ovary on one side and a sliced ovary on the other side (G2C) (n=8), and control animals (G1) (n=8). When the hormone levels were compared between the groups and subgroups, the authors found no differences in values, except in subgroup 2C, which showed higher estradiol levels. After observing the rabbits for nine months, the authors showed that intact or sliced orthotropic allogenic OT without vascular anastomosis was viable in rabbits [2,3]. We noted that the hormone values for allogenic group reported by these authors differed from the values that we observed; their values were especially lower than our measured values for E_2 (4.13 pg/ml versus 135.1 pg/ml), FSH (0.13 IU/l versus 3.02 m IU/ml), and P_4 (0.103ng/ml versus 58.3 ng/ml). We couldn't explain any other reason why our all hormone values were higher than those values in both studies but we think that high estradiol values indicate better functional transplanted ovarian grafts.

OT without the vascular pedicle is much better, especially in small animals because this procedure does not require the difficult microsurgery that is necessary to construct the vascular anastomosis. The increase in the operation time due to the need to construct vascular anastomoses may be more risky for small animals [2].

The levels of serum estradiol and morphologies of autologous transplanted ovarian tissue with or without remote ischemic precondition (R-IPC) in rats were studied by Damous et al.[4]. The authors found that R-IPC led to increased levels of serum estradiol (average 65 pg/ml) in most animals. However, there was no significant difference between the groups, although it showed lower values (36 pg/ml). Generally, the grafts were better preserved in the R-IPC group.

The hormone levels and follicular development after heterotopic ovary transplant without the vascular pedicle were studied in syngeneic Lewis rats [5]. The authors observed the recovery of hormone levels to preoperative values within 28 days, and the lowest values were observed at 4 and 7 days after transplantation. In this study, in only autogenic heterotopic group, the authors reported that the values of E_2 on days 7, 14, 21, 28 were 23.4 pg/ml (versus 163.2 pg/ml), 45.2 pg/ml (versus 146.4 pg/ml), 56.6 pg/ml (versus 147.4 pg/ml) and 83.6 pg/ml (versus 112.3 pg/ml), respectively. Also, our E_2 values were higher than those values in this study [5].

The restoration of ovarian function after transplantation with micro-vascular anastomoses of intact frozen-thawed sheep ovaries was studied by Bedaiwy et al.[6]. They reported that 8 of 11 ovaries were nonfunctional due to thrombotic events in the re-anastomosed vascular pedicles.

Recently, a pregnancy and delivery was achieved after the auto-transplantation of whole cryopreserved sheep ovaries with micro-anastomoses, as reported by Imhof et al.[7].

The first study was performed to evaluate the feasibility of transplanting a whole adult mouse ovary, and to compare the live birth rates of mice in the sham-operated, fresh auto-transplanted ovary and cryopreserved auto-transplanted ovary groups [8].

A study of ovarian auto-transplantation without vascular pedicles in sixteen female rats was reported by Risvanli et al.[9]. In this study, animals were divided three groups. All animals except those in the third group (sham operated group,

n=5) underwent bilateral ovariectomy. The transplanted ovaries were placed completely under the peritoneum in first group animals (n=5) and were placed subcutaneous near the inguinal plexus in the second group (n=6). The authors noted that the estradiol concentrations in rats that had sub-peritoneal transplanted ovaries were higher than the concentrations in the other groups, and the results were statistically significant (P<0.001). In addition, there was no sign of inflammation in the sham group, but the other two groups showed varying degrees of inflammation, but the differences were not statistically significant (P>0.058) [9].

Callejo et al.[10] evaluated long-term ovarian function after the heterotopic auto-transplantation of fresh and frozen-thawed human ovarian tissue without vascular anastomoses in four premenopausal patients. These patients were followed for 1 year and did not receive gonadotropins. The women who received either fresh or cryopreserved ovarian tissues without vascular anastomoses at heterotopic sites regained ovarian function. The authors also reported that there was a significant decrease in the serum E_2 level from normal premenopausal values, and menopausal symptoms were observed within 3 weeks after surgery. The hormone functions of heterologous [subcutaneous in the arm in two patients (patients 1 and 2)], auto-transplanted fresh ovarian tissues and heterologous [in the rectus abdominis muscle in one patient (patient 4)], auto-transplanted frozen-thawed ovarian tissues were regained 3-4 months after transplantation [10].

A successful fertilization and pregnancy were achieved using retrieved oocytes from a primate that had undergone fresh ovarian tissue transplantation without any surgical anastomoses to major blood vessels were reported by Lee et al.[11].

The restoration of endocrine function during 14 weeks was reported by Kim et al.[12] after transplantation in a 37-years-old woman who had undergone heterotopic transplantation of cryopreserved ovarian tissue, but the cessation of ovarian function was verified by very high FSH levels 28 weeks after transplantation.

Camboni and Martinez-Madrid [13] evaluated the structural and ultra-structural morphology and viability of grafted tissue, by using transmission electron microscopy after the orthotropic auto-transplantation of frozen thawed human ovarian tissue for 1 year. They reported that primordial and primary follicles were well-protected throughout the 13 months' post-graft period. These authors confirmed that primordial and primary follicles were perhaps more resistant to freeze-thaw procedures and less vulnerable to ischemia than secondary and antral follicles [13].

In another study, Denjean et al.[14] reported the transplantation of rabbit ovaries with vascular pedicles including the ovarian artery and vein using either orthotropic transplantation by end-to-end anastomoses to the ovarian vessels or heterotopic transplantation to the inferior epigastric vessels. They found that the ovulation rate, if the ovary was enclosed in a peritoneal sac, after heterotopic transplantation to the epigastric vessels was high and comparable to that for orthotropic transplantation [14-16].

In a meta-analysis that included 46 women who underwent ovarian transplantation, and the continuation of ovarian function was documented within 60-244 days in 23 women who had have high FSH level (>30) at the time of transplantation was published. Recurrent ovarian failure was observed in four women within 6 months. The authors emphasized that the findings were not sufficient to assess the functions of transplanted ovarian tissue for longer than 12 months [17].The authors explained that the likelihood of the return of ovarian function for fresh transplanted ovarian tissues was higher than for cryopreserved transplanted tissues and that the likelihood of recurrent ovarian failure was much lower for fresh transplanted tissues than for cryopreserved transplanted tissues. They reported that eight of 25 women who attempted to become pregnant pregnancy had become pregnant within 12 months, giving a cumulative pregnancy rate of 37% [17-19].

The primary drawback of ovarian transplantation without vascular anastomoses is the initial ischemia that occurs to varying degrees, depending on the type of transplanted ovarian tissue (whole ovary or sliced cortical ovarian pieces). According to some authors, the reduction in the number of primordial and antral follicles is expected to be 50-65% in some studies, but one study reported a reduction of >90% [20]. In our study, histologic examination showed severe reduction of ovarian cortical follicles, severe necrosis and weakly vascularization in the both transplant groups. Although we estimated that the reduction in the number of primordial and antral follicles was approximately 90% in our study, hormone values showed good functioning of transplanted ovarian grafts in both groups.

It was reported that the ovarian cortex can tolerate ischemia for at least 3 hour at 4ºC in a study that showed a correlation between ischemic damage and the ischemic period for transplanted ovarian tissue [21].

The auto-transplantation of fresh or frozen-thawed ovarian tissue allows the preservation of the fertility of girls or women whose ovaries are damaged due to treatment for diseases such as cancer.

According to the relevant publications, Donnez et al.[22], up to 2010, 11 live births have been achieved after orthotropic re-implantation of cryopreserved ovarian tissue.

The transplantation of whole ovaries or ovarian tissues without a vascular pedicle requires vascularization, which takes 5 days. Oktay and Karlikaya [23] reported one case in which frozen-thawed ovarian tissue was transplanted laparoscopically into a 29 years-old patient who had under-

gone bilateral oophorectomy due to a nonmalignant disease in 2000 [23]. Donnez et al.[24] reported the first case that resulted in a pregnancy and live birth after successfully transplantation of cryopreserved ovarian tissue in 2004.

A live birth after the orthotropic auto-transplantation of cryopreserved ovarian tissue in a patient who suffered from premature ovarian failure after chemotherapy was also published in by Meirow et al.[25].

Two cases in which allogenic orthotropic vascular (case 1) and avascular (case 2) ovarian transplantation were performed on two girls who had been diagnosed with ovarian dysgenesis were reported by Mhatre et al.[26]. Case 1: The patient exhibited spontaneous menstruation and, ovulation, and excellent secondary sexual characters during the 2.5 year follow-up period. Case 2: Serial measurements of the serum E2 level showed a significant increase from 20 pg/ml to 50 pg/ml. Ovarian grafts showed excellent graft vascularization, with the development of small follicles [26].

In conclusion, our results showed that there is no difference between autogenic and allogenic heterotopic transplanted ovarian tissue, especially in terms of FSH and P_4 levels within period of four weeks. We found that autogenic and allogenic transplanted ovarian tissues had similar endocrine and characteristics.

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RESEARCH ARTICLE

Dissociative Anaesthesia in Foals for Umbilical Herniorrhaphy Under Field Conditions

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Summary

The aim of the present study was to investigate the effects of a dissociative anaesthetic combination of xylazine-tiletamine-zolazepam, administered for the umbilical herniorrhapy of foals under field conditions, on certain cardiorespiratory and clinical anaesthesia parameters. Eleven foals diagnosed with umbilical hernia, of 4-7 months of age (mean age 5.73±0.91 months) and 130-175 kg body weight (mean body weight 152.55±14.35 kg), 7 of which were female and 4 were male, and 8 of which were Arabian horses and 3 were English horses, constituted the material of the study. The anaesthesia protocol was xylazine (1.1 mg/kg, iv), tiletamine-zolazepam (1.65 mg/kg, iv) and half of the indicated doses after observation of the first signs of recovery 3 times at 8-10 min-intervals for sustainment, together with the subcutaneous injection of approximately 12 ml 2% lidocaine peripheral to the hernial sac in a circular pattern for local anaesthesia. In all foals after last drug injection; anaesthesia induction time, operation time, anaesthesia time and standing time were recorded. Quality of induction, anaesthesia/analgesia and recovery were evaluated. Heart rate, respiratory rate, body temparature, arterial oxygen saturation values, mean arterial blood pressure were evaluated before anaesthesia (as a baseline) and after induction of anaesthesia at 15th, 30th, 45th, 60th and 90th min. After induction of anaesthesia cardiopulmonary parameters and body temparature were decreased below baseline values in first stage of anaesthesia and then they were reached to baseline values in late stage of anaesthesia. It was ascertained in the present study that, the supplementation of the combined use of xylazine-tiletamine-zolazepam in foals under field conditions with local anaesthetics induces an anaesthesia of adequate depth for umbilical herniorrhaphy, and sustaining doses enable the prolongation of the anaesthesia period with cardiorespiratory adverse effects remaining within acceptable limits. Therefore, it is considered that, the indicated anaesthesia protocol could be tested for other surgical interventions in foals under both field and clinical conditions.

Keywords: *Xylazine, Tiletamine-Zolazepam, Dissociative anaesthesia, Field condition, Foal*

Saha Şartlarında Taylarda Umblikal Herniorafi İçin Dissosiyotif Anestezi

Özet

Sunulan bu çalışmanın amacı, saha şartlarında taylarda umblikal herniorafi operasyonu için uygulanan ksilazin-tiletamin-zolazepam dissosiyatif anestezik kombinasyonunun bazı kardiyopulmoner ve klinik anestezi parametreleri üzerine olan etkilerini araştırmaktı. Çalışmada, yaşları 4-7 ay arasında değişen (ortalama 5.73±0.91); canlı ağırlıkları 130-175 kg (ortalama 152.55±14.35); 7'si dişi, 4'ü erkek; 8'i Arap, 3'ü İngiliz ırkı olan, toplam 11 adet göbek fıtığı tanısı konulan tay kullanıldı. Taylarda anestezi protokolü olarak; ksilazin (1.1 mg/kg, iv), tiletamin-zolazepam (1.65 mg/kg, iv) ve belirtilen dozların yarısı ilk uyanma belirtisinden sonra ortalama 8-10 dakikada bir 3 kez idame olarak ve lokal anestezik olarak fıtık kesesi etrafına sirküler tarzda deri altı yolla (% 2 lidokain, yaklaşık olarak 12 ml) uygulandı. Araştırmada, taylara uygulanan son ilaç enjeksiyonundan sonra, anestezi indüksiyon, operasyon ve anestezi süresi ile ayağa kalkma zamanı; anestezi indüksiyon, anestezi/analjezi ve uyanma kaliteleri belirlendi. Kalp atım ve solunum sayısı, vücut ısısı, arteriyel oksijen satürasyonu, ortalama arteriyel kan basıncı, anesteziden önce ve anestezi süresince 15, 30, 45, 60 ve 90. dakikalarda ölçüldü. Saha şartlarında, umblikal herniorafi için taylarda uygulanan anestezi protokolü her hangi bir mortaliteye neden olmamıştır. Belirtilen anestezi protokolünün taylarda uygulanması ile klinik anestezi parametrelerine ilişkin uyanma dönemi hariç bir olumsuzluk gözlenmedi. Uyanma döneminde tayların ayağa kalkmak için birden çok atak yaptıkları belirlendi. Kardiyopulmoner parametrelerin anestezi indüksiyonundan sonraki ilk dönemde düştüğü, sonraki dönemlerde ise başlangıç değere ulaştığı gözlendi. Bununla birlikte; taylarda saha şartlarında kullanılan ksilazin-tiletamin-zolazepam kombinasyonunun lokal anestezi ile desteklenmesiyle, umblikal herniorafi için yeterli bir anestezi sağladığı, idame dozlarla anestezi süresi uzatılmasına rağmen, kardiyopulmoner yan etkilerinin de kabul edilebilir sınırlarda kaldığı gözlendi.

Anahtar sözcükler: *Ksilazin, Tiletamin-Zolazepam, Dissosiyatif anestezi, Saha şartları, Tay*

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INTRODUCTION

In veterinary medicine, anaesthesia is performed to maintain analgesia and immobilization in patients for medical and surgical applications. In foals, one of the several cases that require treatment under general anaesthesia on the farm and in the field, is umbilical hernia. Due to the scarcity of publications available on the anaesthesia of foals, generally, reference is made to research carried out in adult horses [1-2].

Injectable anaesthetics have been widely used in combination with sedatives, tranquilizers and analgesics for diagnosis and surgical treatment in horses under field and clinical conditions [3,4]. In the field, short-term general anaesthesia can be induced safely in horses and foals by the use of injectable anaesthetics either alone or in combination with sedatives, tranquilizers and analgesics by intravenous route [3,5,6]. To date, an ideal anaesthetic combination for use in horses and foals under field conditions has not been reported, yet, it is recommended that injectable anaesthetic preparations be used with caution and patients be monitored [2,4].

The combination of tiletamine, a cyclohexamine anaesthetic, with zolazepam, a benzodiazepine tranquilizer, at a weight proportion of 1:1, induces short-term general anaesthesia in horses after premedication with alpha-2 adrenoceptor agonists (xylazine, romifidine, detomidine). Owing to certain features it displays, this short-term general anaesthesia is described as dissociative anaesthesia [3,7-9]. Many researchers have used the tiletamine-zolazepam combination in horses [5,10-13] and foals [8,14], in association with various sedatives, tranquilizers and analgesics, and have reported the induction of safe general anaesthesia, resembling that induced by the combined use of xylazine and ketamine, but longer. However, the majority of these studies have been conducted on horses and foals that were not subjected to nociceptive stimulation in clinical environment with no surgical intervention.

The aim of the present study was to investigate the effects of xylazine-tiletamine-zolazepam anaesthetic combination administered to foals for umbilical herniorrhapy under field conditions, on certain cardiorespiratory and clinical anaesthesia parameters.

MATERIAL and METHODS

Animal Material

Between the years 2002-2011 eleven foals diagnosed with umbilical hernia, of 4-7 months of age (mean age 5.73±0.91 months) and 130-175 kg body weight (mean body weight 152.55±14.35 kg), 7 of which were female and 4 were male, and 8 of which were Arabian horses and 3 were English horses, constituted the material of the study. Clinical physical examination revealed the foals to be healthy. The animals were weighed on a scale. Foals fasted for 12 h and were allowed to drink water only 1 h prior to the surgical operation. Approximately 30 minutes before the start of the operation, an IV catheter (No:14G) was placed in the jugular vein of all animals, followed by the attachment of a 3-way stopcock. All anaesthetic preparations, and during the intraoperative period 5% dextrose-lactated Ringer's solution (at a flow rate of 10 ml/kg/h), were administered through the catheter.

Anaesthesia Protocol

To the test cases (the 2 cases not included in this study) xylazine (Rompun 2%, Bayer) were administered at a dose of 1.1 mg/kg by IV route (initial dose). Once the sedative effect of xylazine manifested itself within 5 min, with the observation of characteristic signs of dropping of the head, lower lip and eyelids, the tiletamine-zolazepam combination (Zoletil 50, Virbac) was administered at a dose of 1.65 mg/kg by IV route in 30 sec (initial dose). Upon the start of the operation (umbilical herniorrhaphy), it was observed that the foals reacted to the surgical manipulations. For this reason, the anaesthesia protocol was further supported by the administration of approximately 12 ml of 2% lidocaine (Adokain, Sanovel) by subcutaneous route, in a circular pattern, peripheral to the hernial sac to maintain local anaesthesia. In order to prolong the duration of anaesthesia, half of the initial doses of xylazine and tiletamine-zolazepam were administered as sustaining doses to the foals upon the observation position of the eyeball (must be central in horse during surgical plane of anaesthesia), and depending on the preference of the surgeon, three times at 8-10 minute intervals. Thereby, the surgical operation was able to be completed without any problem. The anaesthesia protocol developed using the test cases was applied to the 11 cases included in this study. The anaesthesia protocol and surgical operation were performed in the stalls, where the foals were kept (under field conditions). Following the induction of anaesthesia, none of the foals were intubated and ventilated with oxygen. However, an endotracheal tube and oxygen tube were made available in case of an emergency.

Parameters Determined

Following the initial injection with tiletamine-zolazepam, anaesthesia induction time was determined as the interval between the initial injection and the lateral recumbency of the foal; anaesthesia time was ascertained as the interval between the beginning of lateral recumbency and the first spontaneous movement of the foal after the end of the operation; the operation period was determined as the interval between the induction of anaesthesia and the completion of the last skin suture, including the preparation of the operation site; standing time was ascertained as the interval between the induction of anaesthesia and the time the animal maintained standing position. The quality of the induction of anaesthesia, anaesthesia/analgesia and recovery from anaesthesia was scored subjectively by the use of the modification of criteria previously applied by

various researchers [11,12,15]. Accordingly, good, fair and poor were scored with 3 points, 2 points and 1 point, respectively *(Table 1)*.

In order to determine the cardiorespiratory effects of the anaesthetic combinations administered, the heartbeat per minute was counted using a stethoscope, mucosal capillary refill time was ascertained by applying pressure to the oral mucosa or gingivae by a finger, the colour of the mucous membranes was appraised by observing the colour of the oral mucosa, and the number of respirations per minute was determined by observing the costo-abdominal movements of the animals. The mean arterial blood pressure was measured using the non-invasive oscillometric method by placing a cuff (IW1, Omron®, Japan) around the base of the tail (median coccygeal artery). Body temperature was measured from the rectum by means of a digital thermometer. Arterial oxygen saturation values (SpO_2 %) were measured by placing pulse oxymetre probes (Nanox 2, Medlab, Germany) into the ears of the foals and by covering the ears with a surgical drape to provide protection from light. The indicated cardiorespiratory parameters and body temperature were measured prior to anaesthesia (as a baseline value, approximately 10 min before anaesthesia), in a peaceful environment while the animals were calm, and 15, 30, 45, 60 and 90 min after the last injection. Furthermore, any physiological changes and complications (i.e. apnea, apneustic respiration, shock) observed in the foals during the conduct of the study were also recorded.

Statistical Analyses

The Minitab v 11.0 Software was used for statistical analyses. In the present study, data are given as *Mean±SD*. *Descriptive Statistics* were used for the determination of the *Mean±SD* values of the data, whilst data analysis for repeated measurements of cardiorespiratory parameters and body temperature was performed by means of T*wo-Way Anova*. Differences were considered to be significant when the P value was <0.05.

RESULTS

The anaesthesia protocol applied in this study to the foals for umbilical herniorrhapy did not cause any mortality. Excluding recovery from anaesthesia, the anaesthesia protocol followed did not cause any adverse effect on the clinical anaesthesia parameters of the foals. During recovery from anaesthesia, it was observed that the foals made multiple attempts to regain standing position.

Details of the effects of the anaesthesia protocol followed on cardiorespiratory parameters and body temperature are presented in *Table 2*, whilst the effects of the protocol on clinical anaesthesia parameters are given in *Table 3*.

During anaesthesia, the heart rate of the foals displayed moderate (P>0.05) falls at all measurement times, in comparison to the baseline value recorded. Respiratory rates and arterial oxygen saturation (SpO_2) values displayed significant decrease (P<0.05) 15, 30 and 45 min after the last injection, when compared to the baseline values, whilst it was observed that, the values recorded 60 and 90 min after the last injection had drawn closer to the baseline values. Body temperatures measured 30 and 45 min after the last injection were significantly lower than the baseline values, whilst the body temperatures recorded 60 and 90 min after the last injection had drawn closer to the baseline values. Mean blood pressure values measured 15, 30 and 45 min after the last injection were significantly higher than the baseline value (P<0.05), whilst mean values measured 60 and 90 min after the last injection were moderately higher than the baseline value *(Table 2)*.

Table 1. *Scoring criteria for the quality of induction, anaesthesia/analgesia and recovery in foals*
Tablo 1. *Taylarda anestezi indüksiyon kalitesi, anestezi/analjezi kalitesi ve uyanma kalitesini skorlama kriterleri*

GOOD (3 Points)	FAIR (2 Points)	POOR (1 Point)
QUALITY OF INDUCTION		
The animal enters anaesthesia within a short period of time and peacefully. Back and forth movements are very few	The entry of the animal in anaesthesia is prolonged and the animal displays slight incoordination, marked back and forth movements, and convulsions on the ground	Induction of anaesthesia is markedly prolonged than normal, associated with marked incoordination, evident back and forth movements and excessive tremor of the muscles
QUALITY OF ANAESTHESIA/ANALGESIA		
The animal gives no reaction to surgical manipulations. No tremor of the muscles of the body or extremities is observed. Muscle relaxation is satisfactory	The animal reacts to surgical manipulations with very light movements. Slight muscle tremors and moderate muscle relaxation are observed	The animal displays pronounced reaction to surgical manipulations. The animal retracts and shakes its legs. Very evident muscle tremors and marked muscular tonus are observed
QUALITY OF RECOVERY FROM ANAESTHESIA		
No excitation or incoordination is observed. The animal regains standing position calmly and at its first attempt. No marked ataxia is observed in the extremities	Slight excitation and incoordination are observed. The animal regains standing position calmly but only after 2-3 attempts. Marked ataxia is observed while standing	The animal displays convulsions on the ground with an attempt to stand up. The animal makes more than 3 attempts to regain standing position but fails. The animal may hurt itself and attending personnel

Table 2. *Mean values of cardiorespiratory parameters and anaesthesia/analgesia quality in foals (Mean±SD) (n=11)*
Tablo 2. *Taylara ait kardiyopulmoner parametreler, anestezi/analjezi kalitesi ortalama değerleri (Mean±SD) (n=11)*

Parameter	Following Induction of Anaesthesia					
	Baseline	15th min	30th min	45th min	60th min	90th min
Heart Rate (beats/minute)	69.31±5.12^{a}	63.83±2.79^{a}	68.83±9.87^{a}	66.33±7.71^{a}	68.83±4.45^{a}	67.50±5.72^{a}
Respiratory Rate (breaths/minute)	24.50±1.76^{a}	14.00±2.00^{b}	15.67±1.21^{b}	17.67±1.22^{b}	21.17±4.17^{ab}	24.83±3.87^{a}
Body Temperature (°C)	38.05±0.19^{a}	37.53±0.99^{ab}	37.32±0.88^{b}	37.27±1.07^{b}	37.52±0.81^{ab}	37.65±0.71^{ab}
Mean Blood Pressure (mm Hg)	68.30±13.58^{a}	82.49±15.61^{b}	86.22±14.47^{b}	78.17±13.22^{b}	75.27±14.32^{ab}	71.19±12.86^{ab}
SpO_2 (%)	94.33±2.50^{a}	89.00±2.61^{b}	87.17±2.32^{b}	87.50±2.26^{b}	90.17±2.23^{ab}	93.00±2.45^{a}
Quality of Anaesthesia/Analgesia*	ND	2.83±0.41^{a}	2.67±0.52^{ab}	1.83±0.75^{b}	ND	ND

Differences between mean values shown with different superscripts in the same row (a, b) were statistically significant (P<0.05). ***ND:*** *Not Determined,* *** Scored according to the criteria presented in Table 1,* ***good****=3 points,* ***fair****=2 points,* ***poor****=1 point*

Table 3. *Mean values of clinical anaesthesia parameters in foals (Mean±SD) (n=11)*
Tablo 3. *Taylara ilişkin klinik anestezi değerlendirme parametrelerinin ortalama değerleri (Mean±SD) (n=11)*

Parameter	Value
Anaesthesia induction time (sec)	40.17±5.17
Quality of the anaesthesia induction*	2.67±0.52
Operation time (min)	41.67±1.86
Anaesthesia time (min)	46.17±1.72
Standing time (min)	57.17±2.04
Quality of recovery from anaesthesia*	1.83±0.75

*** Scored according to the criteria presented in Table 1,* ***good****=3 points,* ***fair****=2 points,* ***poor****=1 point*

No adverse effects were observed on the refill time of the mucosal capillaries during the sampling periods of the foals. Neither was any disorder observed as regards the colour of the mucosal membranes (i.e. development of cyanosis).

DISCUSSION

Foals differ from adult horses in pharmacodynamics and pharmacokinetics due to physiological differences, including among others, the proportion of extracellular fluid to body weight being high, liver enzyme activities being low, renal functions having not been fully developed, the cardiovascular system being regulated differently, the blood-brain barrier having low efficacy and the autonomous nervous system having not been fully developed [7,16-20]. Therefore, within the context of anaesthesia applications, some researchers [1,16,18,19], classify foals as neonatal foals (≤ 1 month of age) and developing foals (pediatric, juvenile, aged 1-3 months), whilst some other researchers [2,17], include foals up to the age of 6 months in the neonatal-pediatric category and foals aged ≥6 months in the category of adult animals. The latter [2,17] have reported that, foals younger than 6 months bear greater risk for anaesthesia, when compared to adult horses. In the present study, the 11 foals constituting the study material were diagnosed with umbilical hernia at an average age of 1-3 months. However, in accordance with the recommendation of Mair et al.[21], due to the possibility of spontaneous healing, in most of the cases, operative treatment was not performed until 6 months of age. The foals, for which the average age was calculated as 5.73 months, were anaesthetized as described for adult horses [3,5,10-13]. The anaesthesia protocol applied did not cause any mortality or serious complication. Moreover, the protocol applied yielded adequate unconsciousness and immobilisation, satisfactory muscular relaxation and a general anaesthesia characterized by analgesia, which enabled the success of the umbilical herniorrhaphy operations performed.

The anaesthesia protocol followed in the present study caused moderate falls in the heart rate of the foals at all sampling times, when compared to the baseline values *(Table 2)*. Nevertheless, the critical heart rate of ≤25 beats/minute, reported to be a serious bradycardia for horses [22], was observed in none of the foals. The moderate falls determined in the heart rates at all sampling times were attributed to xylazine having reduced sympathetic activity in the central nervous system [3,7,9,23].

In the present study, it was determined that, respiratory rates measured 15, 30 and 45 min after the last injection had decreased significantly compared to the baseline value, consequential to xylazine-tiletamine-zolazepam anaesthesia (P<0.05), leading to decrease in SpO_2 values at the indicated sampling intervals *(Table 2)*. Owing to the sedation it causes by depressing the central nervous system, xylazine causes respiratory depression [3,7,9]. On the other hand, cyclohexamine anaesthetics (ketamine, tiletamine), induce dose-dependent respiratory depression in most animal species [3,8,10,14]. Furthermore, it has been reported that, hypoxaemia may be observed in horses under general anaesthesia, the severity depending on the position of recumbency and the duration of anaesthesia (>60 min), such that oxygen ventilation is recommended for the prevention of the occurrence of such cases [1,4,10]. In the light of these data, the decrease observed in the respiratory rates and SpO_2 values could have arisen due to the drugs administered and the animals being maintained in dorsal

recumbency for the performance of the operation. Furthermore, serious hypoxaemia (SpO_2 <80%) having not been encountered could be attributed to anaesthesia having lasted shorter than 60 min (on average 46 min).

Several researchers [3,17,24] have reported that general anaesthesia causes decrease in body temperature. These researchers have described such decrease as a result of vasoconstriction caused by the anaesthetics administered, heat loss due to the shaving and alcohol disinfection of the operation site, disrupt of thermoregulation due to the inhibition of the limbic-hypothalamic centres and the disrupt of body temperature haemostasis due to reduced metabolic activity. The decrease observed in the body temperatures of the foals in the present study *(Table 2)* could also be attributed to these mechanisms.

Bidwell [2] has reported that, in foals, the average blood pressure should be maintained at a level of ≥70 mm Hg throughout anaesthesia. In the present study, it was observed that the average blood pressure of the foals was higher than the baseline value at all sampling times, yet was maintained at a level of ≥70 mm Hg as suggested by Bidwell [2]. This could be attributed to the peripheral stimulation of alpha-1/2 adrenoreceptors in the early stage of intravenous xylazine anaesthesia [25] and to the sympathomimetic effect of tiletamine [3,10,26].

Matthews and Hartsfield [4] and Hall et al.[3] have recommended the refill time of mucosal capillaries to be determined and the colour of mucosal membranes to be observed for the purpose of monitoring cardiovascular performance in anaesthetized horses and foals. In the present study, no abnormality having been observed in the foals for any of these parameters, and the relevant values having remained stable within normal limits, were considered as indicators of the anaesthesia protocol applied not to have triggered any adverse effect on these parameters.

In previous studies carried out in horses and foals by administering different doses of the anaesthetic combination of xylazine-tiletamine-zolazepam for the induction of general anaesthesia [5,8,10-14], anaesthesia induction time was reported to range between 34-75 sec, the duration of anaesthesia between 22.5-35.7 min, and the period of regaining standing position (standing time) between 30-50 min. Furthermore, it has been indicated that, if required, the duration of anaesthesia could be prolonged by administering half or one-third of the initial doses of the anaesthetics used for sustainment, yet, in such cases, complications such as serious hypoxaemia, myositis and delayed recovery from anaesthesia could be encountered as a result of cardiovascular/pulmonary depression [1,3,4,6,11,27]. In the present study, the clinical anaesthesia parameters resulting from the anaesthesia protocol applied *(Table 3)* was found to be in compliance with the findings of the studies referred to above. In several studies [1,3,8,10-14], it has been reported that, in horses, tiletamine-zolazepam anaesthesia, and in particular anaesthesia prolonged by the administration of sustaining doses, tend to be problematic due to the continuous attempts of the animals to stand up, the standing position of the animals being unsafe owing to ataxia, and consequential falls of the animals. These problems have been indicated to result from the half life elimination time (t ½) of zolazepam from the organism taking a longer time than that of xylazine and tiletamine, thereby, leading to longer muscle relaxation and ataxia during recovery from anaesthesia. In view of the adverse effects that may be encountered during recovery from anaesthesia, in order to prevent the injury of the patient and attending personnel and to allow for adequate time to pass for horses and foals being able to fully regain standing position, it is suggested that the animals be tied and maintained immobilised on the ground for a certain period [3,28].

In a previously conducted study, Short et al.[13], used different doses of xylazine (1.1 mg/kg, IV) and tiletamine-zolazepam (1.1-1.65-2.2 mg/kg, IV) in horses for surgical interventions such as intubation, suturing of lacerations and castration, and reported that the three anaesthetic doses administered induced an adequate anaesthesia for the indicated applications. Furthermore, Ozba et al.[29], in a study in which they administered a combination of tiletamine-zolazepam (3 mg/kg, IM) following premedication with atropine (0.05 mg/kg SC) and xylazine (0.1 mg/kg IM), ascertained that an anaesthesia of adequate depth and duration was induced for the operative treatment of umbilical lesions in calves. However, in the present study, in the test cases, reactions that adversely affected the performance of the surgeon were encountered, and in view of maintaining the safety of anaesthesia, in order to prevent the development of such reactions in the operated foals, instead of increasing the dose of the xylazine-tiletamine-zolazepam combination, it was preferred to modify the anaesthesia protocol by administering lidocaine by subcutaneous route into the periphery of the hernial sac. Gunkel [30] has reported that, as the pain threshold of foals is lower than that of adult horses and other animal species, even under an adequate depth of anaesthesia, foals may react to the first incision. In the present study, it was observed that, rather than to the incision of the skin, the foals reacted to the dissection of the inner hernial sac. Nonetheless, the addition of local anaesthesia to the anaesthesia protocol was in agreement with the report of Burns [17], which suggests that the need for general anaesthetics and, thereby the risk of cerebro-cardiorespiratory depression in foals, could be reduced by the use of local and regional anaesthesia techniques.

It was ascertained in the present study that, the supplementation of the combined use of xylazine-tiletamine-zolazepam in foals under field conditions with local anaesthetics induces an anaesthesia of adequate depth for umbilical herniorrhaphy, and sustaining doses enable the prolongation of the anaesthesia period with cardio-respiratory adverse effects remaining within acceptable

limits. Therefore, it is considered that, the indicated anaesthesia protocol could be tested for other surgical interventions in foals under both field and clinical conditions.

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RESEARCH ARTICLE

Peste Des Petits Ruminants (PPR) Virus Infections in Goats in the Eastern Anatolia of Turkey

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Summary

In this study, a totally 98 materials were used consist of different samples such as blood, oro-nasal swap, lung, spleen and lymph node from goats (n=38) in the 28 flocks suspected the PPRV infection as clinically and macroscopic pathologic remarks. The goats that used in this study housed in the 11 different provinces of Eastern Anatolia. RT-PCR was used for the diagnosis of PPRV infection. PPRV nucleic acid was detected in 50 of 98 materials by RT-PCR. According to the results of RT-PCR, the PPRV nucleic acid was detected in 39.2% (11/28), 44.7% (17/38) and 45.4% (5/11) of the flocks, sampled animals and provinces in eastern anatolia, respectively. Diagnostic value of necropsy materials such as lymph node, spleen, lung and of clinical samples such as oro-nasal swap and blood were determined more valuable diagnostic materials in the diagnosis of PPRV infection by RT-PCR. Data showed that PPRV infections of the goats were widespread in the Eastern Anatolia region. Additionally, it is determined that RT-PCR is sensitive and reliable method in the diagnosis of PPRV infections.

Keywords: *Goat, PPR, RT-PCR*

Türkiye'nin Doğu Anadolu Bölgesin'deki Keçilerde Peste Des Petits Ruminants (PPR) Virus Enfeksiyonları

Özet

Bu çalışmada, klinik ve makroskobik patolojik olarak PPRV enfeksiyonundan şüphelenilen 28 farklı sürüdeki keçilerden (n=38) alınan kan, oral-nazal swap, akciğer, dalak ve lenf nodülü gibi farklı örneklerden toplam 98 materyal kullanıldı. Bu çalışmada kullanılan keçiler Doğu Anadolu Bölgesi'nin 11 farklı ilinde yetiştiriliyordu. PPRV enfeksiyonunun teşhisinde RT-PCR kullanıldı. RT-PCR tekniğiyle 98 materyalin 50'sinde PPRV nükleik asidi belirlendi. RT-PCR sonuçlarına göre, PPRV nükleik asidi sürülerin %39.2'si (11/28), örneklenen hayvanların %44.7'si (17/38) ve Doğu Anadolu Bölgesinde örnekleme yapılan illerin %45.4 (5/11)' inde tespit edildi. RT-PCR ile PPRV enfeksiyonunun teşhisinde oral-nazal swap ve kan gibi klinik örnekler ve lenf nodülü, dalak ve akciğer gibi nekropsi materyali teşhis değeri yüksek materyal olarak belirlendi. Bu bilgiler keçilerin PPRV enfeksiyonunun Doğu Anadolu'da yaygın olduğunu ve PPRV enfeksiyonunun teşhisinde RT-PCR'ın duyarlı ve güvenilir bir metod olduğunu gösterdi.

Anahtar sözcükler: *Keçi, PPR, RT-PCR*

INTRODUCTION

Peste des petits ruminants (PPR) is associated with PPRV, a morbillivirus (family Paramyxoviridae) closely related to the rinderpest virus as well as the viruses of canine distemper in dogs, phocine distemper in seals, and measles in human [1]. PPR, also known as goat plague, kata and pseudo-enteritis complex [2-5], is similar clinically to rinderpest. The diseases occurs mostly in goats and sheep. Infection rates in sheep and goats rise with age, and rapidly fatal in young animals [6,7]. PPRV infection results is an acute, highly contagious disease, and characterized by fever, anorexia, necrotic stomatitis, ulceration of mucous membranes, and inflammation of the gastrointestinal tract leading to severe diarrhoea, purulent ocular and nasal discharges, and respiratory distress [6,8,9]. Morbidity and mortality rates vary but may reach 90-100%.

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These rates are usually lower in endemic areas, where mortality may be 20% or less, and serosurveillance is sometimes the only indicator of infection [6,9]. Four lineages of PPRV have been identified; lineage 1 and 2 viruses in west Africa, lineage 3 in east Africa, Arabia and southern India, and lineage 4 in the Middle East and Asia subcontinent, reaching east as far as Nepal and Bangladesh. PPR was first described [10] in West Africa and for 30 years was thought to be confined to this area. The disease has since been recognized as endemic in West and Central Africa [11] and in the north-east of the continent, Sudan [9,12,13], Kenya and Uganda [14] and Ethiopia [15,16]. In 1987 it appeared in the Middle East and has since then been confirmed in Jordan [17], Pakistan [18], southern India [19,20], Turkey [21-23] and Israel [15].

The existence of PPR infection in Turkey was declared officially in 1999 [8] but, previous reports of the presence of the virus in the country [24,25], and since then many outbreaks have been reported [21-23,26,27]. Ozkul et al.[21] isolated PPR viruses from 2 separate field cases in Turkey. These viruses belonged to lineage 4, to which goats seem to be more susceptible than sheep. The disease results in high mortality, especially among young goats, although the frequency of the disease is higher in older goats [22,28].

In 2004, an outbreak of PPR was detected in Thrace, i.e., the European part of Turkey [29]. As in rinderpest, close contact with an infected animal or contaminated fomites is required for the disease to spread. Large amounts of the virus are present in all body excrections and secretions, especially in diarrheic feces. Infection is mainly by inhalationbut could also occur through the conjuctiva and oral mucosa [1].

Laboratory confirmation of suspected cases is necessitated by the clinical similarity of rinderpest. Enzyme-linked-immunosorbent assay (ELISA) is now routinely used [30,31]. Virus isolation and differential neutralization in cell culture are slow, tedious and of low efficiency. Immunocapture [32] and reverse-transcription polymerase chain reaction (RT-PCR) followed by nucleotide sequencing [33-35] are the current diagnostic methods for all morbillivirus infections.

In this study, we investigated the prevalence of PPRV infection in goats by regions in east of Turkey and to assess the diagnostic values of the virus isolation and RT-PCR techniques in the diagnosis of PPR infections.

MATERIAL and METHODS

From 01.06.2010 to 25.03.2011, a totally 98 materials (24 lymph node, 6 nasal and oral swap, 26 lung, 22 spleen and 20 defibrinated blood) from goats (n=38) in the 28 flocks suspected in the PPRV infection as clinically, housed in the 11 different provinces east of Turkey (Adıyaman, Bingöl, Bitlis, Diyarbakır, Elazığ, Hakkari, Malatya, Muş, Şırnak, Siirt, Tunceli). Collected samples from 28 flocks different suspected to have PPR were submitted to the Elazig Veterinary Control and Research Institute for necropsy and virological examination.

Detection of PPR virus: RT-PCR was performed for the detection of PPR virus. The reaction was carried out with a PPRV-specific primer set (PPRVF1b: AGTACAAAAGAT TGCTGATCACAGT and PPRVF2d: GGGTCT CGAAGG CTAGGC CCGAATA) originally designed by Forsyth and Barrett [34] to amplify a 448-bp cDNA product from the F gene. A lyophilized live PPR vaccine, produced by the Etlik Veterinary Control and Research Institute Ankara, Turkey, was used as the positive control. RNA was extracted from the positive control or tissue homogenate from the field samples using RNeasy Mini Kit [36], (Qiagen, Germany) according to manufacturer's protocol. The RT-PCR was performed with Qiagen One- Step RT-PCR kit (Qiagen, Germany). The 20 µL reaction mixture contained 7 µL Molecular Grade Water, 0.8 µL 10 pmol of forward and reverse primers, 4.0 µL buffer, 0.8 µL dNTP mix, 0.8 µL enzyme mix, 4.0 µL 5x Q-Solution, 2.6 µL template RNA. The thermocycling profile was as follows: reverse transcription at 50°C for 30 min, initial denaturation and activation of polymerase at 94°C for 15 min, followed by 35 cycles of denaturation, annealing and extension at 94°C for 1 min, 50°C for 1 min and 72°C for 2 min, respectively, and final elongation at 72°C for 7 min (Thermal Cycler, Techne Plus). The RT-PCR products were analysed by electrophoresis at 80V for 2 h on 1.5% agarose gel stained with ethidium bromide. PCR products with a molecular size of 448 bp were considered indicative for PPRV [34].

Goats with clinical signs of PPRV infection were develope fever, anorexia, dehydration, dullness, mucoprulent oculonasal discharge, lacrimation, conjuctivitis, dyspnea and diarrhea. Most clinical cases of mouth, erosion, ulceration and necrosis on the lip, gingiva, buccal cavity, tongue and palate.

Blood samples was collected from jugular vein in EDTA containing and nonheparinised vacotainer tubes for analysis RT-PCR and C-ELISA. Blood samples were centrifuged for 10 min at 1500 rpm and destined with pipety the buffy coat than buffy coat stored in -80°C until used. Oro-nasal swaps centrifuged at 3.000 xg for 5 min remove the suspended solids The supernatants were storedat –80°C until used Necropsy was performed on short times the after death, and tissue samples for virologic analyses; fresh samples of the lung, spleen, and lymph nodes. were placed in 2 mL of PBS diluent (1/10 w/v) with MagNA Lyser Green Beads (Roche, Mannheim, Germany) and were homogenized at 3.000×g for 3 min by MagNa Lyser (Roche, Mannheim, Germany). Homogenates were centrifuged in Eppendorf tubes at 12.000×g for 3 min to remove the suspended solids, without removing the beads. The supernatants were storedat –80°C until assayed with reverse transcriptase-polymerase chain reaction (RT-PCR).

Competitive enzyme linked immunosorbent assay (C-ELISA) was used for serological detection of PPRV specific antibodies. Serum was collected from 15 clinically ill kids

analysed for the detection of antibodies against to PPR by a commercial C-ELISA kit (ID vet-veterinary diagnostic kits, France). The ELISA was performed according to the manufacturer's instruction as described elsewhere [37].

RESULTS

Informations belong to focal no and name, the number of sick animals in accepted to laboratory, the number of sampled material and sampling time of the goats in flocks of PPRV positive determinated was showed *Table 1*.

Eleven different provinces brought goat flocks (n=38) with PPRV infection suspicious in the east of Turkey was shown in *Fig. 1*.

PPRV antibodies were detected by C-ELISA in all serum samples of clinically sick kids. *PPRV* was isolated and identified in RT-PCR from the tissue, such as blood samples and oro-nasal swabs collected from all the sick goats. Moreover we can observe the specific 448 bp band obtained from the DNA amplification of F protein-coding gene using the primers PPRVF1b: AGTACAAAAGATTGCTGATCACAG T and PPRVF2d: GGGTCTCGAAGG CTAGGCCCGAATA *(Fig. 2)*.

DISCUSSION

Common clinical signs of infection with PPRV such as high fever, anorexia, necrotic stomatitis, dehydration, ulceration of mucous membranes and inflammation of the gastro-intestinal tract leading to severe diarrhea, purulent ocular

Table I. *Informations belong to focal no and name, the number of sick animals in accepted to laboratory, the number of sampled material and sampling time of the goats in flocks of PPRV positive determinated*

Tablo 1. *PPRV pozitif belirlenen sürülerdeki keçilerin örnekleme zamanları, örneklenen materyal sayısı, laboratuara kabul edilen hasta hayvanların sayısı, odak numarası ve odak isimlerine ait bilgiler*

Focal No	Focal Name	The Number of Sick Animals in Accepted to Laboratory	The Number of Sampled Material	Sampling Time
1	Adıyaman	2	8	01.06.2010
2	Bingöl	1	5	05.09.2011
3	Bitlis	1	3	05.09.2011
4	Bitlis	1	2	22.06.2011
5	Bitlis/Mutki	1	3	23.03.2011
6	Diyarbakır/Çermik	2	10	07.07.2011
7	Elazığ	3	3	30.03.2011
8	Elazığ/Baskil	1	4	20.07.2011
9	Elazığ	1	4	19.09.2011
10	Elazığ/Palu	1	1	10.10.2010
11	Elazığ	1	3	07.02.2011
12	Elazığ/Keban	1	1	26.04.2011
13	Elazığ/Palu	1	1	23.03.2011
14	Elazığ	1	2	02.12.2010
15	Hakkari	3	3	07.07.2011
16	Malatya/Doğanşehir	1	2	05.09.2011
17	Malatya/Yeşilyurt	3	9	21.06.2011
18	Malatya	1	1	20.07.2011
19	Malatya/Arapkir	1	3	30.05.2011
20	Malatya/Arapkir	1	3	25.03.2011
21	Malatya	1	3	13.04.2011
22	Malatya/Yeşilyurt	1	3	25.03.2011
23	Muş	3	3	03.09.2011
24	Şırnak	1	3	30.03.2011
25	Siirt	1	3	30.03.2011
26	Tunceli/Mazgirt	1	3	22.06.2011
27	Tunceli/Hozat	1	5	07.02.2011
28	Tunceli	1	4	25.03.2011
TOTAL		**38**	**98**	**2010-2011**

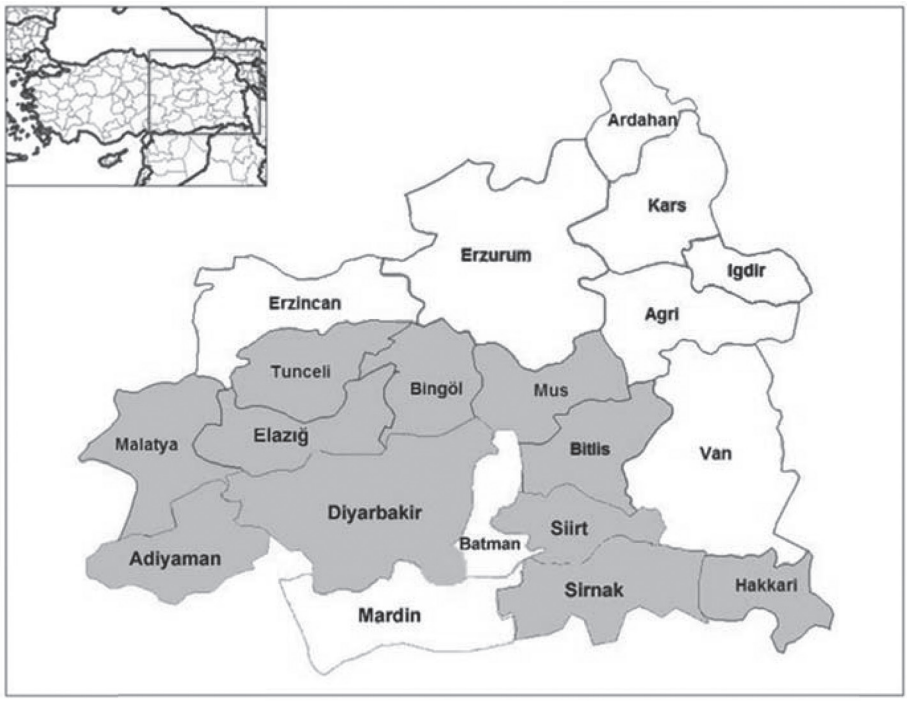

Fig1. Eleven different provinces brought goat flocks (n=38) with PPRV infection suspicious in the east of Turkey (The proviences were shown with bold)

Şekil 1. Türkiye'nin Doğusu'ndaki 11 farklı ilden PPRV enfeksiyonu şüphesiyle getirilen keçi sürüleri (n=38) (İller koyu ile gösterildi)

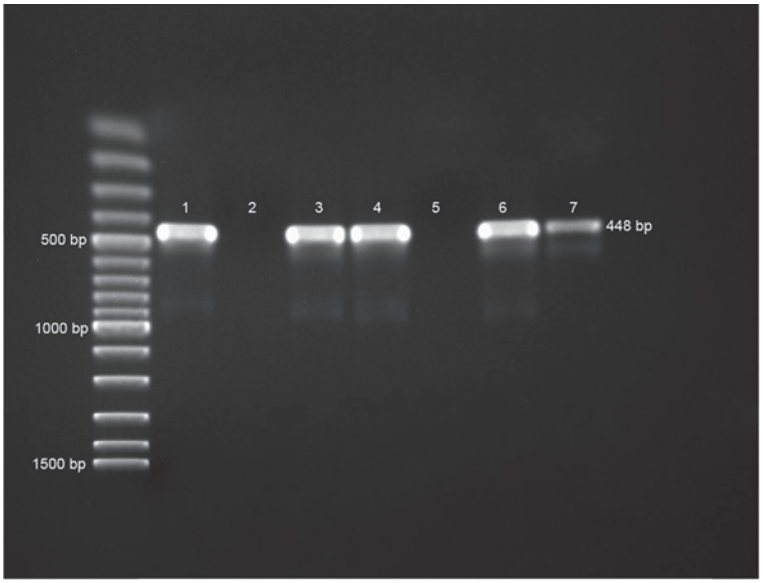

Fig 2. Agarose Gel Electrophoresis of PPRV. PCR Positive and Negative Specimens and Controls. Line 1-Positive Control, Line 2-Negative Control, Line 3- Positive Lymph Node Sample, Line 4- Positive Lymph Node Sample, Line 5-Negative Sample, Line 6-Positive Blood Sample, Line 7-Positive Oral-Nasal Swap Sample

Şekil 2. PPRV' nin Agarose Gel Electrophoresisi. PCR pozitif ve negatif örnekler ve kontroller. Çizgi 1- Pozitif Kontrol, Çizgi-2 Negatif Kontrol, Çizgi 3- Pozitif Lenf Nodülü Örneği, Çizgi 4- Pozitif Lenf Nodülü Örneği, Çizgi 5- Negatif Örnek, Çizgi 6- Pozitif Kan Örneği, Çizgi 7- Pozitif Oral-Nasal Swap Örneği

and nasal discharges, lacrimation and respiratory distress were observed in the present study which were also reported elsevere [38-42].

PPR has been reported in a number of countries in the region including Pakistan, Nepal, Bangladesh, Turkey, Afghanistan, Iran and India [20,24,43,44]. Although the clinical and postmortem findings may be sufficient for the diagnosis of PPR in the endemic areas, yet labaratory confirmation is essential for definitive diagnosis. We were reveal clinical and virologic findings all of animals suspected to have PPR were submitted to the Elazig Veterinary Control and Research Institute for examination.

Forsyth and Barret [34] reported that viral nucleic acid was detected in the samples of blood, conjuctival swap, lymph nodule and spleen, however no viral nucleic acid was detected in the samples of lung, oral swap and nasal swap by RT-PCR. They reported that sample of lymph nodule should be preferred to the other samples as the sample of necropsy. Similarly, Albayrak and Alkan [45] reported that the PPRV nucleic acid from nasal and conjuctival swap samples from the infected animals were detected, but no viral nucleic acid was detected in the samples of blood samples and oral swap by RT-PCR. They reported that the samples of nasal and conjuctival swaps are more valuable as the diagnostic material from animals with clinical symptoms. We determined that the diagnostic value of necropsy materials such as lymph node, spleen, lung, oro-nasal swap

and blood were determined more valuable diagnostic materials in the diagnosis of PPRV infection by RT-PCR. In the our study, different materials such as blood, swap samples (nasal, oral) and tissue samples are used in the diagnosis of PPRV infection. Positivity rates as 54.2% (13/24), 66.6% (4/6), 45.0% (9/20), 46.2% (12/26), 46.2% (12/26) were detected in the samples of lymph nodule, nasal and oral swap, blood, lung, spleen respectively. The results of our study were smiliar reveal with results of other investigations.

The goats seem to be more susceptible than sheep and the disease results in high mortality, especially among young goats, although the frequency of the disease is higher in older goats [6,7,22,28]. Smilarly, the goats of the age was between 7 day and 6 month age in the study. Sarker et al.[46] investigated the prevalence of PPR according to age categories and they have identified as prevalence of PPR in goats with age categories adult (>1 year), young (between 4 to 12 months) and sucklers (between 1 to 3 months) was found to be 10.2%, 31.1% and 13.1%, respectively. In the present study we also have identified as the prevalence of PPR in goats with age categories adult (>1 year), young (between 4 to 12 months) and sucklers (between 1 to 3 months) was found to be 2.6%, 60.5% and 36.8%, respectively. Singh et al.[47] also assessed that the disease is most prevalent in the goats less than one year. According to these report, we though that the increased susceptibility of young goats were might be due to malnutrition, poor immunity and poor examine the affected goats management systems.

In the Turkey, Eastern of Anatolia is one of the regions made intensive of livestock. Region has 34.3% about of small ruminants in the Turkey [48]. As Eastern Anatolia shares a border with five Middle East countries, it is possible that the virus entered Turkey via uncontrolled animal movement from neighboring countries where PPRV is endemic.

Previously, the clinic and laboratory studies in sheep and goats for PPR were applied different province of Turkey such as Afyonkarahisar [49], Burdur [50], Mardin [51], Batman, Denizli, Cihanbeyli (Konya), Amasya, Sakarya Eskişehir, Malatya, Sivas, Isparta, Van, Aydın, Muğla [21,22], Edirne [29], Kırıkkale [52] Samsun, Sinop, Amasya, Tokat, Rize, Trabzon, Giresun, Ordu [45], and gazelle in Ceylanpınar (Şanlıurfa) [53]. Although, the PPR investigation was made in Eastern Anatolia province such as Malatya, Elazığ ve Van [21], this study was more comprehensive related to Eastern Anatolia province such as Adıyaman, Bingöl, Bitlis, Diyarbakır, Elazığ, Hakkari, Malatya, Muş, Şırnak, Siirt and Tunceli.

Albayrak and Alkan [45], PPR nucleic acid detected in 26 of 124 materials by RT-PCR. According to the results of RT-PCR, the PPRV infections were diagnosed in 44.1% (34/51) and 31.5% (18/57) of the flocks and sampled animals, respectively. Smilarly, in the present study, PPRV nucleic acids were detected in 50 of 98 materials by RT-PCR. According to the results of RT-PCR, the PPRV infections were diagnosed in 39.2% (11/28), 44.7% (17/38) and 45.4% (5/11) of the flocks, sampled animals and provinces in Eastern Anatolia, respectively. Data showed that PPRV infections of the goats were widespread in the east anatolia region. Additionally, it is determined that RT-PCR is sensitive and reliable method in the diagnosis of PPRV infections.

PPRV infection in Turkey needs continuous screening by reliable diagnostic systems as pointed out elsewhere. Meanwhile, adequate prophylactic approaches using local virus strains should be considered as part of the control and eradication policies. This will help in immobilizing and suppressing infections before the spread from neighboring countries.

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RESEARCH ARTICLE

24-hour Holter Monitoring and Troponin I Level in Boxers with Arrhythmogenic Right Ventricular Cardiomyopathy

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Summary

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is described as a disease that has an autosomal dominant trait with reduced penetrance, that appears in dogs between two and eight years of age. Boxers are predisposed to ARVC. Some of the symptoms of ARVC are fainting, attacks of tachycardia - especially vetnricular tachycardia and sudden cardiac death. The aim of the study was to estimate the usefulness of 24-h Holter monitoring and the level of troponin I (cTnI) in examining the treatment of Boxers with ARVC. 24-h Holter monitoring and plasma concentrations of cTnI were carried out every three months after introducing anti-arrhythmic treatment (metoprolol prolongatum, sotalol or amiodaron). There was a significant correlation between the number of ventricular premature complexes (VPC) over a 24-h period and the level of cTnI. No correlation was found between the appearance of monitoring ventricular tachycardia (VT) and the level of cTnI. The presented results show the possibility to use cTnI to evaluate the efficacy of anti-arrhythmia treatment in dogs with ARVC.

Keywords: *Arrhythmogenic right ventricular cardiomyopathy, Holter, Troponin*

Aritmojenik Sağ Ventrikül Kardiyomiyopatili Boxerlarda 24 Saatlik Holter Monitorizasyon ve Troponin I Seviyesi

Özet

Aritmojenik sağ ventrikül kardiyomiyopatisi (ARVC) azalmış penetrasyon ile otozomal dominant geçişe sahip bir hastalık olarak tanımlanır, köpeklerde 2 ve 8 yaş arasında görülür. Boxerlar ARVC yatkındırlar. ARVC semptomları bayılma , taşikardi atakları - özellikle ventriküler taşikardi ve ani kardiyak ölümdür. Bu çalışmanın amacı, aritmojenik sağ ventrikül kardiyomiyopatili Boxerlarda 24 saatlik Holter monitorizasyonunun ve tedavinin monitorize edilmesinde Troponin I düzeyinin yararlılıklarını değerlendirmektir. Antiaritmik tedavinin (metoprolol prolongatum, sotalol veya amiodaron) başlatılmasından sonra her 3 ayda bir Holter monitorizasyonu ve cTnI plazma konsantrasyonu uygulandı. 24 saatlik süreçte cTn1 düzeyi ile VPC sayısı arasında önemli bir korelasyon vardı. cTnI düzeyi ile VT görülmesi arasında hiçbir korelasyon bulunamadı. Sunulan sonuçlar ARVC'li olan köpeklerde antiaritmik tedavisinin etkinliğini değerlendirmede cTnI'in kullanılabilirliğini göstermektedir.

Anahtar sözcükler: *Aritmojenik sağ ventrikül kardiyomiyopatisi, Holter, Troponin*

INTRODUCTION

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is one of the heart diseases that can be found both in humans and animals. The first clinical description of this disease was described in 1736 [1], however, a more specific description of the etiology and development of the diseases was defined in the 80s of the twentieth century. ARVC is described as a progressive replacement of myocardium cells with adipose cells and connective tissue cells as well as the emergence of ventricular heart rate disorders that lead to sudden cardiac death (SCD) [2-4].

Boxers are predisposed to have ARVC. ARVC is described as a disease that has an autosomal dominant trait with

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reduced penetrance that appears in dogs two to eight years old [5]. This breed is used as a model for research on ARVC [6]. Single cases of ARVC were noticed in breeds such as Syberian Husky, Labrador Retriever and English Bulldog [7-9]. Cats do not show any predisposition correlated with sex, breed or age [5]. In spite of the development of many new diagnostic techniques like MRI, the foundation to recognize the ARVC is still finding fatty infiltrations in the myocardium of the right ventricle and the developing fibrosis tissue. As a result cardiomyocytes undergo apoptosis. The process initiates in the epicardium of the right ventricle. The highest level of fat (above 40%) is observed in the upper-lateral wall of the right ventricle and the infundibulum [2]. Fatty infiltrations in dogs with ARVC are also observed in the left ventricle, intraventricular septum and even in the walls of the right and left atria [2,10]. The level of fat and fibrous tissue is not correlated with the age, body weight or size of the heart. Lymphocytic infiltrations are also observed. According to Basso et al. they can be found in 60% of Boxers with ARVC and contain CD45, CD45RO-, CD43-positive lymphocytes [2]. Myocardium is obtained for histopathology during endomyocardial biopsies or posthumously. Dogs with ARVC show a reduction in the number of desmosomes in the right ventricle and adherens junctions and gap junctions in the right and left ventricle [11].

The whole process initiates in the epicardium of the right ventricle and may progress to the left ventricle and both atrias. Structural disorders begin in the desmosomes of the cardiomyocytes. The gold standard for ARVC diagnosis is demonstration of transmural fibrofatty replacement in cardiac tissue obtained by autopsy or surgery.

The aim of the study was to estimate the usefulness of 24-h Holter monitoring and the level of troponin I in monitoring the treatment of arrhythmogenic right ventricular cardiomyopathy in Boxers.

MATERIAL and METHODS

The prospective study was performed on 11 Boxer dogs, aged from 5 to 11 years, of both sexes (7 male, 4 female). The observation time was between 4 and 19 months.

All of the dogs were clinically examined, had an echocardiography, electrocardiography, 24-h Holter monitoring, morphological and biochemistry blood tests performed. The echocardiography examination was conducted on the ALOKA 4000+ echocardiograph. A sector 5 MHz and 7.5 MHz probe was used. A right parasternal approach was used to carry out the echocardiography, and a long axis view of the left ventricle in motion mode was used for the observation. All dogs underwent ECG in a right lateral position on the BTL SD08 electrocardiograph machine equipped with a net filter and different frequencies of muscular filters. The electrodes were placed on the: right arm (red electrode), left arm (yellow electrode), right leg (black electrode) and left leg (green electrode) accordingly. The precordial leads were attached as follows: V1 was placed to the right of the sternum at the 5th intercostal space, V2 – was placed directly to the left of the sternum, V4 – was placed to the left at the costochondrial junction at the 6th intercostal space. A 24-h Holter monitoring was performed by an AsPECT 702 3-channel device. Single- use, self-adhesive electrodes were placed on the shaved skin of the thorax *(Fig. 1)*. The unit was stabilized between spatulas by a special protective cloth *(Fig. 2)*.

Blood samples were collected after the clinical examination.

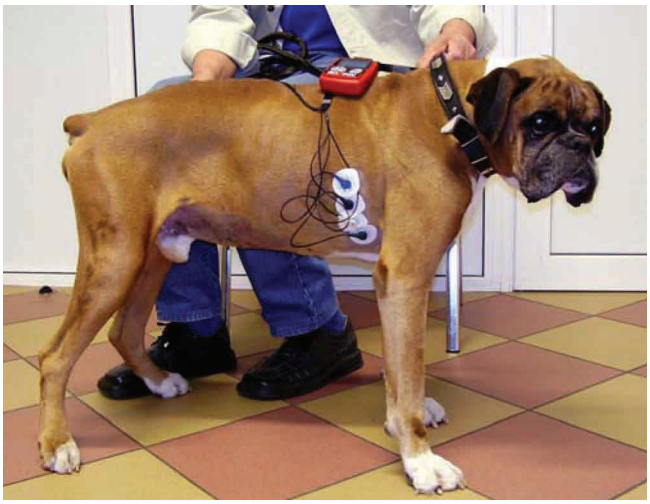

Fig 1. Disposable self-adhesive electrodes sticked to the shaved skin of the chest

Şekil 1. Tek kullanımlık kendinden yapışkanlı elektrotların tıraşlanmış göğüs derisine yapıştırılması

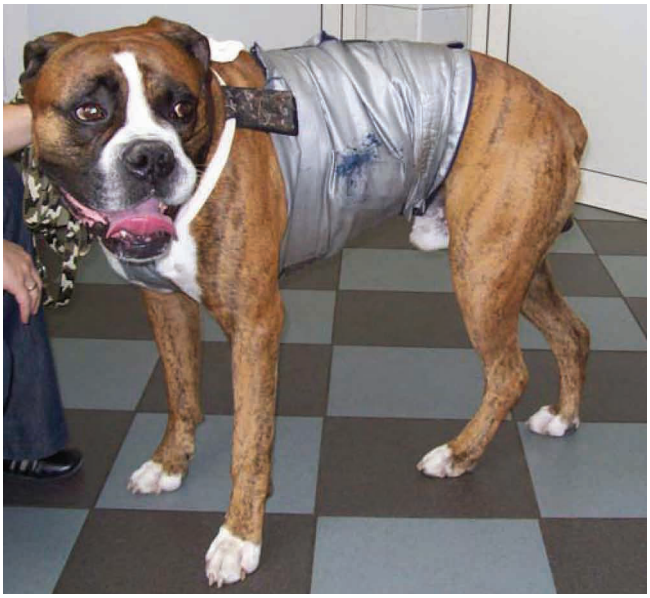

Fig 2. AsPEKT 702 device stabilized between the shoulder blades of a dog by a special protective clothing

Şekil 2. Özel koruyucu kıyafetler ile köpek kürek kemikleri arasında AsPEKT 702 cihazının stabilize edilmesi

Using minimum stasis, cephalic venous blood was obtained with a 21 G disposable butterfly needle and Vacutainer system into serum (6 mL), EDTA (2 mL) tubes. In the morphological blood tests the total level of red blood cells, white blood cells, thrombocytes (PLT), haematocrit (Ht) and haemoglobin (Hb) were measured. Morphological blood tests were performed on an Animal Blood Center abc VET analyzer. Biochemistry tests included the evaluation of the activity of alanine aminotransferase (ALT), asparate aminotransferase (AST), level of urea, creatinine, bilirubin, total protein and albumins, as well as ion levels: Na^+, K^+, Mg^{2+}, total Ca and inorganic phosphorus. Biochemistry tests were performed on the MaxMat PI analyzer. An automated immunoluminescence test was used to evaluate the concentration of Troponin I (cTnI).

The criteria to include the dogs in the study were: presence no less than 500 ventricular premature complexes (VPC) of right ventricular origin during 24 h and syncope [12], no changes in the echocardiography test, no enlargement of the right ventricle, regional hypokinesis of the right ventricle [1]. The foundation of diagnosing ARVC is finding fatty infiltrations in the myocardium of the right ventricle and the development of fibrosis tissue. All the dogs had ARVC confirmed posthumously *(Fig. 3)*. 24-h Holter monitoring and plasma concentrations of cTnI were carried out every 3 months after beginning anti-arrhythmic treatment (metoprolol prolongatum - 2 dogs, sotalol - 7 dogs, 2 dogs did get in the past metoprolol, amiodaron-1 dog, next change to sotalol or mexiletine - 1 dog, before the dog was treated by sotalol).

The correlation between VPC/24 h and cTnI was analyzed by using the R Spearman test. The testing was done based on the significance level of $P<0.05$.

The study was approved by the ethics committee of the University of Environmental and Life Sciences, Wroclaw, Poland.

RESULTS

Fainting was observed in 8 out of 11 Boxers. Seven dogs showed VPC in resting ECG *(Fig. 4)*. In the first Holter monitoring all the dogs had right sided VPC, between 1 473 and 27 651 during 24 h. In 10 out of 11 dogs the VPC doubled or tripled. In 9 out of 11 dogs in the first Holter monitoring ventricular tachycardia (VT) was observed and one of these dogs had a polymorphic tachycardia. In 3 cases there was a historical sudden cardiac death (SCD) in the parents or siblings. Five dogs died suddenly. A moderate enlargement of the right ventricle was observed in echocardiography in 6 out of 11 dogs, hypokinesis in 3 out of 11 dogs *(Fig. 5)*. The results of the 24-h Holter monitoring and the level of cTnI is presented in *Table 1*. There was a significant correlation between the number of VPC during 24 h and the level of cTnI ($r=0.82$; $P<0.05$) in the following tests *(Fig. 6)*. No correlation was found between the appearance of VT and the level of cTnI. There were no changes in the biochemical and morphological blood parameters.

DISCUSSION

The presented results show the possibility to use cTnI to evaluate the efficacy of anti-arrhythmia treatment in dogs with ARVC, especially that for determine the level of cTnI human tests can be used [13].

Some of the symptoms of ARVC are fainting, attacks of tachycardia - especially ventricular tachycardia and SCD [14]. According to Basso et al. 52% of dogs with ARVC show fainting [2]. In the study group 72% of dogs showed fainting. SCD was the first and only symptom of the disease [7]. In the study group 45% of dogs showed SCD and 27% of dogs had a positive anamnesis towards SCD.

Cardiac Troponin I (cTnI) is a protein belonging to the troponin-myosin complex in cardiomyocytes. Its

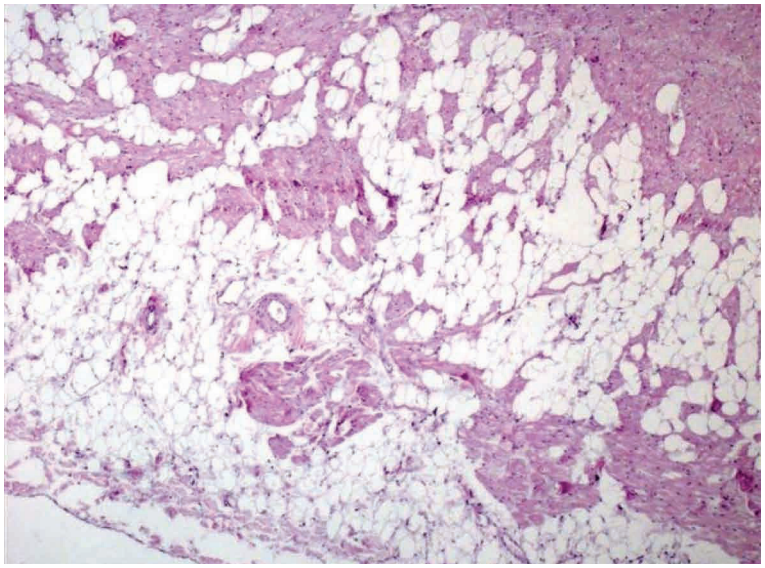

Fig 3. Fatty infiltration in wall of right ventricle. HE, 40x

Şekil 3. Sağ ventrikül duvarında yağ infiltrasyonu. HE, x40

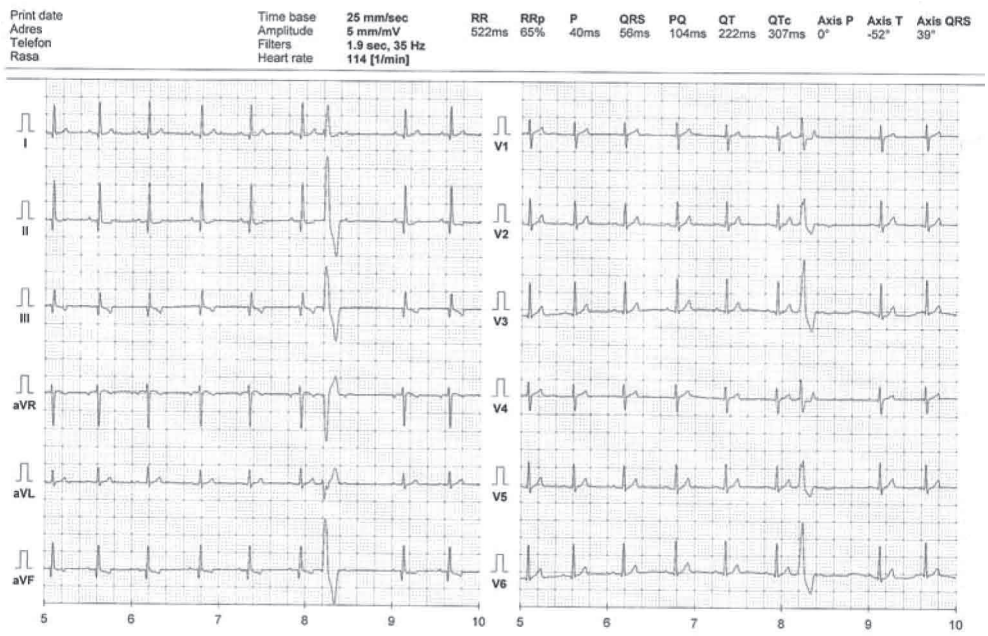

Fig 4. Electrocardiogram with right side origin VPC

Şekil 4. Sağ taraf kökenli VPC elektrokardiyogram

Table 1. *Results of the 24 Holter monitoring and the level of cTnI*
Tablo 1. *24 Holter izleme ve cTnI düzeyi sonuçları*

No Dogs	Parameters	Examination 0	After 3 months	After 6 months	After 9 months	After 12 months	After 15 months	After 18 months
1	cTnI [ng/ml]	1.23	0.2	0.14	0.09	0.23	0.45	1.0
	VPC/24 h	27651	3245	3003	2765	4546	7843	26789
2	cTnI [ng/ml]	0.26	0.11	0.47				
	VPC/24 h	2101	1489	5761				
3	cTnI [ng/ml]	0.76	0.89	0.67				
	VPC/24 h	20567	24789	16456				
4	cTnI [ng/ml]	0.68	1.4					
	VPC/24 h	22435	26900					
5	cTnI [ng/ml]	0.55	0.31	0.2	0.08	0.67	0.92	
	VPC/24 h	11230	5600	3245	2134	23457	26322	
6	cTnI [ng/ml]	0.76	0.77					
	VPC/24 h	23450	24780					
7	cTnI [ng/ml]	0.32	0.45	0.6				
	VPC/24 h	1473	3456	7891				
8	cTnI [ng/ml]	0.22	0.24	0.06	1.1			
	VPC/24 h	3256	5032	1532	25345			
9	cTnI [ng/ml]	0.7	0.05	0.65	0.08			
	VPC/24 h	13021	1789	10024	1568			
10	cTnI [ng/ml]	0.4	0.32	0.25	0.38			
	VPC/24 h	6700	6571	5761	6250			
11	cTnI [ng/ml]	0.56	0.37					
	VPC/24 h	7210	4670					

concentration is very low or hardly detectable in healthy dogs. The level of cTnI increases during cardiomyocytes damage due to hypoxia, toxemia or myocarditis. Dogs with cardiac diseseases have a higher level of cTnI, and the values <0.2 ng/ml are correlated with shorter time of survival [13]. In dogs with ARVD enzyme elevations is connection with myocyte appoptosis [15].The number of premature accelerations can vary in dogs with ARVC, which

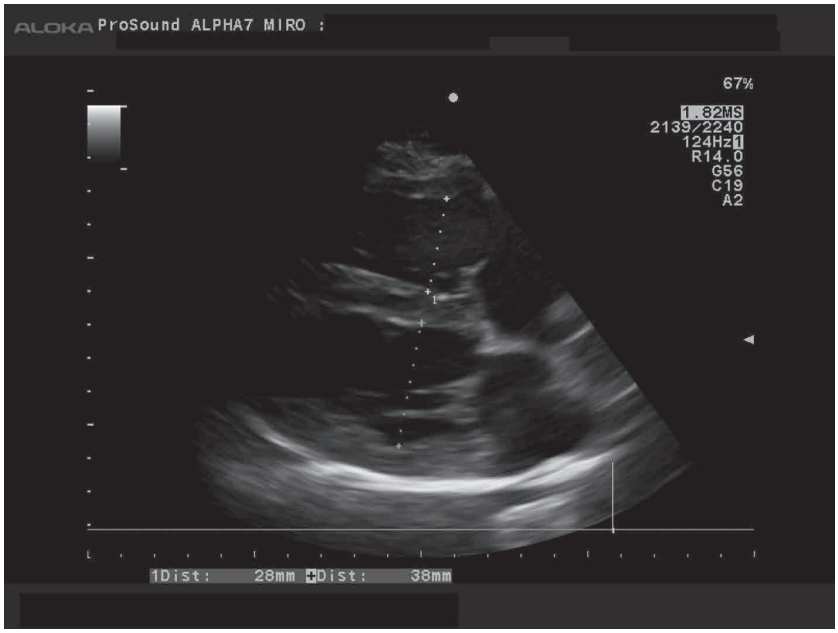

Fig 5. Echocardiogram showing enlargement of the right ventricle

Şekil 5. Sağ ventrikülde genişleme gösteren ekokardiyogram

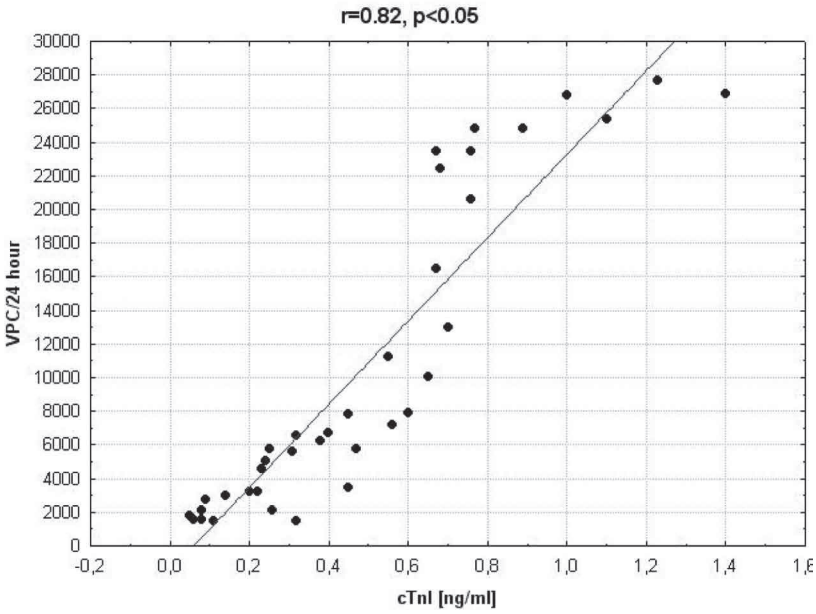

Fig 6. Correlation between cTnI and VPC/24 h

Şekil 6. cTnI ve VPC/24 arasındaki korelasyon

is confirmed in the study [16]. In this study the correlation between VPC/24 h and the concentration of cTnI was observed. Similar observations were done by Baumwart RD et al.[17]. Also, in humans with ARVC increase in cTnI without coronary artery stenosis in coronary angiogram was recorded [18,19]. A significant increase in the concentration of cTnI may also be a signal of structural alteraions during „hot phases" of the ARVC, as described in humans [20].

A lack of correlation between VT and cTnI may be related to a short term prevalence of Troponin I in the serum after its release from the myocardium, which lasts a maximum of approximately 14 days. There is a possibility to „overlook" the increase of cTnI, if the damage of the myocardium was single episode and VT was occasionally. A permanent high level of cTnI is the result of progressive damage of the myocardium, permanent VPC and periodic VT. cTnI level rise occurred five days after the VT episodes [20]. In this study cTnI level was measured once per three month. In this research scheme noticing the growth after an episode of VT is difficult or even impossible. The aim of the treatment is to increase the threshold of cardiomyocytes which will lead to a smaller number of VPCs and the possibility of VT appearance and ventricular fibrillation (VF). In the presented study it is confirmed that if the anti-arrhythmia treatment was efficient, no matter what the acting substance was, it did lower the number of VPC and the level of cTnI.

Polyunsaturated fatty acids and L-carnitine are used as supportive treatment to stabilize the membranes of cardiomyocytes. It is not possible to prevent heart rate disorders during ARVC and if the changes progress, a life

threatening arrhythmia may occur. In humans electrical radio frequency ablation is used to reduce the number of arrhythmogenic centers as one of the ways of treatment [21]. To terminate ventricular fibrillation implantable cardioverter defibrillators are used. This type of therapy to prevent sudden cardiac death in dogs with ARVC was presented by Nelson et al., however the cost of the unit is very high [22]. Latest research indicates the beneficial effect of preload-reducing therapy in progression of ARVC, however, these results need to be confirmed in clinical trials [23]. In case of heart failure adequate treatment is used. The prognosis for dogs with ARVC is not optimistic. New guidelines ARVD 2012: Diagnostic Challenges and Treatment, which pays attention to the fact that excessive exercise appears to exacerbate the manifestation in the disease in experimental animals, were published this year [24].

Monitoring the level of cTnI allows to check the efficacy of anti-arrhythmia treatment by lowering the number of VPC, but does not allow to forecast the risk of SCD related with VT and VF.

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RESEARCH ARTICLE

Antimicrobial Susceptibility of Bacteria Isolated from Uteri of Thoroughbred Mares with Fertility Problems

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Summary

Endometritis is an important cause of subfertility with high economic impact in mares and is mostly associated with bacterial infections. The aim of this study was to investigate bacterial pathogens in uteri and the susceptibility of some clinical isolates against several antimicrobial agents frequently used to control bacterial endometritis in mares in Germany. A total of 247 uterine swabs taken from mares with fertility problems were cultured to isolate bacteria and *in vitro* antimicrobial susceptibility of ß-hemolytic *Streptococcus* spp. and *Escherichia coli (E. coli)* strains was determined. Totally, 151 samples (61.1%) were found culture positive. A total of 332 microorganisms including 331 bacteria and one fungus were isolated from the samples. From the bacteria, 21,9%, 15.9%, 15.4%, 12%, 10.5%, 5.7% and 3.0% of the isolates were identified as *Escherichia coli (E. coli)*, α- hemolytic streptococci, ß-hemolytic streptococci, *Bacillus* spp., γ-hemolytic streptococci, coliform bacteria and *Staphylococcus aureus (Staph. aureus)* respectively. The remaining isolates (15.6%) included 13 other bacterial species and one fungus. ß-hemolytic streptococci and *E. coli* strains were considered as frequently associated with fertility problems and antimicrobial susceptibility of these isolates against 14 antimicrobial agents including penicillin, tulathromycin, tetracycline, erythromycin, florfenicol, ceftiofur, amoxicillin, amoxicillin/clavulanic acid, enrofloxacin, gentamicin, cefquinome, colistin, marbofloxacin and sulfamethoxazole/trimethoprim was determined. All ß-hemolytic streptococci were found to be susceptible to penicillin, ceftiofur, amoxicillin/clavulanic acid, enrofloxacin, cefquinome, marbofloxacin and sulfamethoxazole/trimethoprim, whereas 29.3% of ß-hemolytic streptococci showed resistance against colistin. All *E. coli* strains and 50% of them were resistant to penicillin and erythromycin, respectively, wheres all *E. coli* isolates were sensitive to all other tested antimicrobial agents used in this study.

Keywords: *Mare, Endometritis, Bacterial Isolation, Antibiotic Susceptibility*

Fertilite Problemli Damızlık Kısrakların Uteruslarından Bakteri İzolasyonu ve Antimikrobiyal Duyarlılık

Özet

Kısraklarda endometritis yüksek ekonomik kayba sebep olan önemli kısırlık nedeni olup, çoğunlukla bakteriyel infeksiyonlar sonucunda ortaya çıkmaktadır. Bu çalışmanın amacı, Almanya'da kısraklarda bakteriyel endometritis patojenlerini belirlemek ve klinik izolatların çeşitli antimikrobiyal ajanlara duyarlılıklarını araştırmaktır. Bu çalışmada Almanya'da fertilite problemi olan kısraklardan toplanan 247 uterus numunesi mikrobiyal ve antibiyotik duyarlılığı yönünden değerlendirildi. Yüzellibir (%61.1) numune bakteriyolojik olarak pozitif bulundu. 331'i bakteri ve biri mantar olmak üzere toplam 332 mikroorganizma izole edildi. Bakteri izolatların % 21.9'u *Escherichia coli (E. coli)*, %15.9'u, α-hemolitik streptokok, %15.4'ü ß-hemolitik streptokok, %12'si *Bacillus* spp., %10.5'i γ-hemolitik streptokok, %5.7'i koliform bakteri ve %3'ü *Staphylococcus aureus* olarak identifiye edildi. Geriye kalan suşlar (%15.6), 15 farklı bakteri türü ve bir mantar suşundan oluştu. Kısrakların fertilitesini en sık etkileyen bakteriler olarak ß-hemolitik streptokok ve *E. coli* etkenlerine karşı antibiyogram testi uygulandı. Bu bakterilerin, penisilin, tulatromisin, tetrasiklin, eritromisin, florfenikol, seftiofur, amoksisilin, amoksisilin/klavulanik asit, enrofloksasin, gentamisin, sefkuinom, kolistin, marbofloksasin, sulfametoksazol/trimetoprim olmak üzere toplam 14 antimikrobiyal maddeye karşı duyarlılığı test edildi. Bu çalışmada, ß-hemolitik streptokok suşlarının tamamı florfenikol, seftiofur, amoksisilin/klavulanik asit, amoksisilin, enrofloksasin, sefkuinom, marbofloksasin ve sulfametoksazol/trimetoprim'e duyarlı olduğu, %29.3'ünün de kolistine karşı dirençli olduğu belirlendi. *E. coli* suşlarının tamamı penisillin'e, %50'si de eritromisin'e dirençli bulunurken, tamamının florfenikol, seftiofur, amoksisilin, amoksisilin/klavulanik asit, enrofloksasin, gentamisin, sefkuinom, kolistin, marbofloksasin, sulfametoksazol/trimetoprim'e duyarlı olduğu saptandı.

Anahtar sözcükler: *Kısrak, Endometritis, Bakteriyel izolasyon, Antibiyotik duyarlılığı*

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INTRODUCTION

Bacterial infections of the uterus are known to be an important cause of reduced fertility in mares [1,2]. In the horse industry, endometritis due to uterine infections of bacterial origin brings about 25-60% economical losses, leading to infertility [3-5]. Frequent pathogenic bacteria causing infertility in mares are introduced to the uterus at occasions such as natural breeding, artificial insemination, postnatal infections (pneumovagina infections), and mostly during a delayed postnatal cleaning of the uterus [6-9]. Swabs taken from the lumen of the uterus have been commonly used for diagnosis of endometritis and infertility for long years. Careful evaluation of clinical signs, uterine culture, ultrasonography, cytology and histopathologic examinations are prerequisites for a correct diagnosis and successful cure of uterine infections in mares [9-11].

Bacterial infections are accepted to be the major reason of reproduction failure in mares. Bacterial isolation from the uterus can be shown as an evidence of endometritis [12,13]. Bacterial uterine infections cause conception and embryo survival failures despite many repeated breedings during the season. Moreover, bacterial pathogens of the uterus affect the elasticity and stickiness of the uterine mucus. It is stated that *Klebsiella pneumoniae* and other gram negative bacteria lead to the viscosity of the mucus causing a decrease of mucocilliar activity, while ß-hemolytic streptococci decrease the viscosity of the mucus. If uterus cleaning delays, subclinic endometritis occur [14]. In the same study, it has been mentioned that an increase of prostaglandin (PGE2), leucotriene B4 and arachidonic acide metabolits and a subsequent increase of vascular permeability cause the entrance of *Streptococcus equi* ssp. *zooepidemicus* into the uterus [3].

Aerobic bacteriologic culture from uterine samples of mares is the commonly used method for diagnosis of endometritis. Bacterial agents which are responsible for endometritis are *Streptococcus equi* ssp. *zooepidemicus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus* spp., *Pasteurella* spp., *Taylorella equigenitalis*, *Corynebacterium* spp. and fungi. A previous study has shown, that the degree of endometrial inflammation caused by *Escherichia coli* is more severe than caused by *Streptococcus* spp.[10]. Another study has emphasized that *Escherichia coli* showes an affinity to the urogenital system by uropathogenic virulence factors such as pap, sfa, hlyA, cnf1 and fim [15].

The purpose of this study was to determine bacteriological agents isolated from swabs obtained from the uteri of thoroughbred mares with infertility problems between 2006 and 2008 and to evaluate their antibitiotic susceptibility in Germany.

MATERIAL and METHODS

Between 2006 and 2008, a total of 247 uterine swabs taken from infertile and non-pregnant thoroughbred mares were delivered within the same day to the Bacteriological Diagnostic Laboratory at the Institute for Hygiene and Infectious Diseases of Animals, Justus-Liebig University Gießen. All endometrial samples were collected under sterile conditions either in sterile Phosphate Buffer Saline or Stuart Transport Medium (Oxoid; CM0111). Prior to sampling the external genital region and rima vulva of the mares was cleaned and disinfected several times with paper towels soaked in alcohol based antiseptic. A Polanski speculum was placed into the vagina and the cervix was made visible. Then a disposable swab (Equivet uterine culture swab; Kruse, Marslev, Denmark) or Knudsen catheter was used to take endometrial samples.

Uterine swabs were examined by conventional cultural methods. Bacteriological isolation was performed onto 5% defibrinated Blood Agar (Oxoid; CM0271), Gassner Agar (Merck; 1282) and serum bouillon. All plates and serum bouillon were incubated at 37°C for 24 to 48 h. The number of colonies on primary plates was assessed semi-quantitatively and scored as absent (no colonies), small numbers (1-50 colonies, +), moderate numbers (51-200 colonies, ++) and large numbers (>200 colonies, +++) [16]. Subculturing was performed from suspicious colonies. Biochemical tests and API kits (Biomerieux) were used for identification of subcultures.

Antimicrobial sensitivity test was performed by using the disc diffusion method described firstly by Bauer et al.[17] and evaluated according to the Standards of National Committee for Clinical Laboratory (NCCLS) [18]. Test was performed for *E. coli* and all ß-hemolytic streptococci. Following antibiotic discs were used in the test: Penicillin (10 U) (P), tulathromycin (30 µg) (TUL), tetracycline (30 µg) (TE), erythromycin (15 µg) (E), florfenicol (30 µg) (FF), ceftiofur (30 µg) (EFT), amoxicillin/clavulanic acid (20/10 µg) (AMC), amoxicillin (10 µg) (AML), enrofloxacin (5 µg) (EN), gentamicin (10 µg) (GM), cefquinome (30 µg) (CEQ), colistin (25 µg) (CO), marbofloxacin (5 µg) (MAR), sulfamethoxazole/trimethoprim (23.75/1.25 µg) (SXT).

RESULTS

Out of 247 collected samples, 151 (61.1%) were found to harbor bacteriae by conventional isolation and identification methods. No bacteriologic isolation was achieved from 96 samples (38.9%). More than one species of bacteria was isolated from 90 samples (59.6%) (Data not shown).

A total of 332 isolates were detected from culture positive samples. Most frequently isolated bacteria were *E. coli* (21.9%), α-hemolytic streptococci (15.9%), ß-hemolytic streptococci (15.4%), *Bacillus* spp. (12%), γ-hemolytic *streptococci* (10.5%) coliform bacteria (5.7) and *Staphylococcus aureus* (3%). The other isolates such as *Staph. epidermidis*, *Acinetobacter* spp. and *Corynebacterium* spp. and other species are detailed in *Table 1*.

Table 1. *Microorganisms and their ratios from uterus swabs of Thoroughbred mares between 2006 and 2008*
Tablo 1. *2006-2008 yılları arasında safkan kısrakların uterus svaplarından elde edilen mikroorganizmalar ve oranları (%)*

Microorganism	Number of Isolation (n)	Isolation Rate (%)
ß-hemolytic *Streptococci*	51	15.4
Escherichia coli	73	21.9
Escherichia coli hemolytic	1	0.3
Coliform bacteria	19	5.7
γ- hemolytic *Streptococci*	35	10.5
α- haemolytic *Streptococci*	53	15.9
Bacillus spp.	40	12.0
Actinobacillus equuli	3	0.3
Corynebacterium spp.	7	2.1
Proteus spp.	1	0.3
Staphylococcus epidermidis	14	4.2
Staphylococcus aureus	10	3.0
Pseudomonas spp.	2	0.6
Alcaligenes dentrificans	1	0.3
Enterobacter cloacae	1	0.3
Enterococcus faecalis	1	0.1
Enterococcus spp.	7	2.1
Acinetobacter spp.	9	2.7
Erwinia spp.	1	0.3
Nocardia spp.	1	0.3
Flavobacterium spp.	1	0.3
Fungus	1	0.3
TOTAL	332	100

E. coli and *ß*-hemolytic streptococci strains yielded moderate growth (++) in 40% and 45% of the cultures or abundant (+++) in 50% and 45% of the cultures, respectively (Data not shown).

In antimicrobial susceptibility tests, all *ß*- hemolytic streptococci were found to be susceptible to penicillin, ceftiofur, amoxicillin/clavulanic acid, enrof1oxacin, cefquinome, marbofloxacin and sulfamethoxazole trimethoprim, whereas 29.3% of *ß*-hemolytic streptococci showed resistance against colistin. All *E. coli* strains and 50% of them were resistant to penicillin and erythromycin, respectively, wheres all *E. coli* isolates were sensitive to all other tested antimicrobial agents used in this study *(Table 2)*.

DISCUSSION

Common bacterial agents isolated from uterine disorders and infertility problems are *Escherichia coli*, *ß*-hemolytic *streptococci*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae, Taylorella equigenitalis, Streptococcus equi* ssp. *zooepidemicus* [1,5,6,14]. In this study, 21 different bacteria and one fungus were isolated from uterine swabs of mares. Microbial isolates related to uterine disorders and their resistance and susceptibility to selected antimicrobial agents were discussed in this section.

In previous studies, the rate of mares with fertility problems due to bacterial infection were reported to be 30%, 32%, and 39%, respectively [19-21]. However, a more recent study reported that bacteria could be isolated from 66.2% of mares with fertility problems [22]. In our study, mares were selected from animals with reproductive disorders and 61.1% of them had significant growth of microorganisms.

Table 2. Antibiotic susceptibility of *ß*-hemolytic *Streptococci* and *Escherichia coli* isolates (%)
Tablo 2. *ß*-hemolitik *Streptococci* ve *Escherichia coli* izolatlarının antibiyotik duyarlılığı (%)

Antibiotic Discs	*ß*-hemolytic *Streptococci*			*Escherichia coli*		
	S (%)	MS (%)	R (%)	S (%)	MS (%)	R (%)
Penicillin	100					100
Tulathromycin	93.2	6.8		50.0	50.0	
Tetracycline	81.8	15.9	2.3	100		
Erythromycin	95.4	2.3	2.3		50.0	50.0
Florfenicol	97.7		2.3	100		
Ceftiofur	100			100		
Amoxycillin/ Clavulanic acid	100			100		
Amoxycillin	97.7		2.3	100		
Enrofloxacin	100			100		
Gentamicin	84.6	13.6		100		
Cefquinome	100			100		
Colistin	68.2	2.5	29.3	100		
Marbofloxacin	100			100		
Sulfamethoxazole/Trimethoprim	100			100		

S: *Sensitive* ***MS:*** *Moderate-Sensitive* ***R:*** *Resistant*

The variations between the reports above-mentioned may be due to the prophylactically use of antibiotics, which may also result in increasing resistance of bacterial strains. Antibiotic resistance more and more becomes a health problem of major importance [23,24]. Also sampling technique influences culture results [25,26].

In the present study, most of the isolated nonhaemolytic *E. coli* and *ß*-hemolytic streptococci yielded moderate or abundant growth. This may indicate an infection in the uterus rather than a vulvovestibular contamination. Non-hemolytic *E. coli* and *ß*-hemolytic streptococci were the most frequently isolated microorganisms associated with fertility problems in the mare. In contrast to our study, it has earlier been suggested that non-hemolytic *E. coli* isolates from equine uterine are non-pathogen [27]. However, a similar study has declared that non-hemolytic *E. coli* may cause fertility problems in mares which seems to be more associated with repeat breeding without clinical symptoms than with clinical symptoms of endometritis [6]. *Staph. aureus* is reported to be a rather frequently isolates species from the equine uterus of mares [19,20]. Ten (3%) *Staph. aureus* isolated from the samples may also be responsible for disorder and/or infection in mares' uterus investigated in the present study.

The global rise in antibiotic resistance has been linked to an increased use of antibiotics [28]. In farm applications, antibiotics are used for prophylactic purposes rather than treatment. They are used prophylactically before breeding or treatment of endometritis [19] as well as in semen extenders [29].

In vitro antimicrobial susceptibility test was performed for selected isolates representing pathogenic bacteria such as ß-hemolytic streptococci and *E. coli* which are considered to be responsible for infertility. *ß*-hemolytic streptococci were found to be moderately resistant (29.3%) to colistin, whereas high susceptibility of *ß*-hemolytic streptococci was observed to penicillin, ceftiofour, amoxicillin/clavulanic acid, enrofloxacin, gentamicin, cefquinome, marbofloxacin, and sulfamethoxazole/trimethoprim. All *E. coli* isolates showed resistance to penicillin but were found to be susceptible to florfenicol, tetracycline, ceftiofur, amoxicillin/clavulanic acid, enrofloxacin, gentamicin, colistin, cefquinome, marbofloxacin and sulfamethoxazole/trimethoprim. These results of the study showed that antimicrobial susceptibility of both *ß*-hemolytic streptococci and *E. coli* isolates from uteri of thoroughbred mares in Germany seems to be higher than those of reported in other studies. A similar study reported that *ß*-hemolytic streptococci are susceptible against sulfamethoxazole/trimethoprim, enrofloxacin, gentamicin with a ratio of 100%, 97% and 96%, respectively [6]. In the same study, *E. coli* isolates were found to be susceptible against amoxycilline/clavulanic acid, enrofloxacin, and sulfamethoxazole/trimethoprim with a ratio of 71.9%, 78.1%, and 73.5%, respectively. Another study declared that ß-hemolytic streptococci isolates were susceptible to amoxycilline/clavulanic acid, enrofloxacin and sulfamethoxazole/trimethoprim in rates of 82.7%, 17% and 17%, respectively [7]. Gentamicin susceptibility of *E. coli* strains was found to be 86% and 96% in the studies performed in USA and Sweeden, respectively [6,30].

In conclusion, types and rates of bacteria isolated from the uterus of mares can vary by region during years. Although our results indicated high susceptibility of bacteria, which were responsible for infertility in thoroughbred mares, to antimicrobial agents commonly used for treatment in Germany, systematic bacteriological examination should be performed and positive cultures should be evaluated for resistance of bacteria to determine antibiotics for effective treatment. Infertility factors in mares should also be monitored systematically and regularly to achieve healthy mares.

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RESEARCH ARTICLE

Rectal Catheterization for the Diagnosis of Iatrogenic Descending Colon Injuries During CO_2 Laparoscopy: An Experimental Study

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Summary

Only 35.7% of the bowel injuries occurred during CO_2 laparoscopy is noticeable intra-operatively. Although air insufflations into the rectum is suggested to identify this injuries, increased risk of contamination of the abdominal cavity by bowel contents makes this procedure abolished. In this experimental study we aimed to evaluate the role of rectal catheterization in the diagnosis of small or unnoticed injuries of the intestines during CO_2 laparoscopy. Pneumoperitoneum was created at a pressure level of 12 mmHg in seven white, New Zealand female rabbits. An eight G urinary catheter connected tightly to a urine bag was introduced into the rectum. The descending colon was perforated with the tip of a 21 G needle. Timely CO_2 use and the change of urine bag volume were recorded. Methylene blue was used to demonstrate flow into the bag. Significant amounts of gas accumulated in the urinary bag following the perforation of the large bowel and methylene blue flowed into the catheter. Thus, transanal placement of a catheter connected to a urinary bag may help in the diagnosis of small or unnoticed large bowel injuries during laparoscopy.

Keywords: *Rabbit, Laparoscopic injury, Colon, Rectal catheter, Air accumulation, Methylene blue*

CO_2 Laparoskopisi Sırasında İntraoperatif Barsak Yaralanmalarının Tanısı İçin Rektal Kateterizasyon: Deneysel Bir Çalışma

Özet

CO_2 laparoskopisi sırasındaki barsak yaralanmalarının ancak %35.7'si ameliyat sırasında fark edilebilmektedir. Yaralanmaları teşhis edebilmek amacıyla rektuma hava verilmesi düşünülse de, bu uygulama kontamine barsak içeriğinin yayılma şansını arttırabilir. Bu deneysel çalışmada CO_2 laparoskopisinde rektal kateterizasyonun küçük ya da fark edilmeyen barsak yaralanmalarındaki rolünü belirlemeyi amaçladık. Yedi tane beyaz, dişi Yeni Zelanda tavşanının karnı 12 mmHg basınca erişene kadar gazla şişirildi. Ucu idrar torbasına sıkıca bağlanmış 8 G idrar sondası tavşan rektumuna yerleştirildi. Bir 21 G iğnenin ucuyla inen kolon delindi. Deney süresince CO_2 kullanımı ve idrar torbasında izlenen hacim değişiklikleri kaydedildi. Torbaya akışı göstermek için metilen mavisi kullanıldı. Kolonun delinmesinden sonra anlamlı miktarda gaz idrar torbasında birikti ve metilen mavisi kateter içine aktı. Bu bulguya göre, transanal uygulanan ve idrar torbasına bağlanan bir kateter, laparoskopik cerrahi sırasında oluşabilecek küçük ya da fark edilmeyen barsak yaralanmalarının tanısında yardımcı olabilir.

Anahtar sözcükler: *Tavşan, Laparoskopik yaralanma, Kolon, Rektal kateterizasyon, Hava akümülasyonu, Metilen mavisi*

INTRODUCTION

Laparoscopic surgery has some potential advantages over laparotomy including less postoperative pain, fewer complications and earlier discharge. However, it has the similar complication risks observed in laparotomy, involving injury to a nearby vital structure, bleeding, and infection [1-4]. The best known risks of laparoscopic procedures are

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related to abdominal cavity access techniques, creation of pneumoperitoneum, use of intra-abdominal energy, and increased anesthesia risks potentialized by the increased intra-abdominal pressure [5-8].

Intra-operative diagnosis of a complication increases the chance of avoiding further hazardous injury to the effected tissue. Although gross vascular injuries are diagnosed instantaneously, minor bowel injuries may be unnoticed and result in the contamination of the abdominal viscera. Intra-operative diagnosis of a bowel injury, either provide the excellent chance for an immediate repair or prevent the late complications involving ileus, abscess formation or sepsis. In a study aiming to specify the circumstances under which the gastrointestinal injuries occur, the researchers [9,10] reported that only 35.7% of the injuries could be noticed intra-operatively. An additional 48.3% of the injuries could be diagnosed in the first post-operative week. Moreover, the remaining 16% of the injuries could be diagnosed after the first postoperative week [9]. The time of the diagnosis was most likely to elongate if the injury was in the bowel and resulted from the use of electro surgery [9,10].

Although some authors [7] suggest the routine insufflations of air into the rectum to identify small injuries in cases of extensive adhesions, the procedure may increase the chance of the dissemination of the contaminated bowel contents into the abdominal cavity.

Previous studies demonstrated the role of prompt intra-postoperative diagnosis of the bowel perforations which may significantly decrease the rates of morbidity and mortality. In this experimental study we aimed to evaluate the role of intra-operative rectal catheterization in the diagnosis of small or unnoticed injuries of the intestines during CO_2 laparoscopy. Our study may aid in the early diagnosis of intestinal injuries occurred during CO_2 laparoscopy and change the standard diagnostic strategy depended on expectant management. In addition, evidence based diagnostic approach may also prevent many unnecessary major surgical procedures.

MATERIAL and METHODS

The study was carried out in the Surgery Department of Kafkas University Faculty of Veterinary Medicine between 15th and 21st of August 2011. Before starting the experiments the study was approved by the Animal Ethics Committee of Kafkas University (KAU-HADYEK/2011-26). The participating animals were provided by University of Kafkas Faculty of Veterinary Medicine.

We used seven white New Zealand female rabbits in the experiment. All the rabbits were 2 years old and weighted between 3130 and 3460 g. Before anticipating the experimental procedure all the rabbits were fed as usual until the last six hour of the preoperative period. Oral alimentation and hydration were prohibited six hour before the induction of anesthesia. The rabbits received no special medication for bowel preparation. In order to achieve prophylaxis against intra-operative infection, a single dose of 80 mg/ kg cefazoline sodium was administered intramuscularly one hour before the start of the surgical procedure. All operations were performed between 5 and 7 p.m. on separate days by the same surgical team.

In all 7 rabbits intramuscular 10 mg/kg xylazin HCl (Rompun® 2% 50 ml Bayer) and 30 mg/kg ketamin HCl (Ketasol 10% inj, 10 ml vial Richter Pharma) were used for the induction of the anesthesia. Sevoflurane 2-2.5% mixed with dry air was used for the maintenance of the anesthesia.

Anterior abdominal wall was routinely prepared by shaving and the surgical area was prepared with 10% polyvinylpyrrolidone iodine. Through the 2-3 mm sub-umbilical incision the Veress needle was introduced into the abdominal cavity, and following the insufflations of CO_2 pneumoperitoneum was maintained at an intra-abdominal pressure level of 12 mmHg. The skin incision was enlarged to 6 mm and a 5 mm trocar was introduced into the abdominal cavity. Through the 5 mm trocar the telescope was introduced and the abdominal cavity was visualized. A second paramedian 2 mm port was inserted to manipulate the intra abdominal tissues. At an intra-abdominal pressure level of 12 mmHg the prevention of gas leakage was checked by the observation of the automatic cease of the gas inflow.

The initial phase of the experiment was mainly observational. An eight G urinary catheter connected tightly to an empty urine bag was introduced 10 cm into the rectum and the balloon of the catheter was inflated with 5 ml of saline *(Fig. 1)*. Other than moving the catheter slightly (1-2 cm up and down) along its longitudinal axe and spinning it around its transverse axe, the operative setting was not changed for 5 min and the changes of gas flow, intra-abdominal pressure and the volume of the urine bag were recorded.

In the second phase of the experiment, the line of the rectal catheter was clamped first. Under telescopic view a 21 G needle was inserted into the abdominal cavity. The tip of the needle was oriented to the anti-mesenteric wall of the descending colon and the colon was perforated approximately 10 cm above the point of insertion of the rectum into the pelvic base *(Fig. 2)*. The needle was taken outside the abdominal cavity. Prevention of gas leakage was checked as defined previously. At the point where the gas flow ceased along with an intra-abdominal pressure of 12 mmHg, the rectal catheter was unclamped. Other than moving the catheter slightly (1-2 cm up and down) along its longitudinal axe and spinning it around its transverse

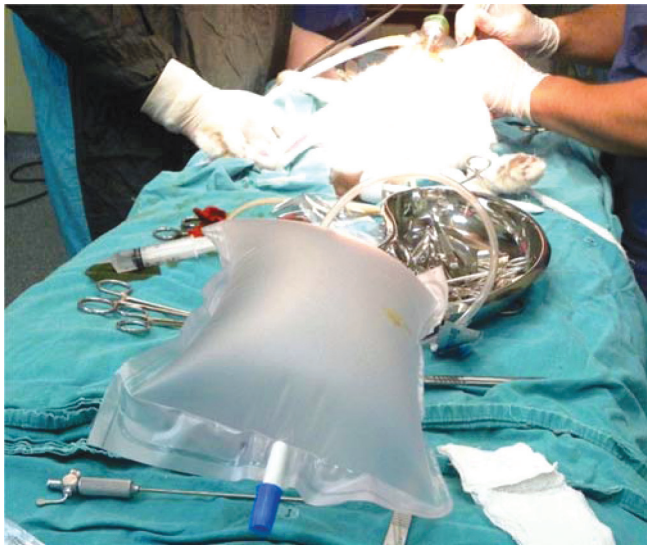

Fig 1. Experimentation in a rabbit during CO_2 laparoscopy. Perforation of the descending colon with the tip of a 21 G needle caused the distension of the urinary bag connected to the catheter inserted into the rectum of the rabbit

Şekil 1. CO_2 laparoskopisi sırasında bir tavşandaki deney. 21 G iğnenin ucu ile inen kolonun delinmesiyle rektuma yerleştirilen kateter yoluyla kateter ucuna bağlı idrar torbasının şişmesi

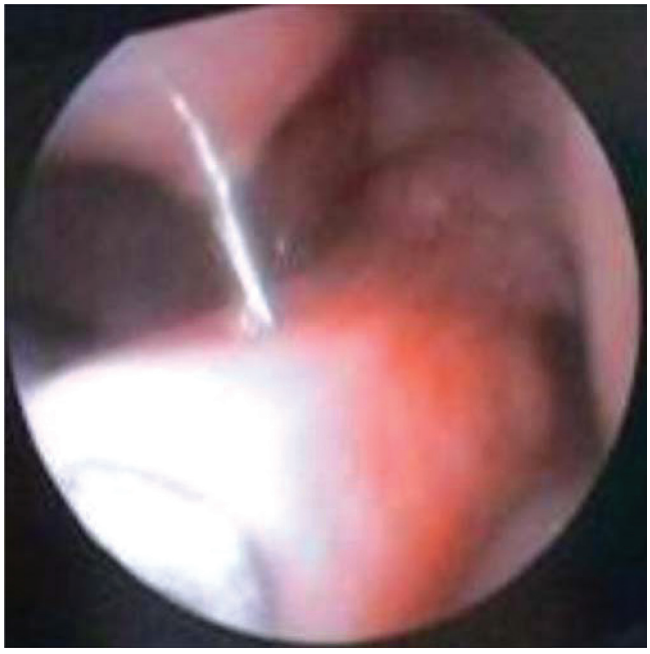

Fig 2. Perforation of the descending colon under telescopic view by using the tip of a 21 G needle

Şekil 2. Teleskopik görüntü altında 21 G iğnenin ucu kullanılarak inen kolonun delinmesi

axe, the operative setting was not changed for 5 min and the changes of gas flow, intra-abdominal pressure and the volume of the urine bag were recorded for the next 5 min.

In the third phase of the experiment 50 ml of methylene blue was injected into the abdominal cavity and the rectal catheter was observed for 5 min.

At the end of the experiment the abdominal cavity was deflated and the incision of the abdominal entry site was closed with number 2-0 delayed absorbable sutures.

The first two operated rabbits died the next day after the operation with the clinical symptoms of generalized peritonitis and sepsis, thus in order to prevent unnecessary suffering of the animals, the remaining five rabbits were sacrificed at the end of the experiment by the administration of 100 mg intramuscular xylocaine and 200 mg/kg intra-cardiac ketamine.

Statistical analyses were performed using SPSS version 16.0 software (SPSS Inc, Chicago, IL). The amounts of CO_2 use at each phase and the weights of the rabbits were evaulated by using the Kolmogorov-Smirnov Z test. The amounts of the used CO_2 and the estimated changes of the volume of the urinary bags during each three phases of the study were compared by using Friedman's and Wilcoxon's tests. Correlations between the study parameters were analysed by using Spearman's test. A P value of <0.05 was considered significant.

RESULTS

In five of the cases, although the gas accumulation in the urinary bags was observed initially, the gas flow ceased due to the obstructions of the catheters. Slight movements of the catheter around itself and upward or downward reconstructed the gas flow into the urinary bag in four of the cases; however in one case we had to take the catheter, clean the obstructed tip and reinsert into the rectum in order to reconstruct the gas flow into the urinary bag.

The weights of the rabbits and the initial amount of CO_2 used for the creation of pneumoperitoneum did not differ among the participating rabbits ($P>0.05$). We did not observe any CO_2 flow at the first phase of the experiment, thus there was not any CO_2 use or CO_2 accumulation in the urinary bags. However, we observed CO_2 flow and CO_2 accumulation in the urine bags *(Fig. 1)* in all the participating animals in the second and third phase of the study *(Table 1)*. In addition methylene blue flow into the line of the urinary bag was observed in all seven cases.

Statistical analysis by using Friedman's and Wilcoxon's tests showed that the amounts of used CO_2 and accumulated CO_2 in the urinary bags were significantly different ($P<0.05$). The used CO_2 was significantly highest in the second phase and lowest in the first phase ($P<0.05$). Similarly, the accumulation of gas in the urinary bags was significantly highest in the second phase and lowest in the first phase ($P<0.05$). Both the amount of CO_2 use and urinary bag accumulations were significantly different in each phase of the study *(Table 2)*.

Table 1. *Comparison of some selected parameters of seven rabbits. The values are presented as mean ± standard deviation or percent (%)*
Tablo 1. *Yedi tavşana ait bazı seçilmiş verilerin karşılaştırılması. Değerler, ortalama ± standart sapma ya da yüzde (%) şeklinde sunulmuştur*

Characteristics	Mean ± Standard Deviation	P Value*
Weight of the rabbits	3.27±1.11	0.540
Initial amounts of CO_2 used to maintain the abdominal pressure of 12 mmHg (ml)	857.14±1.81	0.564
CO_2 use in the 1st phase ǂ of the experiment (ml)	0	N/A
CO_2 use in the 2nd phase ǂǂ of the study (ml)	1271.43±149.60	0.423
CO_2 use in the 3rd phase ǂǂǂ of the study (ml)	814.28±121.50	0.675
Increase of estimated urinary bag volume in the 1st phase (ml)	0	N/A
Increase of estimated urinary bag volume in the 2nd phase (ml)	785.71±94.49	0.726
Increase of estimated urinary bag volume in the 3rd phase (ml)	471.43±111.27	0.457
Methylene blue in the urinary catheter or bag (%)	100	N/A

** Kolmogorov-Smirnov Z test,* ***ǂ 1st phase:*** *5 min interval following the placement of rectal catheter,* ***ǂǂ 2nd phase:*** *5 min interval following the perforation of the large bowel with the tip of a 21 G needle,* ***ǂǂǂ 3rd phase:*** *5 min interval following the intra abdominal administration of methylene blue*

Table 2. *Comparison of the parameters at different phases of the study. The values are presented as mean ± Standard deviation*
Tablo 2. *Çalışma parametrelerinin farklı fazlarda karşılaştırılması. Değerler ortalama± standart sapma olarak sunulmuştur*

Parameter	1st Phaseǂ	2nd Phaseǂǂ	3rd Phaseǂǂǂ	P Value
CO_2 use (ml)	0	1271.43±149.60	814.28±121.50	0.001*
Estimated urinary bag volume increase (ml)	0	785.71±94.49	471.43±111.27	0.001*
	1st Phase	**2nd Phase**		
CO_2 use (ml)	0	1271.43±149.60		0.018**
Estimated urinary bag volume increase (ml)	0	785.71±94.49		0.017**
	1st Phase	**3rd Phase**		
CO_2 use (ml)	0	814.28±121.50		0.017**
Estimated urinary bag volume increase (ml)	0	471.43±111.27		0.017**
	2nd Phase	**3rd Phase**		
CO_2 use (ml)	1271.43±149.60	814.28±121.50		0.017**
Estimated urinary bag volume increase (ml)	785.71±94.49	471.43±111.27		0.017**

** Friedman test, **Wilcoxon signed ranks test,* ***ǂ 1st phase:*** *5 min interval following the placement of rectal catheter,* ***ǂǂ 2nd phase:*** *5 min interval following the perforation of the large bowel with the tip of a 21 G needle,* ***ǂǂǂ 3rd phase:*** *5 min interval following the intra abdominal administration of methylene blue*

Correlation analysis showed that the weight of the rabbits, initial CO_2 use for creating pneumoperitoneum, and the CO_2 use in the second and third phase of the experiment correlated with each other ($P<0.05$), however the amount of CO_2 accumulated in the second and third phase of the experiment (although correlated with each other) did not correlated with the abovementioned parameters ($P>0.05$).

DISCUSSION

Principal Findings

In this experimental study we demonstrated that a complete penetrating injury of the large bowel (even as small as the tip of a needle) during laparoscopic surgery causes the diffusion of CO_2 into the large bowel. Trans rectal insertion of a urinary catheter tightly connected to a urinary bag may help diagnosing the bowel injury after observing the inflation of the urinary bag. The accumulation of the intra-abdominally administered methylene blue in the urinary bag makes the diagnosis certain.

Strengths

To our knowledge this is the first study offering the insertion of a urinary catheter into the rectum in order to diagnose the small or unnoticed bowel injury during CO_2 laparoscopic surgery. Although some authors suggested transanal insufflations or methylene blue administrations [7,9], both procedures may enhance the dissemination of the contaminated contents of the bowel, particularly the large one. In contrast our technique requires the flow of gas from intra-abdominal cavity into the intestines and the catheter placed in the rectum, thus reasonably may decrease the rates of contamination. Although first two rabbits participated in our study died with the findings of peritonitis and sepsis, there was no bowel preparation at the beginning of the study and the contamination at the time of the bowel perforation may be the reason. In order to compare the effect of our technique on bacterial

contamination with the previous ones we need further studies.

Another favorable characteristic of our technique is its simplicity and easily accessible nature in every operative theatre setting.

Limitations

Rectally placed catheter may identify the bowel injury which affects all layers of the intestinal wall. However abrasions, lacerations and cautery burns that may cause delayed perforations or obstructions are missed. In addition, it is almost impossible to find the exact place of the injury in where the injury site is very small. Intra abdominal administration of methylene blue also seems unhelpful for this purpose.

Although we presented the amounts of CO_2 used and accumulated in the urinary bag in every phase of the study, the reader should notice that the values were mostly estimated. The used CO_2 values were red from the insufflator device which only showed one decimal and the accumulated CO_2 in the urinary bags were estimated by the appearance and the covered scale of the bags.

The perforation was simulated on the descending colon, thus we can not state the findings for the perforations located on the proximal parts of the intestines.

Comparison with the Previous Studies

Creation of pneumoperitoneum during laparoscopy is critical because more than half of the complications occur at this stage [9,10] and one-third (34.5%) of the complications are related with the gastrointestinal system. In addition 48.4% of the gastrointestinal complications involve the large bowel [8]. However, only one-third (35.7%) of the complications are noticed intra-operatively. Moreover, the mortality rate of unrecognized bowel injuries was found 21% [12].

Upper bowel injuries may be free or contained within the surrounding structures, however, lower bowel injuries are nearly always free and the intestinal contents spill into the abdominal cavity. In addition the foul smell of the intestinal contents and bleeding at the site of injury may help in intra operative diagnosis. However, elevated intra abdominal pressure in CO_2 laparoscopy may cause the leakage of the gas into the perforated bowel and obscure the signs of perforation, particularly in small injuries. Reasonably the chance of spill of the bowel contents and sensing of the foul smell is less during laparoscopy. Thus, for the small and unnoticed perforations the diagnosis depends on the suspicion. Obesity, endometriosis, malignancy, dense adhesions and extensive adhesiolysis are the predisposing factors associated with bowel injury [11,13-18]. However, high suspicion and laparoscopic exploration do not guarantee the diagnosis of a small perforation.

In order not to miss an intra operative iatrogenic bowel perforation, some authors suggest the routine insufflations of air into the rectum [7] and/or transanal injection of 200 ml methylene blue [9] using a Foley catheter to identify small injuries in cases of extensive adhesions. However, these procedures carry the risk of the dissemination of the contaminated bowel contents into the abdominal cavity. In addition, in order to provide the flow of transanal gas or methylene blue into the abdominal cavity the pressure inside the bowel must exceed the intra abdominal pressure which in turn may enlarge the injury dimensions and obscure the surgical view after the dilatations of the intestines. In our technique the flow of the gas or methylene blue is from inside towards outside and it does not increase the risk of contamination or the rate of bowel dilatation. However our technique may be insufficient in diagnosing the exact location of the injury.

In our study intra abdominal methylene blue was used for demonstrative purposes. It was not a part of the technique. Thus in the diagnostic workup of an intra -operative bowel injury, we think that insertion of a catheter into the rectum and observation of the distension of the connected urinary bag should be the first step. If the operators are convinced on the diagnosis of perforation and the exact location of the perforation is not demonstrable, then transanal injection of methylene blue may be considered.

In a study [19] performed to evaluate the feasibility of methylene blue as a marker to detect the gastric perforations, the researchers demonstrated the extra-vasations of methylene blue during laparoscopic surgery with the perforations of 1.2 mm and greater with or without air insufflations. Air extravasation was seen with perforations of 2.0 mm and greater in the same study. In our study we did not measure the specific diameter of the each individual injury; however we created the injury by using a 21 G needle with an outer diameter of 0.8 mm. Although we did not perform a colonoscopic examination to determine the extravasations of methylene blue or air into the intestinal lumen, we demonstrated both the air and methylene blue in the urinary bag connected to the rectal catheter. The amounts of the used CO_2 and accumulated air in the urinary bag were significantly less after using methylene blue in comparison before using methylene blue ($P<0.05$). Probably, the slower extravasation of the methylene blue with a higher viscosity compared to CO_2 was the explanation for the phenomena.

Meaning of the Study and Clinical Implications

Our experiment demonstrated that bowel injury during CO_2 laparoscopy may be diagnosed by inserting a catheter tightly connected to a urinary bag. However, it should be remembered that the injury should involve the whole intestinal wall connecting the intestinal lumen and the intra abdominal cavity. According to our study settings

the technique is helpful with an injury diameter of 0.8 mm or greater.

Although the diagnostic technique worked in our experimental study, we do not have evidence about its use in human beings. Further clinical studies performed on human beings may provide the evidence about the efficiency of the technique in routine daily practice. In our study we used an eight G urinary catheter and did not have preoperative bowel preparation. In human beings the bowels are usually prepared preoperatively and it is possible to use larger rectal catheters with larger lumens. Thus, it is reasonable to think that the technique will be more efficient in humans.

According to our study the distension of the urinary bag defines the perforation of the large bowel. However, we do not know whether the technique is applicable for upper intestinal injuries or not. Another important issue is encountered in cases where the urinary bag does not distend. Does it exclude perforation? Does it exclude perforation only at large bowel. We believe that large bowel perforations at the time of the applications may be diagnosed. However, incomplete injuries or necrotic burn injuries which lead to delayed perforations may be incorrectly diagnosed as if there is not an injury. Thus in clinical practice and studies patients with negative tests should be followed for an adequate period of time.

Transanal placement of a catheter connected to a closed system may help in the diagnosis of small or unnoticed large bowel injuries during laparoscopy.

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RESEARCH ARTICLE

The Importance of Concentrations of Sorbitol Dehydrogenase and Glutamate Dehydrogenase and B-Mode Ultrasonographic Examination in The Diagnosis of Hepatic Lipidosis in Dairy Cows [1]

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Summary

The aim of this study is to determine the importance of B-mode ultrasonography in the diagnosis of hepatic lipidosis in dairy cows and compare this mode of diagnosis with both the histologic examination of liver biopsy samples and investigation of some biochemical parameters associated with hepatic lipidosis. 15 Holstein cows with moderate hepatic lipidosis and 15 cows with severe hepatic lipidosis and 6 healtyh cows were used as a metarilas. Blood samples were obtained from all cows and analyzed. Liver samples were obtained by biopsy in cattle. The ultrasonographic examination of liver was performed on animal. Serum glutamate dehydrogenase (GDH) and aspartate amino transferase (AST) concentrations were increased in cows with moderate hepatic lipidosis. Serum sorbitol dehydrogenase (SDH), GDH, and AST concentrations were increased in cows with severe hepatic lipidosis. Ultrasonographic examination revealed an increase in diffuse echogenicity of the liver in cows with moderate and severe hepatic lipidosis cows, but the increase was little in moderate hepatic lipidosis. Both serum GDH and SDH levels were found to be increased in severe hepatic lipidosis. However, only the serum GDH level was elevated in moderate hepatic lipidosis. Therefore, both ultrasonographic examination and measurement of specific liver enzymes seem to be beneficial in the diagnosis of hepatic lipidosis.

Keywords: *Hepatic lipidosis, Liver enzymes, Liver ultrasonography, Dairy cow*

Sütçü Sığırların Hepatik Lipidozisinin Teşhisinde B-Mode Ultrasonografik Muayene ve Serum Sorbital Dehidrogenaz ve Glutamete Dehidrogenaz Düzeylerinin Önemi

Özet

Bu çalışmanın amacı; sütçü sığırların yağlı karaciğer sendromunun teşhisinde B-mode ultrasonografinin önemini belirlemek, hastalığın tanısında ultrasonografik muayene ile karaciğer biyopsi örneklerinin histolojik bulguları ve bazı biyokimyasal parametre sonuçlarını karşılaştırmaktır. Bu çalışmada 15 orta hepatik lipidozisli, 15 şiddetli hepatik lipidozisli ve 6 sağlıklı Holştein ırkı sütçü sığır kullanıldı. Bütün sığırlardan kan alındı ve analiz edildi. Sığırların karaciğerinin ultrasonografik muayenesi yapıldı. Hepatik lipidozisli sığırlardan karaciğer biyopsisi alındı. Orta dereceli hepatik lipidozisli sığırlarda serum glutamate dehidrogenaz (GDH) ve aspartat aminotransferaz (AST) seviyeleri artarken, şiddetli hepatik lipidozisli sığırlarda serum sorbital dehidrogenaz (SDH), GDH ve AST seviyeleri arttı. Ultrasonografik maueyenede; orta ve şiddetli hepatik lipidozisli sığırlarda karaciğerde diffuz ekojenite artışı gözlemlenmekle birlikte, orta dereceli hepatik lipidozis olgularında ekojenite artışı daha azdı. Şiddetli hepatik lipidozisde SDH ve GDH enzim düzeylerinde, orta dereceli hepatik lipidozisde ise sadece GDH enzim düzeyinde artış belirlendi. Hem ultrasonografik muayene hem de karaciğerin spesifik enzim ölçümü hepatik lipidozisin teşhisinde faydalı olduğu düşüncesine varıldı.

Anahtar sözcükler: *Hepatik lipidozis, Karaciğer enzimleri, Karaciğer ultrasonu, Sütçü sığır*

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INTRODUCTION

Liver plays a pivotal role in provision of energy for periparturient dairy cows from various precursors [1]. A combination of a deep negative energy balance and fatty liver is a common feature in high producing dairy cows after parturition [2]. Fatty liver syndrome or hepatic lipidosis is characterized by infiltration of triacylglycerol (TAG) in the liver [3]. Fatty liver is a metabolic disorder that is caused by excessive mobilization of body fat, which occurs in high yielding dairy cows in which the demands for energy exceed the supply [4]. Over conditioning is a major risk factor for developing fatty liver [5]. Fatty liver is associated with decreased health status, well-being, milk yield, reproductive performance, and reduced immune response. Severe and moderate fatty liver develops in approximately 15% and 35% of dairy cows, respectively [6]. Fatty liver occurs primarly in the 1st month of lactation in dairy cows [7,8].

Blood profiles and changes in body condition score have been used to monitor metabolic imbalance around parturition and, in early lactation, to investigate problem [9,10] as well as to predict the risk of diseases such as displaced abomasum [11]. Parameters used to monitor imbalance in energy metabolism have included glucose, non-esterified fatty acids (NEFA), betahydroxybutyrate (BHBA), and change in body condition score [12,13]. Fatt liver develops when hepatic availability of lipogenic and glucogenic products is imbalanced. Thereby the oxidation capacity of fatty acids is exceeded and since hepatic secretion of lipids is inherited, low excess hepatic lipids are stored as TAG in the liver tissue [6,14]. In domestic animals, serum enzyme test are grouped into those that indicate hepatocelluler leakage due to hepatocyte damage [15-17]. Hepatocelluler enzymes, particularly AST, GDH and SDH may be useful in monitoring the hepatic lipidosis that commonly occurs at parturition [5,18]. The hepatocelluler leakage enzymes-AST and cholestatic enzymes-GGT activities have been used to evaluate the liver [19]. Blood cholesterol concentration is related to feed intake and low cholesterol concentrations have been associated fatty liver post-partum [20].

Fatty liver can be diagnosed as reliable only by determining TAG content biochemical or histological analysis of a liver puncture biopsy sample. Biopsies are impracticable for on-farm diagnosis because they cause temporary discomfort to cow, pose risk of infection, and can be lethal if a major blood vessel is punctured [21]. Therefore, a non-invasive technique would be very useful. Ultrasonograph has been used routinely for about long time as a diagnostic procudure in hepatic disease of cows [22]. Ultrasound imaging followed by digital analysis of sonograms has potential to non-invasively detect fatty liver and estimate liver TAG content [23]. Haudum et al.[24] reported that ultrasonography has proven useful for the evaluation of hepatic triacylglycerol content in dairy cows. However, detection of fatty liver is more difficult because it results in smaller cages of hepatic echostructure [22].

The aim of this study is to evaluate the importance of B-mode ultrasonography in the diagnosis of hepatic lipidosis in dairy cows and compare this mode of diagnosis with both the histologic examination of liver biopsy samples and investigation of some biochemical parameters (Especially SDH and GDH) associated with hepatic lipidosis.

MATERIAL and METHODS

Animals and Clinical Examination

The institutional ethical committee approved this prospective study. In this study, 15 Holstein cows with moderate hepatic lipidosis and 15 Holstein cows with severe hepatic lipidosis in the first 4 weeks of lactation were used. Cows were aged 3-7 years, and the mean daily milk yield was 25 kg. In the reference group, 6 clinically healthy postparturient Holstein cows from a local dairy farm were used. These cows were aged 3-6 years, were in the first 2-4 weeks of lactation, and were reported by the owner to have a daily milk yield ranging from 23 to 27 kg on milking twice daily. Of 15 cows with moderate and severe hepatic lipidosis, 10 showed left abomasal displacement, and of 10 cows with moderate and severe hepatic lipidosis, 5 had ketosis. Routine physical examination, including simultaneous auscultation and percussion of the abdomen, ballottement of the abdomen for a splashing sound to indicate the presence of an air-fluid interface in a large viscus, palpation per rectum, urinary examination, and ultrasonographic examination, were performed on each cow.

Ultrasonographic Examination of Abomasum and Liver

The ultrasonographic examination of the abomasum is performed on the left side of the standing animal. Ultrasonographic examination of the abomasum was performed at the 10th and 13th intercostal spaces on the left side, and the area was examined ventrally to dorsally using a real-time 3.5-5.0-MHz convex transducer [25,26]. The liver was examined by ultrasonography from the caudal to the cranial region, beginning from the caudal region to the last rib on the right side and ending at the fifth intercostal space, and from the dorsal to ventral region in every intercostal space using a real-time 3.5-5.0-MHz convex transducer [22]. Ultrasonographic examination of the liver was performed in all cows.

Detection of Ketosis

Detection of ketosis; urine was collected with a catheter or free flow urine was collected from all cows and a drop was applied to reagent strips (Multistix® 10 SG; Bayer). Results were scored as positive or negative depending on whether or not there was a change in color from white to purple.

Treatment

A right flank laparotomy was performed under regional analgesia on cows with a presumptive clinical diagnosis of LDA, and the diagnosis was confirmed during surgery using established criteria [27]. Cows with LDA were hospitalized for 1 day after surgery and then discharged. The clinical outcome was investigated at least 2 weeks after surgery by communicating with the owner via telephone. Cows with ketosis were treated. The treatment protocol for cows with ketosis was as follows: intravenous administration of 1.000 mL of 30% serum dextrose (Dekstrose®, Eczacibasi, Baxter) for 3 days, administration of 200 IU insulin (NPH, Humulin®, Lilly Ilac), intramuscular administration of 10 mg of dexamethasone (Devamed®, Topkim Ilac) for 3 days, and oral administration of 150 mL of propylene glycol (Bovical®, Bioteknik) for 4 days. Cows with moderate and severe hepatic lipidosis received ancillary treatment for hepatic lipidosis.

Blood Sample Collection

Blood samples were collected from the jugular vein immediately before surgery in cows with displaced abomasum and before treatment in cows with ketosis and from healthy cattle. An aliquot of blood was placed into glass tubes for serum biochemical analysis. The tubes were centrifuged after clotting, and the serum was harvested and stored at -20°C until analysis.

Biochemical Analyses

Serum SDH concentration was determined by sandwich ELISA (USCN Life Science Inc. Cat No: E91495 Bo, Wuhan, China). The manufacturer of this assay reported the limit of detection in bovine serum as 20 µL and the reference range as 1.56-100 U/L. Serum GDH concentration was determined by sandwich ELISA (USCN Life Science Inc. Cat No: E90293Bo,Wuhan, China). The manufacturer of this assay reported the limit of detection in bovine serum as 100 µL and the reference range as 7.8-500 U/L. Serum AST, ALT, GGT, and ALP activities, as well as serum glucose, cholesterol, triglyceride, blood urea nitrogen (BUN), creatinine, total protein, albumin, Mg^{+}, P^{+}, and Ca^{++} concentrations were measured with an automatic analyzer (BT 3000 plus, Biotecnical Inc, SPA, Via lizenca, 18 00155, Rome, Italy). The serum insulin level was measured by an immunoassay system (Invitrogen immunoassays kit # PL2820085, Advia Center XP, Siemens, REVM). Blood Na^{+}, K^{+}, and Cl^{-} concentrations were measured by using ion-selective electrodes.

Liver Sample Collection and Histological Examination

Liver biopsy samples were obtained preoperatively in cows with LDA and cows with ketosis percutaneously through the right 11^{th} to 12^{th} intercostal space. Liver biopsy samples were not obtained from healthy cattle. Liver biopsy samples were placed in Baker's formal-Ca solution and fixed in paraffin for at least 16 h. From each fixed liver sample, 12-mm sections were cut and stained with oil Red O and Sudan Black B. The sections were examined under light microscopy as described [28], and the percentage volume of visible fat in hepatic parenchymal cells was estimated by a stereological point counting method. The extent of fat infiltration in the liver was categorized as mild (<10%, <10 μm^2/100 μm^2), moderate (10%-20%, 10-20 μm^2/100 μm^2), and severe (>20%, >20 μm^2/100 μm^2) on the basis of the percentage volume of visible fat [29].

Statistical Analyses

Statistical analyses were performed using a package program (SPSS 15.0), as reported by Akgül [30]. One-way analysis of variance (ANOVA) and Tukey's test were used for comparing the data ($P<0.05$).

RESULTS

Twenty cows with LDA did not have evidence of any other clinical disease. Cows with LDA had fair to moderate appetite and decreased rumen contraction frequency, defecation, and milk production. Ten cows with ketosis had depressed appetite, decreased rumen contraction frequency and milk production, dry feces, ketonuria, and ketonemia.

Serum GDH, and AST concentrations were increased in cows with moderate hepatic lipidosis and severe hepatic lipidosis as compared with in the controls *(Table 1)*; however, serum SDH concentration was increased in cows with severe hepatic lipidosis. In addition, serum SDH, GDH, AST, and cholesterol levels were higher in cows with severe hepatic lipidosis than in cows with moderate hepatic lipidosis. In contrast, serum cholesterol, Ca^{++}, Cl^{-}, Na^{+}, and K^{+} levels were decreased in cows with moderate hepatic lipidosis and in cows with severe hepatic lipidosis as compared with in the controls *(Table 1)*. Serum total protein level was increased in cows with severe hepatic lipidosis as compared with in cows with moderate hepatic lipidosis and in the controls.

Increase in diffuse echogenicity of the liver was observed in cows with severe hepatic lipidosis *(Fig. 1)* and cows with moderate hepatic lipidosis, but the increase in the latter was little *(Fig. 2)*. The liver appeared white on ultrasonograms, and it was difficult to differentiate the liver from the surrounding tissue. Echogenicity of the liver was normal in healthy cows *(Fig. 3)*. There was no significant difference between cows with ketosis and cows with LDA in terms of the liver fat percentage. There were 15 cows with moderate (10% - 20%) hepatic lipidosis and 15 with severe (20% - 48%) hepatic lipidosis.

DISCUSSION

The main finding of the present study was that SDH, GDH, and AST enzyme activities were increased in post-

Table 1. *Serum biochemical parameters healthy lactating cows (control) and lactating cows with moderate and severe hepatic lipidosis.*
Tablo 1. *Sağlıklı, orta ve şiddetli hepatik lipidozisli sütçü sığırların serum biyokimyasal parametreleri*

Parameters	Groups			P
	Control N=6	Moderate N=15	Severe N=15	
SDH (UI/L)	18.93±4.32 [a]	42.63±5.28 [a]	75.78±9.75 [b]	0.000
GDH (UI/L)	101.33±8.78 [a]	132.08±9.93 [ab]	149.50±12.18 [b]	0.040
AST (UI/L)	71.33±6.30 [a]	139.33±13.28 [b]	155.07±15.19 [b]	0.007
ALT (U/L)	26.17±2.65	25.31±2.91	28.30±3.17	0.762
GGT (UI/L)	31.50±8.43	46.85±6.29	38.30±5.26	0.297
ALP (UI/L)	47.17±5.15	41.38±3.82	37.30±5.72	0.302
Insulin (µU/L)	0.35±0.07	0.42±0.29	0.50±0.34	0.752
Cholesterol (mg/dL)	176.10±89.03[a]	125.23±68.74[ab]	78.00±11.7[b]	0.36
Tryglyseride (mg/dL)	16.17±2.86	19.92±4.65	22.50±9.30	0.183
Glucose (mg/dL)	101.67±6.64	102.31±13.79	79.90±18.49	0.515
Urea (mg/dL)	29.50±0.99	39.00±4.17	32.70±4.96	0.329
Creatinin (mg/dL)	1.82±0.18	1.30±0.12	1.37±0.12	0.051
Total protein (g/dL)	7.08±0.14 [a]	8.65±0.31 [a]	10.81±0.84 [b]	0.001
Albumin (g/dL)	3.38±0.26	3.86±0.22	4.18±0.15	0.089
Magnesium(mg/dL)	2.00±0.05	1.67±0.17	2.09±0.11	0.101
Calcium (mg/dL)	10.05±0.31 [a]	8.49±0.25 [b]	9.12±0.27 [ab]	0.004
Phosphorus (mg/dL)	6.40±0.66	5.22±0.47	4.68±0.32	0.096
Chlor (mmol/L)	101.50±1.61[a]	94.23±1.76 [b]	96.10±1.66 [ab]	0.045
Sodium (mmol/L)	144.33±0.84 [a]	137.00±0.72 [b]	140.75±1.15 [c]	0.000
Potassium (mmol/L)	3.90±0.18 [a]	3.06±0.22 [b]	3.41±0.15 [ab]	0.043

SDH: *Sorbitol dehydrogenase,* ***GDH:*** *Glutamate dehydrogenase,* ***AST:*** *Aspartate amino transferase,* ***ALT:*** *Alanin amino transferase,* ***GGT:*** *gamma-glutamyl transferase,* ***ALP:*** *Alkaline phosphatase, [a-c] means within row with different supercript differ (P<0.05)*

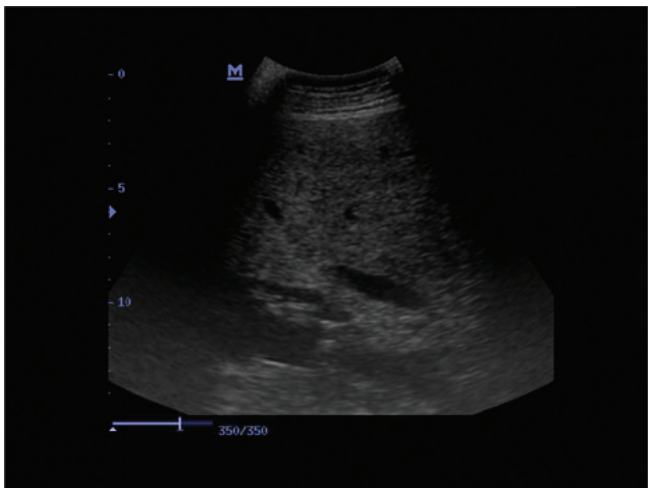

Fig 1. Ultrasonogram of liver of a cow with severe hepatic lipidosis
Şekil 1. Şiddetli hepatik lipidosisli bir inekte karaciğer ultrasonogramı

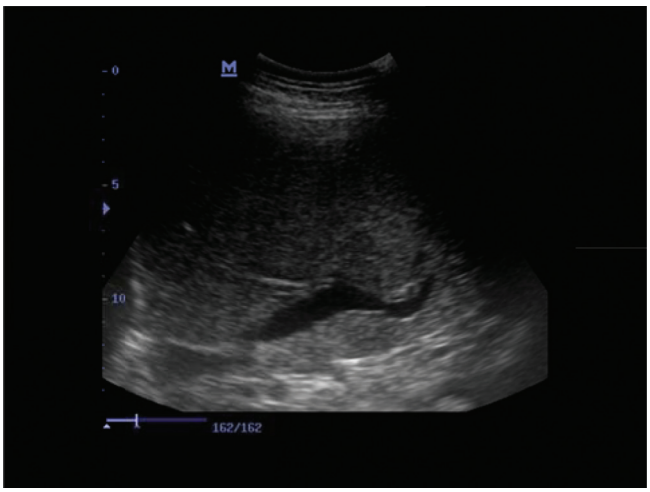

Fig 2. Ultrasonogram of liver of a cow with moderate with hepatic lipidosis
Şekil 2. Hafif şiddetli hepatik lipidosisli bir inekte karaciğer ultrasonogramı

parturient dairy cows with hepatic lipidosis. The second finding was that an increase in diffuse echogenicity of the liver was observed on ultrasonographic examination in cows with severe hepatic lipidosis and cows with moderate hepatic lipidosis, but the increase was little in the latter.

Hepatocelluler enzymes, particularly AST and GDH may be useful in monitoring the hepatic lipidosis that commonly occurs at parturition [5,31]. SDH and GDH are liver-specific enzymes [16,17,32,33]. Increased serum SDH and GDH activity is suggestive of either hepatocyte death or sublethal hepatocyte injury. Sorbital dehydrogenase and GDH have been regarded by many as enzymes of choice for

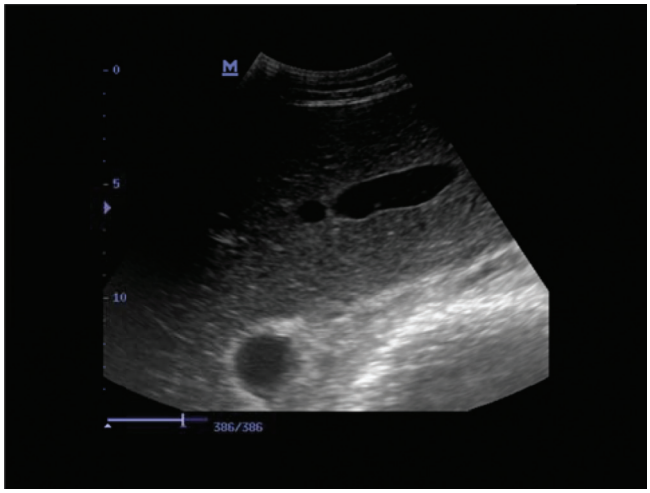

Fig 3. Ultrasonogram of liver of healthy a cow
Şekil 3. Sağlıklı bir inekte karaciğer ultrasonogramı

use as indicators of the degree of hepatic necrosis in sheep, goats, and cattle [16,17,33-35]. Braun et al.[36] reported that SDH, GDH, and GGT concentrations are increased in cows with liver tumors. Kalaitzakis et al.[33] mentioned that AST, SDH, GDH and ornitine carbamobyl transferase, activity was increased in cases of fatty liver syndrome because of the destruction of liver cells. The activities of hepatocellulerr leakage enzymes such as AST and cholestatic enzymes such as GGT have been used to evaluate the liver [19]. Bogin et al.[37] found significantly increased AST levels in cows with severe fatty liver. Sevinc et al.[28] showed that cows with severe fatty liver had high AST and GGT activities. Hepatic lipidosis may contribute to the pathogenesis of abomasal displacement. Komatsu et al.[38] indicated that high levels of AST in cows with LDA were found in fatty liver degeneration. Kalaitzakis et al.[39] reported that OCT and GDH enzymes might be useful in diagnosis fatty liver in downer cows. In this study, serum GDH and AST concentrations were increased in cows with moderate and severe hepatic lipidosis compared with in the controls. However, only the serum SDH concentration was increased in cows with severe hepatic lipidosis. Increased SDH and GDH concentrations in cows with hepatic lipidosis may be related to hepatocyte death or sublethal hepatocyte injury. SDH, GDH, and AST levels were high in cows with moderate and severe hepatic lipidosis compared with in the control group. Although higher levels of SDH and GDH are generally reported in acute liver damage (within 4 to 24 hours of hepatic injury) [19,32]. We found that higher levels of these enzymes were found in cows with hepatic lipidosis. We understand that these enzymes are increased not only in acute liver damage but also in chronic liver damage [15,17,33].

Blood cholesterol concentration is related to feed intake [40] and low cholesterol concentration have been associated fatty liver post-partum [20]. Several authors [1,28,33] reported that triglyceride, cholesterol and HDL- cholesterol concentrations are decreased in the cows with fatty liver. Sevinc et al.[41] found that serum triglyceride and cholesterol concentrations were decreased in the cows with moderate and severe fatty liver. In the present study, serum cholesterol level was decreased in cows with moderate hepatic lipidosis and in cows with severe hepatic lipidosis as compared with in the controls, but triglyceride level in cows with moderate and severe hepatic lipidosis was not different from healthy cows. The low cholesterol level can be thought to be caused by a fat infiltration in the liver and a low output of lipoprotein.

Madison and Trout [42] found that calcium when present at a level of <1.2 mmol/L had a reducing effect on abomasal motility. They also brought attention to the fact that hypocalcemia cannot be a major causative factor for decreased abomasal motility with respect to the development of abomasal displacement. Some authors reported that hypocalcemia is a risk factor for abomasal displacement [43-45]. Metabolic alkalosis is mentioned as a risk factor for abomasal displacement [44]. The fluid accumulating in the distended abomasum indicates continuous secretion of hydrochloric acid. This sequestration of the chloride ion in the abomasum along with some abomasal reflux into the rumen results in metabolic alkalosis [46]. During the dilatation phase, which commonly lasts for several days, there is continuous secretion of hydrochloric acid, sodium chloride, and potassium into the abomasum; thus, the abomasum becomes gradually distended and does not evacuate its contents into the duodenum. This leads to dehydration and metabolic alkalosis with hypochloremia and hypokalemia [47]. In this study, Ca^{++}, Cl^{-}, Na^{+}, and K^{+} levels were decreased in cows with moderate hepatic lipidosis and in cows with severe hepatic lipidosis as compared with in the controls. Decrease in the Ca^{++}, Cl^{-}, Na^{+}, and K^{+} concentrations may be related to metabolic alkalosis resulting from abomasal displacement.

Nutritional stress in dairy cattle can be evaluated by determining the serum NEFA, BHBA, acetoacetate, cholesterol, and glucose and liver fat percentages, with the latter being regarded as the most accurate indicator of nutritional stress [15,16,48,49]. Liver fat percentage is increased in cows with abomasal displacement [50-52] and ketosis [48]. The liver fat percentage in healthy cattle is typically 5%, and is increased to 8% in healthy cows shortly after calving and to 33% in cows with postparturient ketosis [48]. In the present study, cows with abomasal displacement and ketosis had moderate (10% - 20%) and severe (20% - 48%) hepatic lipidosis according to histopathologic evaluation. Increase in serum AST activities is consistently related to fatty liver [41,53].

Ultrasonography has been used routinely for a long time as a diagnostic procedure in hepatic disease of cows [22]. Ultrasound imaging followed by digital analysis of sonograms has the potential to non-invasively detect fatty liver and estimate liver triacylglycerol content [23,24]. Braun [22] reported the ultrasonographic features of fatty livers as an

increase in the size of the liver, round margins, hyperechoic hepatic parenchyma near the abdominal wall, and decrease in the strength of the echo with increase in the distance from the abdominal wall, and poor visualization of hepatic blood vessels. In this study, increase in the diffuse echogenicity of the liver was observed in cows with severe hepatic lipidosis *(Fig. 1)* and cows with moderate hepatic lipidosis *(Fig. 2)*, but the increase in the latter was little. There was a good relationship between the ultrasonographic image findings and 20% and over fatty liver. Large vessels of the liver in cows with moderate or severe hepatic lipidosis were observed, but the small vessels of the liver were poorly imaged or not seen in cows with severe hepatic lipidosis *(Fig. 1)* and only faintly seen or poorly imaged in cows with moderate hepatic lipidosis *(Fig. 2)*. The poor images of the small vessels may be attributable to the deposition of fat on the surface of vessels and the swelling of hepatocytes due to fat deposition in the cells. Otherwise, the cause of increase in diffuse echogenicity of the liver may be the diffuse fat deposition in the cells. Mohamed et al.[54] reported that ultrasonography may provide good images of focal fatty infiltration of the liver. There was also a significant relationship between serum liver-specific enzymes (SDH, GDH, and AST), histopathologic findings, and ultrasonographic imaging findings of the liver.

The results of our study showed that B-mode ultrasonography is a valuable tool in the diagnosis of hepatic lipidosis. Both serum GDH and SDH levels were found to be increased in severe hepatic lipidosis. However, only the serum GDH level was elevated in moderate hepatic lipidosis. Therefore, both ultrasonographic examination and measurement of specific liver enzymes (SDH and GDH) seem to be beneficial in the diagnosis of hepatic lipidosis.

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RESEARCH ARTICLE

Inhibition of Corneal Neovascularization by Subconjunctival Injection of Ranibizumab and Bevacizumab in Rabbit Cornea

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Summary

The aim of this study was to evaluate the effects of subconjunctival ranibizumab and bevacizumab injection on angiogenesis in the rabbit cornea. The corneas of 24 New Zealand rabbits were cauterized with silver nitrate to induce neovascularization. The eyes were irrigated with 10 ml of 0.9% saline solution. The alkaline burns were similar in all the rabbits. At the 24 h after cauterization, the rabbits were divided into three groups of eight animals each: The first group (GC) received 0.02 ml 0.9% saline solution as a control group whereas second (GR) and third (GB) groups received 0.5 mg ranibizumab and 1.25 mg bevacizumab by subconjunctival injection, respectively, on days first and 7 after lesion. The rabbits' corneas were extracted on the 14th day. Digital photographs of the corneas were obtained and the newly formed vessels were analyzed in a computerized system (google sketch-up program). The rates of these vessels were compared between the groups. Ranibizumab and bevacizumab were both effective on inhibition of angiogenesis, in comparison to 0.9% saline solution ($P<0.05$). Ranibizumab was found to be statistically more effective to reduce corneal neovascularization than bevacizumab ($P<0.05$). Bevacizumab and ranibizumab were found to be effective in inhibiting the corneal neovascularization in the rabbit cornea. Ranibizumab seemed more effective than bevacizumab on inhibiting corneal neovascularization in the rabbit cornea.

Keywords: *Ranibizumab, Bevacizumab, Rabbit cornea, Corneal neovascularization, Angiogenesis*

Korneal Neovaskularizasyonun Subkonjonktival Ranibizumab ve Bevacizumab Enjeksiyonu ile Tavşan Korneasında İnhibisyonu

Özet

Bu çalışmanın amacı; subkonjunktival olarak uygulanan ranibizumab and bevacizumab'ın tavşan korneasında anjiyogenezis üzerine etkilerini değerlendirmekti. Yirmi dört adet Yeni Zelanda tavşanının korneaları neovaskülarizasyon oluşturmak için gümüş nitrat ile koterize edildi. Gözler 10 ml %0.9 salin solusyonu ile yıkandı. Tüm gözlerde eşit miktarda kimyasal yanık oluşturulmasına dikkat edildi. Koterizasyon sonrası 24. saatte tavşanlar üç gruba ayrıldı: Kontrol grubuna (GC) (n=8) subkonjunktival 0.02 ml %0.9'luk salin solüsyonu; Grub Ranibizumab'a (GR) (n=8) subkonjunktival olarak 0.5 mg ranibizumab ve Grup Bevasizumab'a (GB) (n=8) ise subkonjunktival olarak 1.25 mg bevacizumab 1. ve 7. günlerde uygulandı Topical antibiotik tedavisi sonrası, tavşan korneaları 14. günde çıkarıldı. Korneaların digital fotoğrafları alınarak yeni oluşmuş damarlar bilgisayar programı (google sketch-up programı) yardımıyla analiz edildi. Yeni oluşan damar yapılarının alanının kornea alanına oranları gruplar arasında değerlendirildi. Ranibizumab ve Bevasizumab'ın her ikisi de anjiyogenez inhibisyonunda %0.9 salin solüsyonuna kıyasla ($P<0.05$) daha etkili olduğu saptandı.Ranibizumab'ın korneal neovaskülarizasyon azaltıcı etkisi Bevasizumab'tan istatistiksel olarak anlamlı ($P<0.05$) derecede fazla bulundu. Bevasizumab ve Ranibizumab'ın tavşan korneasında neovaskularizasyonu inhibe etmekte etkili olduğu tespit edildi. Ranibizumabın tavşan korneasında neovaskülarizasyonu inhibe edici etkisinin Bevasizumab'tan daha fazla olduğu saptandı.

Anahtar sözcükler: *Ranibizumab, Bevasizumab, Tavşan korneası, Korneal neovaskularizasyon, Anjiyogenez*

INTRODUCTION

Corneal avascularity is an essential element of corneal transparency and optimal vision [1]. The cornea has the unique feature of being normally avascular, but under pathologic conditions vessels invade the cornea from the limbal vascular plexus. A wide variety of insults including infection, inflammation, ischemia, degeneration, trauma, and loss

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of limbal cell barrier can cause corneal neovascularization (CNV) [2].

Corneal neovascularization is under the control of local, pro- and anti-angiogenic factors [3]. The natural balance of these factors maintains corneal avascularity. The overall process of angiogenesis involves the degradation of the extracellular matrix and the vascular basement membrane by matrix metalloproteinases (MMP), allowing endothelial cells to invade and form vessels [4]. Under inflammatory conditions, the invasion of endothelial cells into the cornea is largely stimulated by the actions of macrophages which enhance inflammation through the recruitment of additional macrophages while also producing pro-angiogenic factors [5]. The most significant role of macrophages in CNV is their secretion of Vascular Endothelial Growth Factor (VEGF) [6]. VEGF factor plays a key role in angiogenesis in the human cornea.

Comprised of 5 isoforms, VEGF promotes several steps within normal vascular growth including the induction of angiogenesis, endothelial cell proliferation, enhanced inflammatory response, proteolytic activities and increased vascular permeability [2]. Several cellular components within the human cornea have been found to excrete VEGF when under duress or inflammation, including corneal endothelial and epithelial cells, fibroblasts, macrophages, and limbal vascular endothelial cells [7]. VEGF antagonists disrupt these pathways, thus preventing and regressing corneal neovascularization. Blockage of VEGF with bevacizumab and ranibizumab has been a successful treatment in decreasing visual morbidity associated with abnormal vascular conditions of the choroid and retina [8].

More recently, topical anti-VEGF agents have been used to treat abnormal vascular conditions of the cornea. Vascularization from conditions such as chemical injury, Stevens-Johnson Syndrome, ocular cicatricial pemphigoid, interstitial keratitis, post infectious keratitis, and corneal graft failure has been shown to regress with the use of topical and subconjunctival bevacizumab [9-12]. It is presented that subconjunctival bevacizumab treatment to chemical burns of rat corneas decreased inflammatory cell infiltration and cytokines [13].

The aim of this study was to evaluate the effects of subconjunctival injection of ranibizumab 0.5 mg and bevacizumab 1.25 mg on angiogenesis in the rabbit cornea that was chemical injury by cauterized with silver nitrate crystal.

MATERIAL and METHODS

Animals

A total of 24, ten-twelve month-old, New Zealand white albino male rabbits (average weight: 3-3.5 kg) supplied by the Experimental Animals Unit at Kafkas University (Kars, Turkey), were used in the study.The study was carried out in accordance with the Animal Ethical Guidelines for Investigations in Laboratory Animals and was approved by the Kafkas University Animal Care and Use Committee (Approval Number: KAÜ-HADYEK/2011-002). The rabbits were kept under standard conditions (20±1°C, 12 h light/12-h dark cycles) and were fed 160 g pelleted rabbit diet (Ankara Feedstuff Industry, Ankara, Turkey) daily and water was available *ad libitum*. After alkaline burns formed, they were randomly divided into three groups of eight animals each: control group (GC), bevacizumab group (GB) and ranibizumab group (GR), respectively. A complete blood count was performed for each rabbit on days 0, 7, and at the end of the study.

Anesthesia Procedure

The animals were withheld food 12 h prior to operation. General anesthesia was performed with combination of xylazine HCl (Rompun® vial, 23.32 mg/ml, Bayer Turkish Chemistry Industry, Istanbul, Turkey) (5 mg/kg, IM) and ketamine HCl (Ketalar® vial, 50 mg/ml, Pfizer, Istanbul, Turkey) (50 mg/kg, IM) intramuscularly. Before corneal burns were achieved, propacaine HCl 0.5% (Alcaine®, Alcon) as a topical anesthetic eye drop was also applied to form a more analgesic effect as defined by Mahoney and Waterbury [14].

Creating the Alkaline Burn Model

Alkaline burn was achieved in the right eye by contacting the silver nitrate applicator sticks (75% silver nitrate, 25% potassium nitrate) (*Flexible Caustic Applicator 6", Bray Group Ltd., UK*) to all corneal surface for 2 min. Thus, alkali-induced corneal neovascularization model was performed as decscribed by Mahoney and Waterbury [14] with some modifications. Excess silver nitrate was removed by rinsing the eyes with approximately 10 ml of a 0.9% saline solution. A single investigator (M.E.) cauterised all animals for similarity of process model *(Fig. 1)*.

Treatment Protocol

On the first and seventh days after the formation of alkaline corneal burn, subconjunctival anti-VEGF agent ranibizumab 0.5 mg (0.05 ml) (Lucentis®, Genentech/Novartis, South San Francisco, California, USA) and bevacizumab 1.25 mg (0.05 ml) (Avastin®; Genentech/Novartis, South San Francisco, California, USA) were injected to the righ eye of rabbits in group GR and GB, respectively. Group GC (n=8) received a subconjunctival injection of 0.05 ml of 0.9% saline solution. After the formation of alkaline burn, tobramycin topical antibiotic ointment once a day and tobramycin eye drop four times a day (Tobrased®, Bilim İlaç, Istanbul-TURKEY) were used for 14 days in all groups.

Evaluation of Corneal Neovascularization

On the 14th day of the study, following the clinical

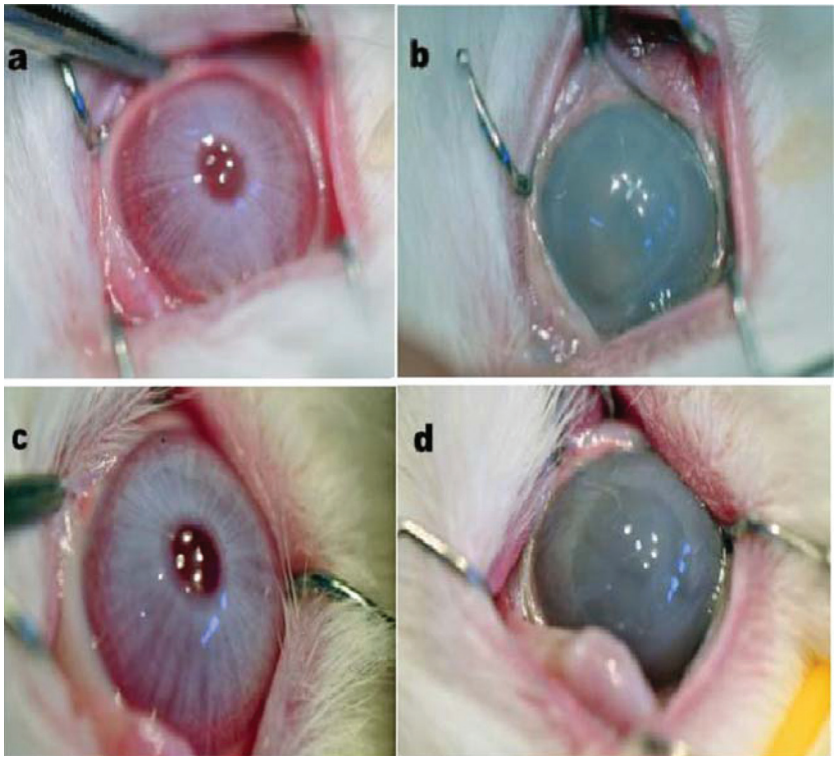

Fig 1. The cornea samples before and after cauterization **a,c-** Normal corneas, **b,d-** Corneas after alkali burn was performed

Şekil 1. Korneaların koterizasyondan önce ve sonrasında görünümü **a,c-** Normal kornealar, **b,d-** Alkali yanık oluşturulduktan sonraki kornealar

examination, the rabbit corneas were totally extracted with 360 degree incision under general anesthesia, as described above. After then, eviseratio bulbi was done and conjunctival tissue was stitched with 6/0 polyglactin 910 suture (*Vicryl*® Ethicon Inc. UK) and antibiotic ointment was applied for 5 days in all animals. The surgery was performed by the same surgeon. The animals in all the study groups were delivered to the Laboratory Animal Resource Center, Kafkas University (Kars, Turkey) to be used for other experimental studies. The corneas in each group were digitally photographed for the status of corneal neo vascularization under standard conditions by Nikon D90 SLR camera, with camera lens distance of 85 mm, shooting distance of 29 cm, digital zoom rate of 100%, and with artificial light source. Then, corneas samples were passed all of fixed procedure and sections (thickness 5 μm) stained with Hematoxylen & Eosin for histological studies. H&E stain slides evaluated and photographed under ligth microscope (Olympus BX-51). The ratio of the neovascularization zone to all corneal area was calculated via Google sketch-up program in all groups *(Fig. 2)*. The corneas were placed in concordance to their anatomical curvatures on a round shaped light source *(Fig. 2A)*. Total corneal areas were measured *(Fig. 2B)*. The neovascularized areas were marked with the Google sketch-up program *(Fig. 2C)*. The total neovascularization areas were calculated and the ratio of the neovascularized areas to total corneal areas were obtained.

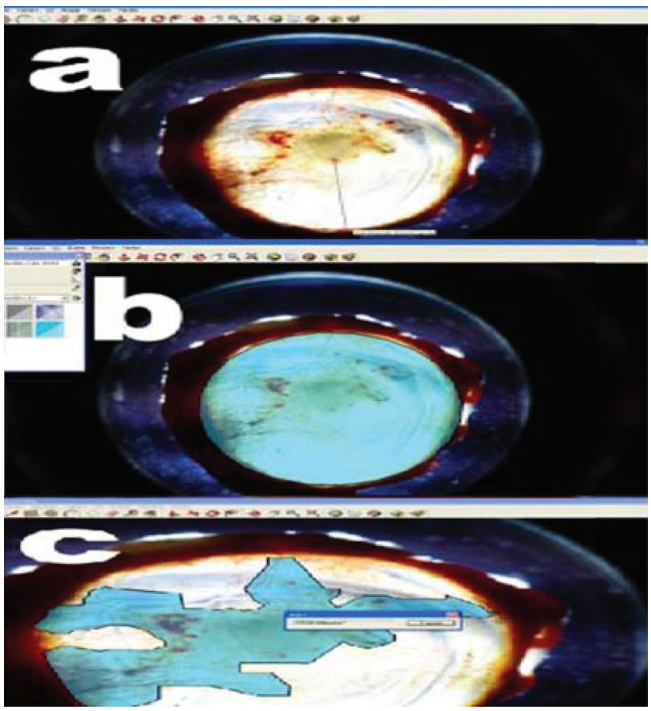

Fig 2. The application scheme of the Google SketchUp program **a-** The photograph of the cornea on the round shaped light source, **b-** The measurement of the total corneal area, **c-** The calculation of the total neovascularization area with the Google SketchUp program

Şekil 2. Google SketchUp programının uygulanış şeması **a-** oval aydınlatılmış yüzeye konmuş korneanın görünümü, **b-** Total korneal alanın ölçülmesi, **c-** Google SketchUp programı ile total neovaskularizasyon alanının ölçülmesi

Statistical Analysis

After assessing the normality in groups by using One Sample Kolmogorov Smirnov test, comparisons between groups were completed using a non-parametric Kruskal-Wallis Test for continuous data. $P<0.05$ was considered statistically significant.

RESULTS

Corneal neovascularization that induced by chemical cauterization with silver nitrate was supported with the histological findings such as the presence of intense inflammatory cells (neutrophil leukocyte and eosinophil leukocyte) and newly formed blood vessels *(Fig. 3)*.

The digital photographs of the corneas which were taken on the 14th day after treatment for all studygroups were presented in *Fig. 5*. When the corneal neovascularization rates were assessed by using Google sketch-up program, the mean rate of control group was found as 69.54±16.48% (40.83-92.00), 31.56±3.06% (25.41-35.25) in the ranibizumab group and 42.95±5.94% (34.02-51.29) in the bevacizumab group *(Table 1)*.

The percentage of CNV in ranibizumab (GR) and bevacizumab (GB) groups were statistically significant difference lower than the control group (GC). On the other hand, ranibizumab was found to be statistically more effective to reduce CNV than bevacizumab ($P<0.05$).

No adverse effects such as corneal melting, descemetocele, or corneal perforation were observed in external ophthalmic examination in all study groups.

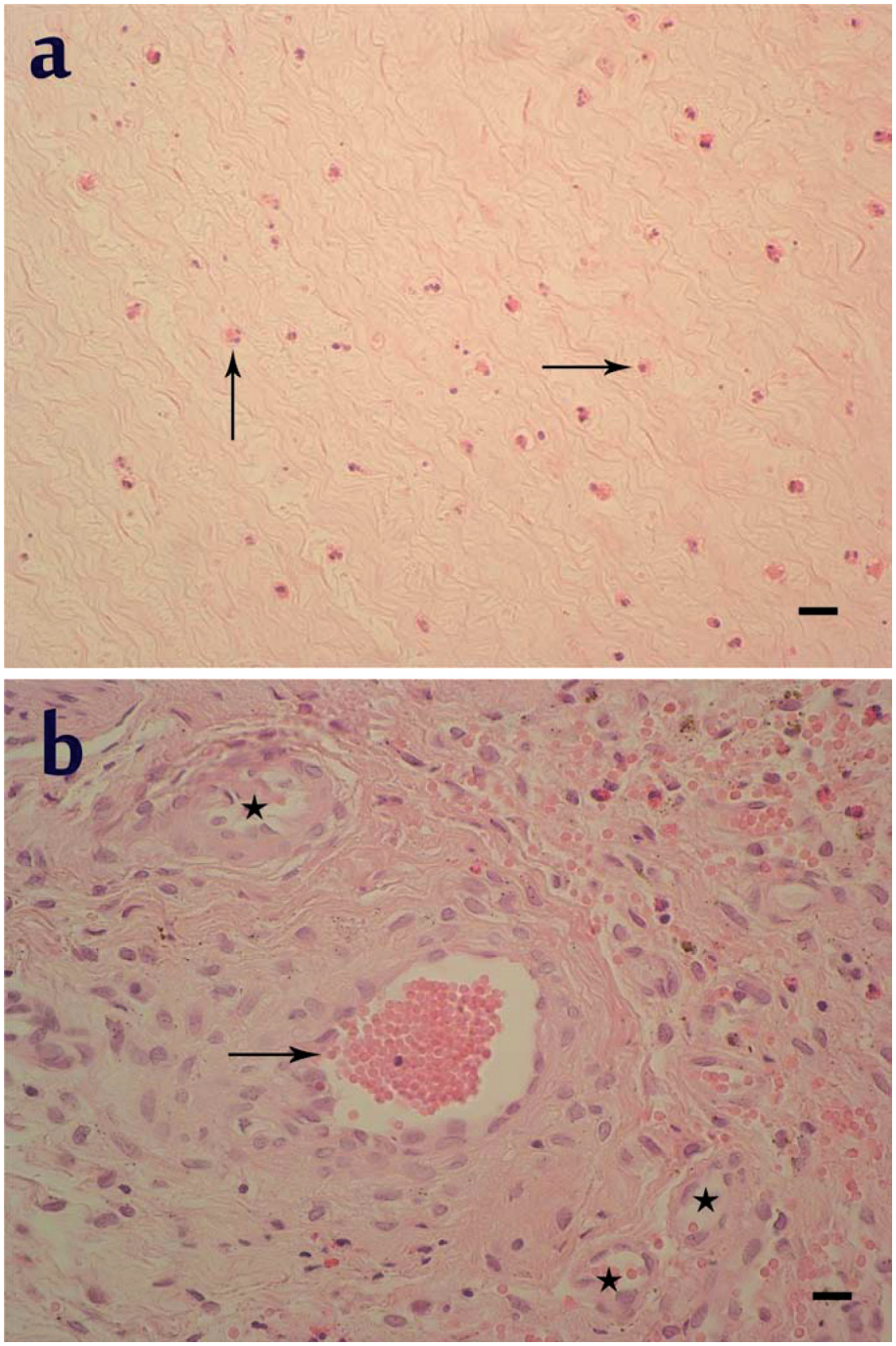

Fig 3. Histological sections of the neo vascularized rabbit corneas **a-** Neutrophil leukocytes *(vertical arrow)* and eosinophil leukocytes *(horizontal arrow)*, **b-** Newly formed vein *(arrow)* and arteries *(stars)*, H&E, Bar=50 μm

Şekil 3. Neovaskularize tavşan korneasından histolojik kesitler **a-** Nötrofil lökositler *(dikey ok)* ve eosinofil lökositler *(yatay ok)*, **b-** Yeni oluşmuş ven *(ok)* ve arterler *(yıldız)*, H&E, Bar=50 μm

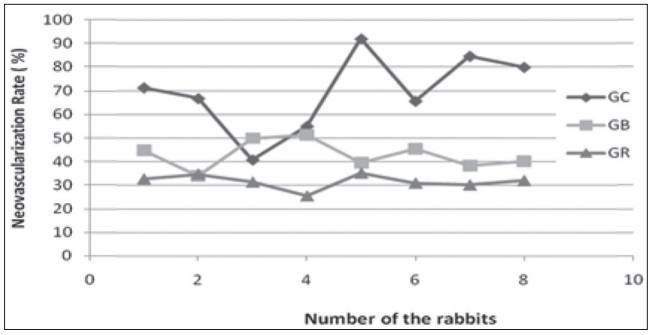

Fig 4. The corneal neovascularization rates in the study groups **GB-** Bevacizumab Group, **GR:** Ranibizumab Group, **GC-** Control Group

Şekil 4. Çalışma gruplarında korneal neovaskularizasyon oranları **GB-** Bevacizumab Grup, **GR:** Ranibizumab Grup, **GC-** Control Grup

and bevacizumab in corneal neovascularization induced by alkali burn. Although we found that both agents were effective on reducing CNV, ranibizumab was found to be more effective than bevacizumab ($P<0.05$). Recently, both anti-VEGF agents are commonly used for retinal vascular disorders and macular diseases, such as macular neovascular degeneration, diabetic retinopathies, retinal neovascularization due to retinal vascular disorders, retino-pathy of prematurity, and neovascular glaucoma [15]. Even though there are many reports involving the effect of bevacizumab on inhibiting angiogenesis of the anterior segment of the eye in the literatüre [16-21], studies about the use of ranibizumab for the same purpose are rare [22,23].

Table 1. *The corneal neovascularization rates in the study groups*
Tablo 1. *Çalışma gruplarındaki korneal neovaskularizasyon oranları*

Groups	Number of Eye	Neovascularization Rate (%)		
		Minimum	Maximum	Average±Standard Deviation
GB	8	34.02	51.29	42.95±5.94
GR	8	25.41	35.25	31.56±3.06
GC	8	40.83	92.00	69.54±16.48

GB: *Bevacizumab Group,* ***GR:*** *Ranibizumab Group,* ***GC:*** *Control Group*

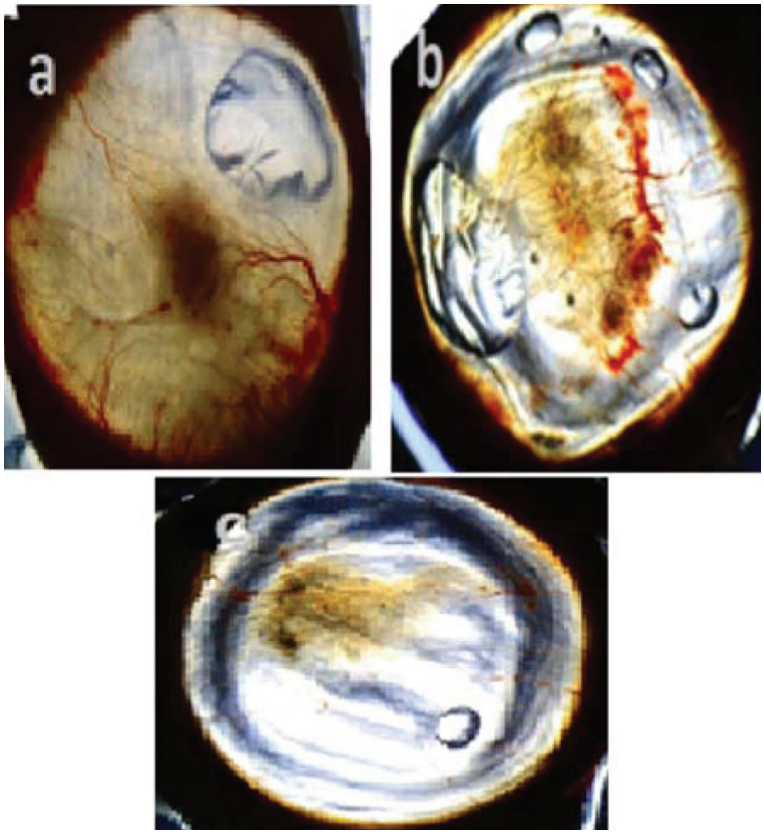

Fig 5. The digital photographs of the rabbit corneas taken on the 14th day of the experiment **a-** Control Group, **b-** Bevacizumab Group, **c-** Ranibizumab Group

Şekil 5. Tavşan korneasından çalışmanın 14. gününde alınmış dijital fotoğraflar **a-** Kontrol Grup, **b-** Bevacizumab Grup, **c-** Ranibizumab Grup

DISCUSSION

This experimental study was designed to evaluate and compare the effects of anti-VEGF agents, ranibizumab

As the optimal vision can only be achieved with an avascular and transparent cornea, to totally prevention or minimizing is an essential step in corneal neovascularization. Corneal avascularity requires low levels of angiogenic factors

and the high levels of anti-angiogenic factors. The angiogenic factors include basic and acidic fibroblast growth factor (FGF), VEGF, angiogenin, transforming growth factor, interferon, tumor necrosis factor-α and platelet derived growth factor [24]. Anti-angiogenetic factors include interferon-α, thrombospondin-1, angiostatin, endostatin, and pigment epithelium-derived factor. The imbalance between the angiogenic and anti-angiogenic factors lead to corneal neovascularization and scar formation. Steroids, methotrexate, heparin, thalidomide, artepillin, C-caffeic acid phenetylester (CAPE), and anti-VEGF agents are proposed as inhibitors of CNV [24].

Bevacizumab is a full-length, recombinant humanized monoclonal immunoglobulin G1 (IgG1) that binds to and inhibits the activity of VEGF-A, thereby inhibiting angiogenesis [8]. A related compound, Ranibizumab (Lucentis), is a high affinity recombinant monoclonal antibody derived from the same parent murine antibody as bevacizumab and also neutralizes all isoforms of VEGF-A [6].

It has been reported that; no retinal damage was observed and the histopathologic studies yielded similar after repeated intravitreal injections of bevacizumab and ranibizumab in rabbit eyes [25]. Ranibizumab has a high, but 40-fold reduced, affinity towards rabbit VEGF in comparison with human VEGF. But bevacizumab binds rabbit VEGF with a 7.27-fold lower affinity than for human VEGF [26,27].

There are main differences between these 2 anti-VEGF agents. First difference is their molecular weights; ranibizumab is a 48-39-kDa Fab fragment, whereas bevacizumab is a complete 149-kDa antibody [28]. As second difference, ranibizumab is not glycosylated, resulting in a 140-fold higher binding affinity of a single site compared to bevacizumab [15,29].

Other differences are the development in different cell lines: bevacizumab is developed in Chinese hamster ovary mammalian cell expression system; while ranibizumab is produced by an *Escherichia coli* bacterial expression system; and the formulation for intraocular use: ranibizumab was formulated for intraocular use, while bevacizumab was formulated for intravenous use [30]. In addition, the pharmacokinetics of these 2 agents are different: the vitreous half-life of 1.25 mg intravitreal bevacizumab is 4.32 days, while ranibizumab (0.5 mg) has a vitreous half-life of 2.9 days with minimal systemic exposure in a rabbit eye [31,32].

In another study Christoforidis et al. were defined that there was no significant escape of bevacizumab and ranibizumab from the vitreous cavity after intravitreal injection [33]. This period in primates is 6.9 days for bevacizumab and 3.5 days for ranibizumab. Peak serum concentration after intravitreal injection is 3000 ng/ml in bevacizumab and 0.3 ng/ml in ranibizumab. Systemic intravenous bevacizumab treatment in primates leads to a half-life of 21 days. On the other hand, half-life of ranibizumab applied in the same way is less than 1 day [34]. To achieve detectable accumulation of bevacizumab in the vitreous of the injected rabbit, the dosing interval should be shorter than four half-lives [27].

This fact leads us to set the dosage regimen subconjunctivally on days 1 and 7 of the study to minimize the effect of the difference in pharmacokinetics between these 2 agents.

It has been demonstrated that, at clinical doses, ranibizumab and bevacizumab are equally potent in neutralizing VEGF in a porcine retina-retina pigment epithelium-choroid organ culture and retina pigment epithelium cell culture[35]. Another in vitro study showed that bevacizumab and ranibizumab targeted several of the steps required during the angiogenic process, namely endothelial cell proliferation, migration and assembly into capillary-like structures [36]. Akova et al.[37] (escrs.org/vienna2011/programme/poster presentation) studied to determine and compare the effects of subconjunctival ranibizumab, bevacizumab and pegaptanib injections on CNV in a rat model. Bevacizumab showed the highest inhibitory effect on corneal neovascular vessels between three anti-angiogenic agents. The anti-neovascular effect of pegaptanib was higher than ranibizumab. It was observed that ranibizumab was effective in the inhibition of corneal NV seconder to alkali burn. Chan et al.[38] were observed that in different corneal NV rabbit models; subconjunctival injection of bevacizumab was effective in inhibiting corneal NV in several rabbit models. Also in their study Sener et al.[39] observed that bevacizumab, ranibizumab, pegaptanib, and trastuzumab were found effective for the inhibition of corneal NV. In this study, it was determined that the most effective agent was bevacizumab. Stevenson et al.[40] emphasized that ranibizumab and bevacizumab are safe and effective treatments for corneal NV and their results suggest that ranibizumab may be modestly superior to bevacizumab in terms of both onset of action and degree of efficacy. In contrast to Stevenson's study Dursun et al.[41] observed that both subconjunctival bevacizumab and ranibizumab treatments may be effective methods in reducing corneal NV; furthermore, bevacizumab is more effective than ranibizumab in the inhibition of corneal NV. In their study Christoforidis et al.[42] were defined that there was no statictically significant difference in terms of effect on peripheral wound healing between intravitreal injected ranibizumab and bevacizumab.

These studies indicate that bevacizumab is more effective to inhibit CNV than ranibizumab. In contrary to previously reported results, we have noted that ranibizumab was more effective. This fact was related to the repeat injection frequency and the non- glycosilated molecular structure, which made it 140 times more specific than bevacizumab. Early studies on bevacizumab explained that bevacizumab was not capable of neutralizing mouse and rat VEGF-A. Lu et al.[43] studied efficacy and reliability of intravitreal injection of bevacizumab, ranibizumab, and

pegaptanib in choroidal NV treatment in a rat model and they indicated that these three anti-VEGF agents had no efficacy in stopping leakages in choroidal neovascularization. However, efficacy of these agents for choroidal neovascularization in humans has been demonstrated [44]. Based on these results, we think that the affinity of anti-VEGF agents for VEGF-A in muridae family may really below. This situation may explain the difference between the earlier studies' results and our results.

In conclusion, bevacizumab and ranibizumab were found effective for the inhibition of corneal neovascularization, whereas ranibizumab was found more effective to reduce the percentage of corneal neovascularization than bevacizumab. As ranibizumab was produced specifically for human ophthalmic use, we think that further studies are needed to detect the minimal effective dose, injection repeat frequencies, and the way of the drug administration for ranibizumab to inhibit corneal neovascularization.

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RESEARCH ARTICLE

Clinical, Radiological and Computed Tomographic Evaluations of the Effect of Triple Pelvic Osteotomy for Treatment of Canine Hip Dysplasia [1] [2] [3]

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Summary

Triple pelvic osteotomy (TPO) is one of the main surgical procedures to prevent or modify the progress of degenerative joint disease (DJD) related with hip dysplasia in young dogs. The aim of this study was to evaluate the effects of TPO in dogs. In this study, TPO were performed in 7 dysplastic cross-breed dogs in different gender (6 female and 1 male) and body weight (13.42±2.29 kg). In all cases, 20 degrees canine TPO plates were used. Before the operation, all cases were examined clinical, radiological and by computed tomographic. Postoperative 2nd and 6th weeks dogs were examined clinical, radiological and by computed tomographic and after 6 month both clinical and radiological examinations were performed. The joint laxity decreased 2 weeks after the operation. Dogs could walk on the treated leg after 2 weeks. The joint laxity decreased 2 weeks after the operation. Postoperatively dogs could walked on the treated leg after 2 weeks. The postoperatively measured Norberg angles ($P<0.01$) and acetabular anteversion angle ($P<0.05$) were significantly higher and distance between caput femoris and acetabulum was significantly lower ($P<0.05$) than preoperatively measurements. So, contact surface between the femoral head and the acetabulum had increased and the subluxation had disappeared. In radiographs obtained 6 months later, degenerative joint disease was not encountered. It was decided that canine hip dysplasia, could be treated successfully in young dogs via TPO, carried out before degenerative changes begin within the hip joints.

Keywords: *Hip dysplasia, Triple pelvic osteotomy, Radiological, Computed tomography, Dog*

Köpeklerde Kalça Displazisi'nin Sağaltımında Triple Pelvik Osteotomi Etkilerinin Klinik, Radyolojik ve Bilgisayarlı Tomografi ile Değerlendirilmesi

Özet

Triple pelvik osteotomi (TPO) genç köpeklerde kalça displazi ile ilgili dejeneratif eklem hastalığı (DJD)'nın ilerlemesini önlemek ve değiştirmek için uygulanan cerrahi bir işlemdir. Bu çalışmanın amacı, köpeklerde TPO'nun etkilerini araştırmaktır. Bu çalışmada, farklı cinsiyet (6 dişi, 1 erkek) ve vücut ağırlığındaki (13.42±2.29 kg) displazik 7 köpekte TPO yapıldı. Bütün olgularda, 20° canin TPO plağı kullanıldı. Operasyon öncesi tüm olguların klinik, radyolojik ve bilgisayarlı tomografi ile muayeneleri yapıldı. Köpekler operasyon sonrası 2. ve 6. haftalarda klinik, radyolojik, bilgisayarlı tomografik muayene ve 6 ay sonra hem klinik hem de radyolojik muayeneleri yapılarak takip edildi. Operasyondan 2 hafta sonra eklem gevşekliği azaldı. Köpekler operasyon sonrası 2 haftada tedavi edilen bacakları üzerinde yürüyebiliyorlardı. Postoperatif ölçülen Norberg açıları ($P<0.01$) ve asetabular antreversiyon açısı ($P<0.05$) preoperatif değere göre yüksekti ve kaput femoris ve asetabulum arasındaki mesafe belirgin olarak düşük bulundu. Bu nedenle, asetabulum ve kaput femoris arasındaki temas yüzeyi artarak, subluksasyon kayboldu. Altı ay sonra elde edilen radyografilerde, dejeneratif eklem hastalığına rastlanmadı. Kalça displazili genç köpeklerde kalça ekleminde dejeneratif değişiklikler başlamadan yapılan TPO ile başarılı bir şekilde tedavi sağlanabileceği sonucuna varıldı.

Anahtar sözcükler: *Kalça displazisi, Triple pelvik osteotomi, Radyoloji, Bilgisayarlı tomografi, Köpek*

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INTRODUCTION

Canin hip dysplasia (CHD) is an inherited, developmental orthopedic disease of the hip joint, affect the puppies of large and giant breed dogs [1-4]. The dogs are born with normal hip joint. During growth, there may be incoordination between the skeleton and the supporting muscle system [1]. The earliest clinical sign of the disease is hip joint laxity. In the later stages of the disease, clinical symptoms such as swinging, exercise intolerance, atrophy of the muscules of the hind limbs are seen [3,5]. In the treatment of hip dysplasia, conservative or surgical methods are used depending on the age and body weight of the animal, the physical and radiologial finding and the financial status of the patient owner. Many surgical procedures (total hip replacement, excision arthroplasty, triple pelvic osteotomy, juvenil pubic symhysiodesis etc.) for treatment of HD have been reported [6-9]. Triple pelvic osteotomy is frequently used for the treatment of hip dysplasia in immature dogs. The ideal candidates for triple pelvic osteotomy should have minimal to no degenerative osteoarthritis seen on radiographs and positive Ortolani sign [8,10]. The primary objective of this surgery is to improve joint stability by rotating the dorsal rim of the acetabulum laterally, thus providing greater dorsal coverage of the head.

MATERIAL and METHODS

A total of 110 hip joint of 55 cross-breed dogs, of different ages and gender, were examined in this study. Following clinical, radiological and tomographic examinations *(Fig. 1)*, hip dysplasia was diagnosed in a total of 7 dogs (6 females, 1 male) and those which degeneration had not yet begun in the joint, were selected for surgery. General anaesthesia was applied with 1.1 mg/kg xylazine HCl (Alfazine®, 20 mg/ml, Ege-Vet, Izmir, Turkey) and 10 mg/kg ketamine HCl (Alfamine®, 100 mg/ml, Ege-Vet, Izmir, Turkey) intramuscularly after premedication with 0.04 mg/kg atropine sulphate (Atropan®, 2 mg/ml, Vetas, Istanbul, Turkey) subcutaneously. Operation site was shaved and disinfected in a routine manner. For the ostectomy of the pubis, the related leg was held at a position of 90º abduction and a 4-5 cm skin incision was made starting from pubis on the inside of the leg between pectineus muscle and gracilis muscle, perpendicular to the median line. The origin of the pectineal muscle was isolated by dissection and after separation, including the periosteum, of the part attached to the area of pubis to be ostectomized together with abductor magnus muscle and other soft tissue, the pubis was cut and a 1-2 cm long piece of bone was removed from the pubis ramus. For the ischial osteotomy, a 4-6 cm long skin incision was made parallel to tuber ischiadicum, from the lateral aspect to the starting point of arcus ischiadicum. A part of the internal obturator muscle connection to tuber ischiadicum was elevated and the foramen obturatorium was reached. After sufficient space

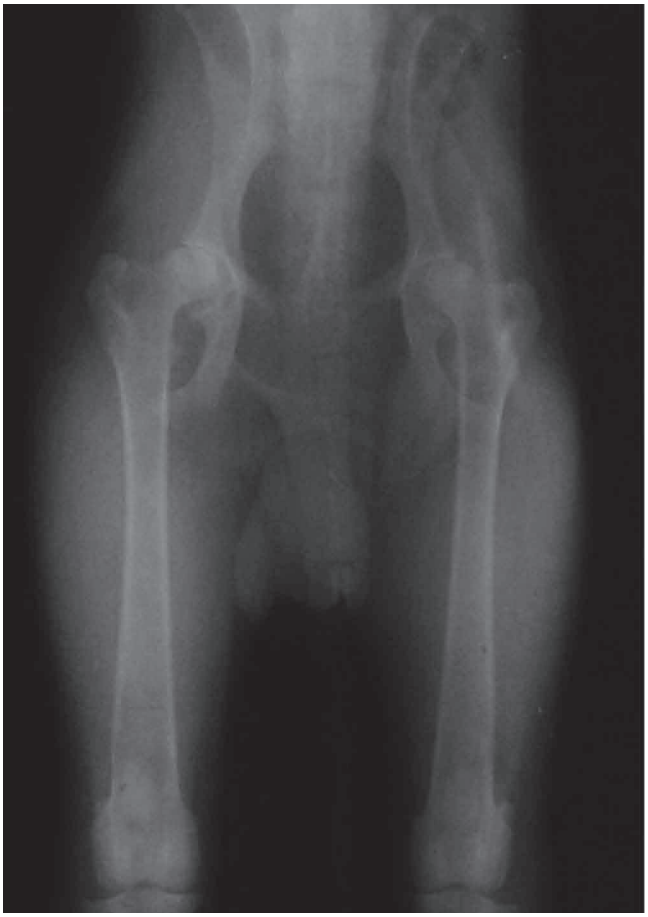

Fig 1. Ventrodorsal radiograph of pelvis before TPO (case 3)
Şekil 1. TPO'dan önce pelvis'in ventrodorsal radyografisi (olgu 3)

was achieved, an osteotomy was done on the ischii from the outside towards the inside, parallel to the longitudinal axis of the pelvis. For the iliac osteotomy and placement of the Canine Osteotomy Plate, a 10-15 cm skin incision was made between the wing of the ilium to the greater trochanter. Subcutaneous adipose tissue and gluteal fascia were dissected, the middle and the deep gluteal muscles were elevated. The body of the ilium was reached. An ilial osteotomy was carried out immediately on the caudal of the sacroiliac joint, protecting isciadic nerve.

After osteotomy, Canine Osteotomy Plates with an angle 20º were placed. Following placement of the plate, ischial osteotomy line was fixed using cerclage wire [6]. The operated areas were surgically closed. Immediately after the operation, symmetrical VD radiographs were taken. All cases were given penicilline G 20.000 IU/BW/day (Iecilline 400.000 İÜ, İ.E. Ulagay® Istanbul) during a week after the operation. Weekly clinical and radiological examinations, and computed tomographic examinations at the 2nd and 6th weeks were performed.

Radiographs examinations were taken in standart ventrodorsal (VD) positions. Degenerative changes on the caput femoris and acetabulum, Norberg angle and implant status

were evaluated. Computed tomographical examinations were taken in ventro-dorsal positions. Acetabular anteversion angle (AAA**)**, the perpendicular distance from the center of the femoral head and midpoint of the line which connecting the cranial and caudal corner of the acetabulum (a), the perpendicular distance from the most lateral point of the femoral head and midpoint of the line which connecting the cranial and caudal corner of the acetabulum (b) were measured from computed tomographic (CT) scans.

Kruskal Wallis Test was used for statistical comparison of data obtained radiological and Computed tomographical examinations performed in preoperative, early and late postoperative periods, while Duncan test was used to determine group/groups causing difference among groups.

RESULTS

TPO were performed in 7 dysplastic cross-breed dogs in different gender (6 female and 1 male) and body weight (13.42±2.29 kg). In preoperative clinical examination, physical findings such as difficulty in walking upstairs, a swinging gait, bunny hopping and laxity in the hip joint were observed. Positive and negative ortolani test were determined in 4 and 3 dogs respectively.

Dogs could able to used treated leg after 2 weeks. The joint laxity decreased 2 weeks after the operation. Postoperative complications were observed such as skin sutures opening in the area of the ischial osteotomy (cases III, VI) and sciatic paralysis (cases II, IV, VII). The wounds presented no further complications after having been reopened, cleaned and sutured again. Sciatic paralysis cases were improved within two weeks in two cases.

Radiographical *(Fig. 2, 3* and *4)* and computed tomography

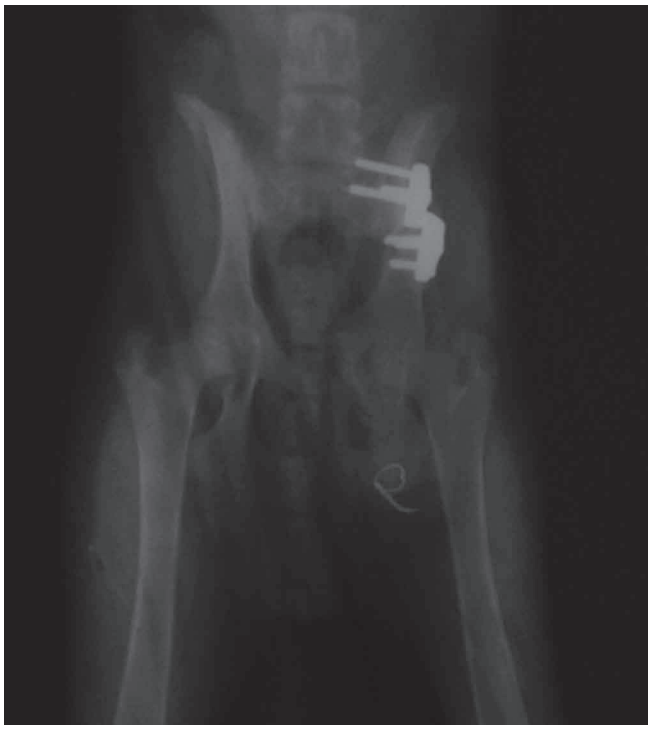

Fig 3. Postoperative 2nd week (case 7)
Şekil 3. Postoperatif 2. hafta (olgu 7)

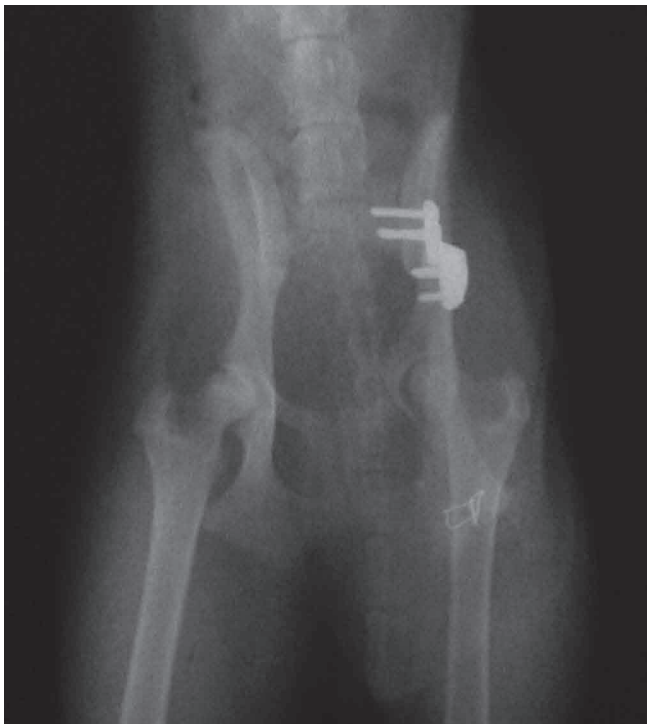

Fig 2. Postoperative 1st day (case 4)
Şekil 2. Postoperatif 1. gün (olgu 4)

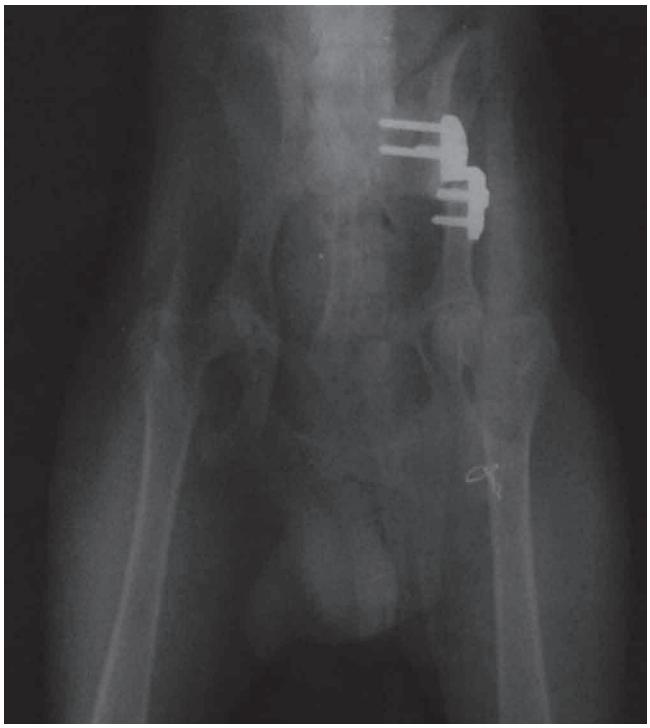

Fig 4. Postoperative 6th week (case 3)
Şekil 4. Postoperatif 6. hafta (olgu 3)

Table 1. *Measurements taken from CT Scans*
Tablo 1. *Bilgisayarlı Tomografi taramalarından elde edilen ölçümler*

Parameters	Preoperative X±sX	Postoperative 2nd week X±sX	Postoperative 6th week X±sX	X²
Norberg Angle (Radiographical)	99.14±0.59[b] (96-100)	120.71±3.85[a] (105-130)	124.29±2.97[a] (110-130)	14.36**
Acetabular Anteversion Angle	27±2.36[b] (19-34)	52.71±6.67[a] (28-70)	54.57±5.72[a] (30-74)	8.83*
Acetabular Coverage(%)	43.74±2.30 (31.93-50.0)	46.18±1.39 (41.56-52.41)	47.69±1.04 (44.05-51.79)	1.99
A (mm)	2.64±0.28[a] (1.60-4.0)	1.97±0.14[b] (1.50-2.70)	1.67±0.23[b] (0.4-2.20)	6.510*
B (mm)	8.95±0.42 (8.0-11.30)	8.54±0.19 (7.90-9.20)	8.40±0.19 (7.80-9.40)	1.078

*** P<0,01 * p<0.05;* ***A (mm):*** *The perpendicular distance from the center of the femoral head and midpoint of the line which connecting the cranial and caudal corner of theacetabulum;* ***B (mm):*** *The perpendicular distance from the most lateral point of the femoral head and midpoint of the line which connecting the cranial and caudal corner of the acetabulum;* ***a,b:*** *There is istatistical difference between groups in the same line with different letters*

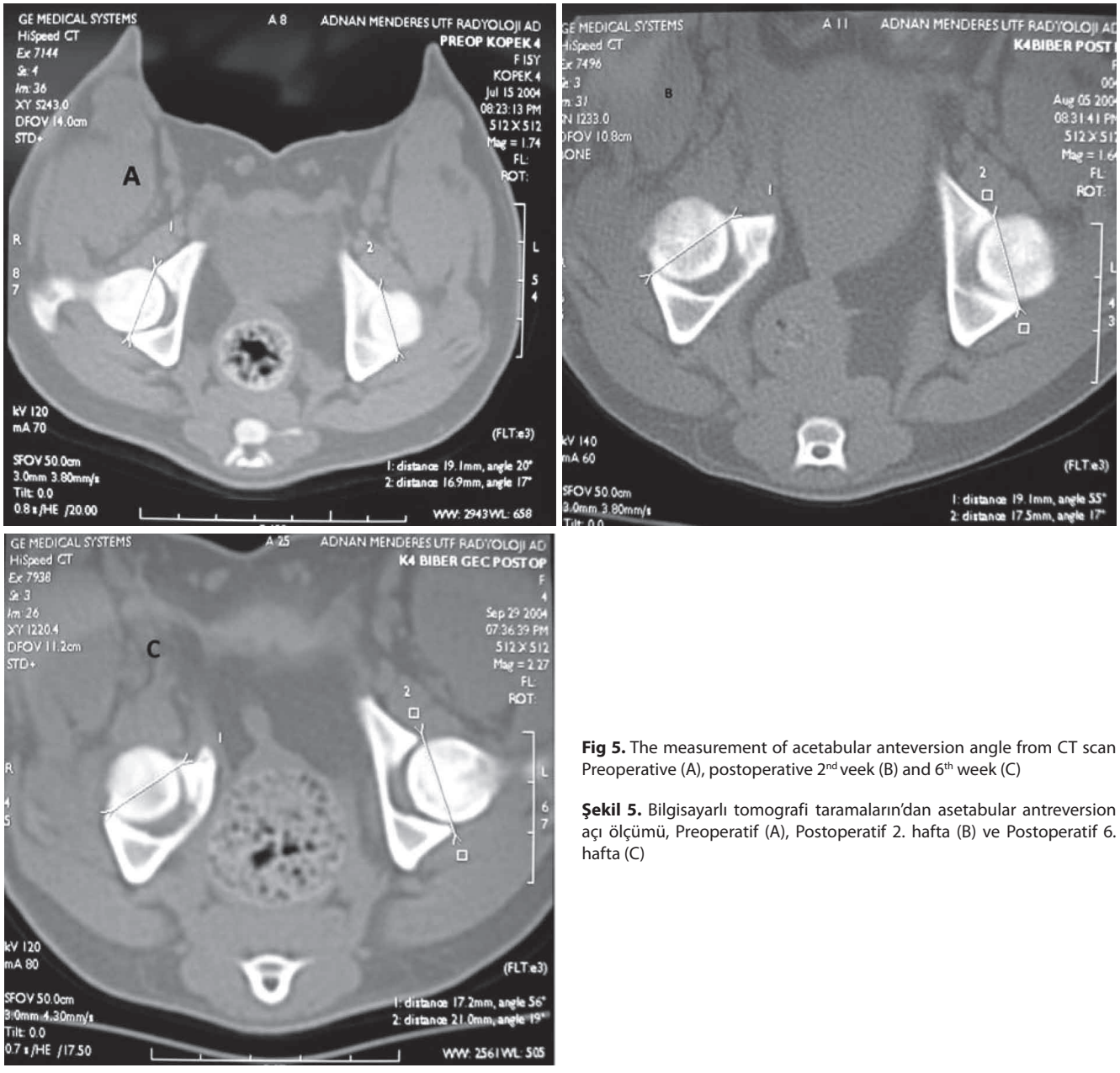

Fig 5. The measurement of acetabular anteversion angle from CT scan Preoperative (A), postoperative 2nd veek (B) and 6th week (C)

Şekil 5. Bilgisayarlı tomografi taramaların'dan asetabular antreversion açı ölçümü, Preoperatif (A), Postoperatif 2. hafta (B) ve Postoperatif 6. hafta (C)

findings *(Fig. 5* and *6)* were shown *Table 1*. The mean Norberg angles were 99.14±0.59 (96-100) preoperative, 120.71±3.85 (105-130), 124.29±2.97 (110-130) postoperative, 2nd and 6th weeks, respectively.

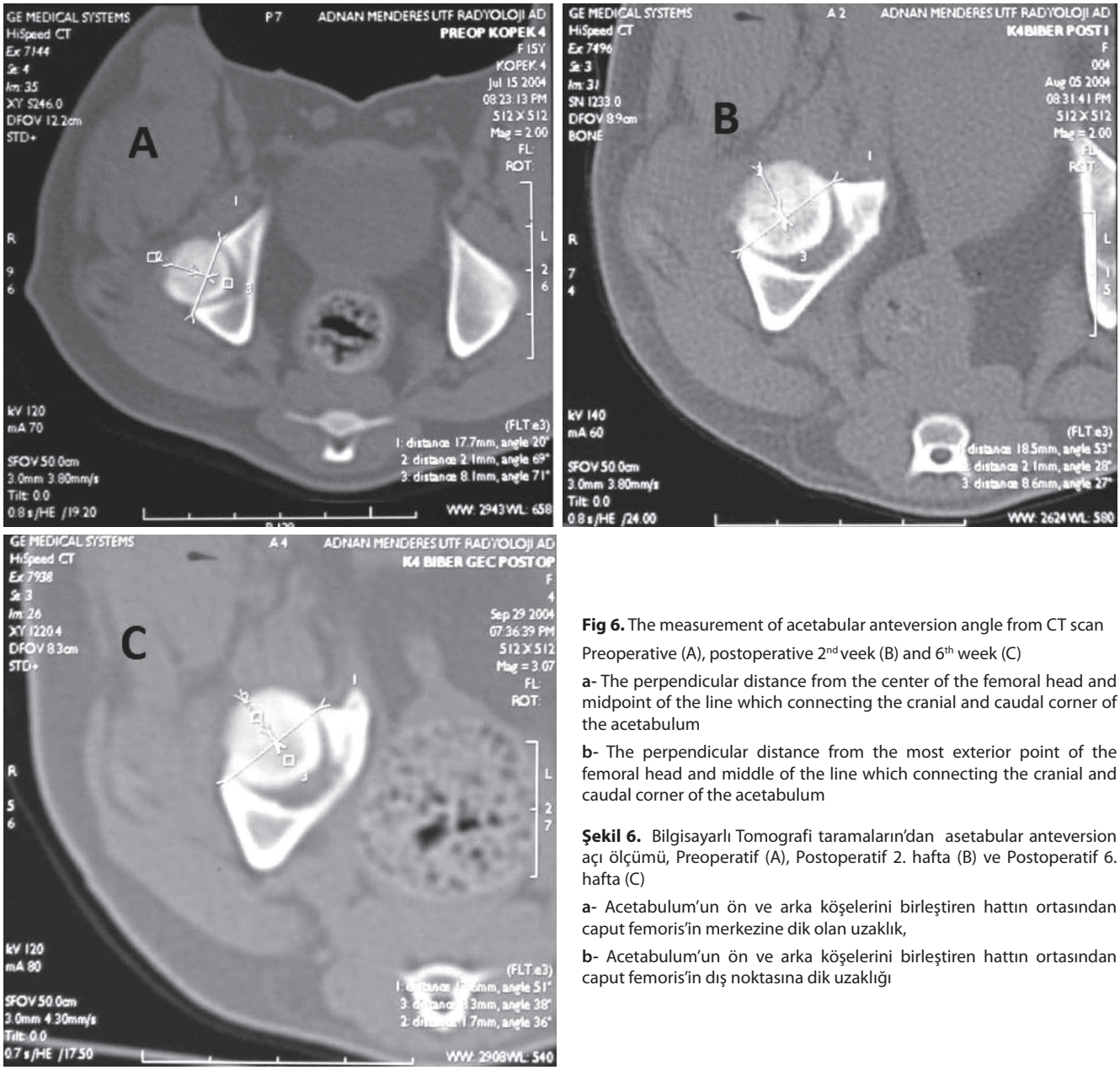

Fig 6. The measurement of acetabular anteversion angle from CT scan

Preoperative (A), postoperative 2nd veek (B) and 6th week (C)

a- The perpendicular distance from the center of the femoral head and midpoint of the line which connecting the cranial and caudal corner of the acetabulum

b- The perpendicular distance from the most exterior point of the femoral head and middle of the line which connecting the cranial and caudal corner of the acetabulum

Şekil 6. Bilgisayarlı Tomografi taramaların'dan asetabular anteversion açı ölçümü, Preoperatif (A), Postoperatif 2. hafta (B) ve Postoperatif 6. hafta (C)

a- Acetabulum'un ön ve arka köşelerini birleştiren hattın ortasından caput femoris'in merkezine dik olan uzaklık,

b- Acetabulum'un ön ve arka köşelerini birleştiren hattın ortasından caput femoris'in dış noktasına dik uzaklığı

The mean percentage of acetabular coverage were 43.74±2.30% (31.93-50.0), 46.18±1.39% (41.56-52.41), 47.69±1.04% (44.05-51.79), in same periods. The post-operatively measured Norberg angles ($P<0.01$) and acetabular anteversion angles ($P<0.05$) were significantly higher and distance between femoral head and acetabulum was significantly lower ($P<0.05$) than preoperatively measurements.

In radiographic examination performed 6 months later postoperatively, DJD was not encountered.

DISCUSSION

While several methods are known for the surgical treatment of hip dysplasia, TPO appears to be increasingly used in the last years [11-14]. In order to obtain desired results with TPO, patient should be young and free from degenerative changes of the hip joint, or at the very least with only a minimal level of such changes [11,12]. All dogs in this study had no clinical and radiological finding related with hip joints at the beginning of the study.

There is no consensus about sex predisposition on CHD. Hara et al.[15] reported an incidence of 25% females 75% in the male dogs. Bakır [16] have been reported that hip dysplasi were 29.56% in males, 39.06% females in dogs. A total of 55 dogs were examined in this study. 85% of the dysplasia dogs were female and 15% were male. These results were reported by Bakır [16]. We considered that it would be inappropriate to formulate an opinion, considering the small number of cases.

Dogs with hip dysplasia are generally described as having trouble walking upstairs, bunny happing, standing up with difficulty and showing a laxity of the hip joint. It is also reported that laxity of the hip joint is established in the case of a positive Ortolani test, while a negative test does not indicate that the hip is healthy because of the occasional false positive and false negative results [11,17,18]. Similar clinical results were found in all dogs in this study. The Ortolani test was positive in nine hips and negative in five. A parallelism between these findings and the Norberg angle was also noted.

Radiological examination is used in the diagnosis of hip dysplasia. In symmetrical ventro-dorsal (VD) radiographs, joints have a Norberg angle over 105 and those with more than 50% of the femoral head inside the acetabulum are considered to be normal [19]. A definite post-operative increase in both the Norberg angle and the femoral head portion that fits in the acetabulum is reported for dogs undergoing TPO surgery [12,20]. In this study, the post-operatively measured Norberg angles ($P<0.01$) and acetabular anteversion angles ($P<0.05$) were significantly higher and distance between caput femoris and acetabulum was significantly lower ($P<0.05$) than pre-operatively measurements. In all cases, both the clinical and the radiological findings showed an improvement when compared to the pre-operative status.

In the light of data on the tomographic appearance of the hip joint in both normal and dysplastic hip [21], dogs with hip dysplasia in this study had been evaluated by computed tomography as well as in the early and late post-operative periods. When basing the evaluation on length measurements, some changes were seen also in the unoperated hip joints. It was thought that this difference may be due to the fact that the at slice sections, which are 3 mm apart, did not correspond to the same points in the different examinations. The distance from the center of the femoral head to the midpoint of the line that joins the anterior and posterior corners of the acetabulum (a) was seen to be statistically smaller than before the operation both at the 2-week and the 6-week follow-up measurements ($P<0.05$). As for the distance from the midpoint of the line between the anterior and posterior corners of the acetabulum (b), there was no significantly change even though there was a certain post-operative decrease. Statistically significant increase ($P<0.01$) was established at both post-operative follow-ups in the acetabular ante-version angle (AAA). These findings lead us to think that the use of angular measures, and even numeric values resulting from the comparison of certain structures within the field of interest which may remain stable, may be subject in the dogs is seen.

Complications reported for TPO surgery include diarrhea, bloody feces, hematuria, incision discharge, scrotal swelling and sciatic nerve paralysis [6,12,22]. Altunatmaz et al.[12] reported that paralysis of sciatic nerve was observed in 3 cases and a collection occurred at the ischii osteotomy In 1 case of 22 cases. The wounds presented no further complications after having been reopened, cleaned and sutured again. As for the sciatic nerve paralysis, two of these cases improved within the week while the third dog (case 7) showed no such improvement.

Borostyankoi et al.[23] report that the majority of their 95 dogs could stand 24 h after undergoing a bilateral TPO; the average length of hospitalization was 7.5 days. In this study, we observed that, owing to the unilateral nature of the interventions, all cases could stand without help 24 hours after surgery. The dogs continued to be observed for a longer period because of the study protocol. The prolonged observation of the animals facilitated post-operative care while allowing early recognition and early treatment of the complications. It was concluded that prolonged observation of patients following this, or similar, interventions.

Following TPO surgery male dogs may experience difficulties in lifting their hind leg to urinate, a situation that may be due to the friction of the femoral head on the dorsal acetabular wall [24], or to excessive tension of the pectineus muscle [12]. Even though it is not possible to conclude with the single male subject in our study, similar problem was observed in this patient during the first two post-operative days; the animal was later able to easily lift its hind leg. This may also have been caused by the operation's site pain.

Following TPO in dogs, the most frequently complications are observed pelvic canal narrowing and screw loosening [6,22-26]. Screw loosening being encountered in 33-36% of cases [22]. Certain investigators report that hemicerclage of the ischial osteotomy line reduces screw loosening [22], while others indicate that applying cerclage to either the ischial or ilial osteotomy lines has no such effect [27]; Altunatmaz et al.[12] propose that 20 days or longer movement restriction may be helpful in this circumstances. All cases undergoing TPO in our study were kept in individual cages with their movements restricted; none presented any problems related to the screws or plates. These considerations show that good postoperative observation and follow-up are imporatl for reduce post-operative complications.

Contrary to some investigators who reported that DJD change continues to progress following TPO [11,23], others indicate that such changes stop after the operation [12,28]. No degenerative changes were observed in any of the animals in our study either before the operation, or at the early or late postoperative follow-up.

Most commonly used canine pelvic osteotomy plates are available in three angles 20°, 30°, 40°. However, several authors suggests that the use of TPO procedure should be avoided where plates with extreme angles of rotation are

required [29-31]. The more extreme angle of rotation of the acetabulum, the more risk there is of interference between the femoral neck and the dorsal acetabular rim, and also reduces congruency, due to articular cartilage being thicker centrally on the femoral head and peripherally in the acetabulum [32]. Altunatmaz et al.[11] report that the animals in whom 40º plates were experienced obvious narrowing of the pelvic canal, while this effect was rather mild following use of 20° plates. This narrowing, however, did not cause any inconvenience to the subjects. In our study, we observed that the pelvic canal narrowing observed in the early post-operative follow-up improved in the later stages. This result may be due to a combination of the unilateral nature of the operation in all cases, and the uniform use of 20º plates.

The result of this research suggested that canine hip dysplasia, could be treated successfully in young dogs via TPO, carried out before degenerative changes begin within the hip joints.

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RESEARCH ARTICLE

Rotavirus Diarrhea Outbreaks in Arabian Thoroughbred Foals in A Stud Farm, Turkey

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Summary

In this study, the aetiological agent of diarrhea in Arabian thoroughbred foals housed in a stud farms and it's molecular characterization were reported. Out of sampled seven foals with diarrhoea, five were detected positive by RT-PCR depending on the amplification of the VP6 gene of rotavirus. Then the aetiological agent was characterized genetically by sequence analysis of the genome segments encoding VP6, VP7, VP4 and NSP4 of rotavirus. Findings revealed that the Turkish equine rotavirus circulating within this stud farm belongs to G3 and P[12] genotype with E2 NSP4 and I6VP6. This is the first study to report the G and P genotypes of equine group A rotaviruses in Turkey.

Keywords: *Rotavirus, Equine, Genotyping, Diarrhoea, Turkey*

Türkiye'de Bir Arap Atı İşletmesindeki Taylarda Rotavirus İshali Salgını

Özet

Bu çalışmada, bir at yetiştiriciliği işletmesinde bulunan safkan arap taylarındaki ishal olgusunun etiyolojik ajanının tespiti ve moleküler karakterizasyonu bildirildi. İshal semptomlu yedi taydan sağlanan materyallerin beşinde RT-PCR tekniği ile rotavirus VP6 geni yönünden pozitiflik saptandı. Daha sonra enfeksiyona neden olan virusun VP6, VP7, VP4 ve NSP4 gen bölgelerini kodlayan gen bölgelerinin dizi analizi yapıldı. Elde edilen verilere dayanılarak söz konusu at yetiştiriciliği işletmesinde enfeksiyona neden olan ERV saha suşunun E2 NSP4 ve I6VP6 ile ilişkili G3P[12] genotipinde olduğu belirlendi. Bu çalışma, Türkiye'de atlardaki grup A rotavirusların G ve P genotiplerinin bildirildiği ilk çalışmadır.

Anahtar sözcükler: *Rotavirus, At, Genotip, İshal, Türkiye*

INTRODUCTION

Group A rotaviruses (GARV) are one of the most important causative agents of severe diarrhea resulting in dehydration in human infants and the offspring of many animal species including cattle, equine, goat, etc. [1-3] and have severe economic impact on stud farming [4,5]. The rotavirus genome consists of 11 segments of double-stranded RNA (dsRNA), which encode 12 viral proteins in which 6 structural (VP1-VP4, VP6 and VP7) and 6 non-structural proteins (NSP1-NSP6). The structural protein, VP6, bears group spesific antigenic determinants. As genotyping analysis of VP6 gene, 16 different VP6 gene were recognized [6]. The nonstructural transmembrane glycoprotein NSP4 from group A rotaviruses, the viral enterotoxin, have been genetically classified into 14 genotypes, E1 and E14, recently [6]. The virus has two outer capsid proteins, VP7 and VP4, which are independently associated with the serotype specificities for G serotype (for glycoprotein) and the P serotype (for protease-sensitive protein), respectively [1]. In human and animal rotaviruses 27 G genotypes and 35 P genotypes have been identified so far [6].

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Equine GARVs have been identified in foals with diarrhea in a lot of countries as Ireland [7], Australia [8,9], India [10], Greece [5], Germany [11], Italy [12], Argentina [13] and Japon [14-16]. Reports reveals that the majority of rotavirus strain from foals are either G3P[12] or G14P[12] [5,7,9,11-14,16,17]. The other strains detected in foals belonging to genotype G5, G8, G10, G13 and P[18], P[3], P[11], P[1], P[7] types [4,13,17-21]. But there is no report on the G and P genotypes of equine rotavirus in Turkey.

In this study, it is aimed i-) to investigate of rotavirus as causative agent in Arab thoroughbred foals with diarrhea, houses in a stud farm and ii-) to report of the molecular characterization of the VP6, VP4, VP7 and NSP4 genes of a rotavirus detected in these foals.

MATERIAL and METHODS

Field Sample

In the present study, faecal samples from 9 to 24 days old foals (n=7) with clinical symptoms as mild diarrhea, loss of appetite and intestinal cramping, bred on a stud farm consisting of approximately 350 mares with different age, were used for the detection of rotavirus. Outbreaks of diarrhea during the foaling period in 2010-2011 were detected and foals tested in this study were sampled in February 2011. Foals were lactated naturally from their mother. As a knowledge given the veterinarians working this stud farm, diarrhea cases in foals had also been seen in the foaling period in the years before. In this farm, to prevent to the some viral, bacterial and parasite infections, regular administration of drugs and vaccination programs have been subjected to the farmed mares, except rotavirus vaccine.

RNA Extraction, RT-PCR and Genotyping of ERV

The extraction of rotavirus genomic RNA was performed using a QIAamp Viral RNA Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. All faeces samples were analyzed for rotavirus presence by VP6 RT-PCR with the primers [22]. Then, all positive samples for the amplicons (379 bp) for VP6 genes were characterized rotavirus G and P types by seminested PCR. For amplification of full length VP7 (1062 bp) gene [23] with Beg9-End9 primers and VP4 (876 bp) specific regions [24] with Con2-Con3 primers were used. For G typing, primers for G3, G13, G14 types [14] and End9 [23] were used [14]. For P typing, the second round PCR was performed with primer Con3 [24] and primer specific for genotypes P[12] and P[18] [15]. Additionally, the expected size amplicons were produced for NSP4 gene region from all samples positive for rotavirus with primers and protocols elsewhere [25]. Either full-length or partial sequences of the VP4, VP6, VP7 and NSP4 genome segments of the Turkish equine rotavirus strain, RVA/horse-wt/TUR/Eskisehir/2011, were determined after RT-PCR amplifications with specific primer sets. The list of the primer sets used in this study and their detailed specifications including expected product sizes, etc. were given in *Table 1*. The results from sequencing of amplicons compared with cognate sequences of reference viruses available in the databases. Sequence editing and multiple alignments were performed with Bioedit software package version 2.1 [26]. Phylogenetic analysis was carried out with the software package MEGA version 5.0 [27], using the Neighbor-Joining model with Kimura 2-parameter correction and bootstrap analysis (1.000 replicates).

RESULTS

Out of 7 faces samples, 5 were found positive for rotavirus by RT-PCR based on the detection the expected sizes (379 bp) of amplicon for VP6 gene of rotavirus. The RT-PCRs used for the detection and molecular characterization of equine rotaviruses detected (n=5) revealed expected sizes of DNA products (876 bp for VP4, 1062 bp for VP7 and 743 bp for NSP4) in five faces samples in concordance with reference viruses. As results of PCRs for G and P

Table 1. *Primers used for RT-PCR and sequence analysis of VP4, VP6, VP7 and NSP4 gene region of rotavirus*
Tablo 1. *Rotavirusun VP4, VP6, VP7 ve NSP4 gen bölgelerinin RT-PCR ve dizin analizinde kullanılan primerleri*

Procedure	Primer Sequence (5'→3')	Specifity	Location	Product Size (in bp)	Reference
VP4 gene amplification	TGGCTTCGCTCATTTATAGACA ATTTCGGACCATTTATAACC	P-F(con3) P-R(con2)	11-32 887-868	876	[24]
P genotype	CCATTATAAACCCATAGCTG ATGCACCATCTAATGTTTGC	P12 P18	545-526 463-444	535 452	[15]
VP7 gene amplification	GGTCACATCATACAATTCTAATCTAAG GGCTTTAAAAGAGAGAATTTCCGTCTGG	G-R(end9) G-F(beg9)	1062-1036 1-28	1062	[23]
G genotype	CAATCGAAGAGATTGCGACAG GGAGTAAATCACAAAATAAATCTC GACGAAGCATTGCAATTA	G3 G13 G14	683-703 742-765 481-498	374 321 582	[14]
VP6 gene amplification	GACGGVGCRACTACATGGT GTCCAATTCATNCCTGGTGG	VP6-F VP6-R	747-766 1126-1106	379	[22]
NSP4 gene amplification	GGCTTTWAAAAGTTCTGTTCCGAGAGAG TAAGACCRTTCCYTCCATTAAC	151 152	1-28 743-722	743	[25]

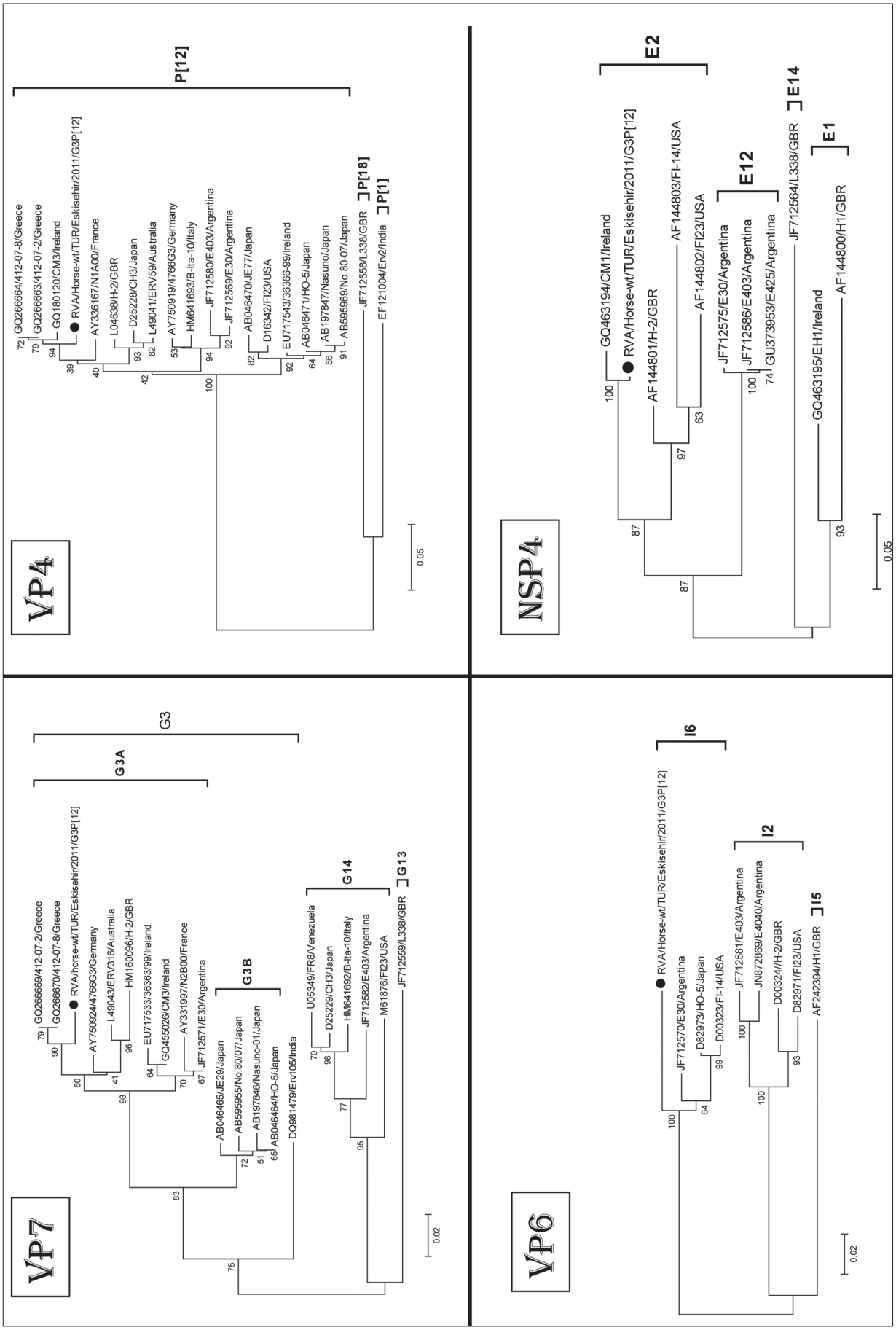

Fig 1. Phylogenetic analysis (Neighbor Joining method) of the VP4, VP6, VP7 and NSP4 genes of RVA/horse-wt/TUR/Eskisehir/2011 and some rotavirus strains deposed in GenBank.

Şekil 1. Gen Bankasından alınan birkaç rotavirus suşu ile RVA/horse-wt/TUR/Eskisehir/2011 adlı suşun VP4, VP6, VP7 ve NSP4 gen bölgeleri düzeyinde, Neighbor Joining metoduna göre yapılmış filogenetik analizi

typing, the binary combination of rotavirus isolates were characterized as G3P[12].

According to the results of phylogenetic analysis using the Neighbor Joining method, Turkish equine rotavirus, RVA/horse-wt/TUR/Eskisehir/2011, have been characterized as G3P[12] with E2 NSP4 and I6 VP6 genotypes *(Fig. 1)*. The accession numbers of the sequences are: JQ687223, JQ687220, JQ687221 and JQ687222 for NSP4, VP4, VP6, VP7 of a Turkish equine rotavirus, RVA/horse-wt/TUR/Eskisehir/2011/G3P[12], analyzed in this study, respectively.

DISCUSSION

The Arabian horses are worldwide, including the United State and Canada, The United Kingdom, continental Europe, some South American countries and Middle East, and also they have a importance for the equestrian activity. In Turkey, races for two equine breed (Arabian and English horses) has been organized for a long time in a seven different places/city according to the climatic conditions/seasons. Then, the breeding of horses has economical importance for the private breeders and also state stud farms responsible to protect the genes and pedigree of Arabian thoroughbred horses in Turkey. To date, there is no report about rotavirus or other viruses causes diarrhea in foals housed in stud farm sampled in this study, and also other state or private stud farms, althought owners reported the diarrhea cases in newborn foals when we asked them to be presence these events.

Equine rotavirus belong to genotype G3 was predominantly detected throughout the world. This genotype followed the G14 strains isolated in different countries (UK, Japon, Australia, Venezuela and US, etc. [9,14,17,28] while the other G genotypes (G5, G8, G10, G13) from foals were detected sporadically [4,13,17-19]. While the P genotypes of rotavirus from human and other some animal species as bovine and porcine especially had been classified into 35 P types, equine rotaviruses are predominantly in P [12] genotype. At this date, at least six different P types (P[12], P[3], P[11], P[1], P[18] and P[7]) of equine rotaviruses has been reported [4,13,17,18,29]. The known binary combinations in group A equine rotaviruses are especially G14P[12] [14] and G3P[12] [14], followed P[1] with combination G10 and G8, P[7] with combination G13 [17,20].

In this paper, Turkish equine rotavirus were clustered in phylogenetic analyses based on VP4 and VP7 gene regions of viral genom. The sequence analyses of rotavirus defined in this study (RVA/horse-wt/TUR/Eskisehir/2011/G3P[12]) shown that the mentioned virus belongs to G3A and P[12] genotypes *(Fig. 1)* as most of the rotaviruses from horses deposited in GenBank, detected in an European countries.

The vaccination programs had been used to the prevention of the rotavirus infection in human and bovine worldwide. Similarly, some inactivated vaccines for equine rotavirus had been developed and licenced USA and some European contries (the vaccine including H2 strain) and Japon (the vaccine including H0-5 strain), except Turkey [13,29,30]. However it is remained that the insufficient of the vaccine have been documented on several occations if foals are not supported by keeping the stable clean and by outsiding of their mares on the pastures. Additionally, it is reported that the differences of the genotypes of field strains can be causes the vaccine breakdown. Thus, it is reported that the vaccine (RotaCli Equina®) including the prototype ERV H2 (G3P[12]), simian rotavirus (SRV - SA11 G3P[2]), and bovine rotavirus (BRV- NCDV-Lincoln G6P[1]) strains had been used in Argentina since 1996 and also ERV diarrhea incidence reduced in the high level depend on the this vaccine application to the pregnant mares [13]. In the same paper, the researchers reported that the rates of the G14 rotavirus detection and the evaluating of the incidence of rotavirus cases at the last years studied and that the ERV vaccine should be updated according to the detected G and/or P type rotaviruses in countries, as previously described for human and bovine rotavirus vaccines.

It is known that risks for horses to acquire rotavirus are high because of the increased movement of horses from one farm to another for different reason or their presence in the race area . The stud farm sampled in current study is one of the three state stud farms which are the producer (for Arabian horses) of thoroughbred foals in Turkey. These stud farms have an exchange procedure of horses for reproductive reasons especially. To date, there is no investigation based on the molecular characterization of VP4 and VP7 gene regions of GARVs from horses while the knowledge were reported about GARVs from other species in Turkey [31,32]. It's possible reasons are the inadequate knowledge on the rotavirus infection in foal diarrhea cases, disregarding of the mild diarrhea. In the future studies, we will investigate the presence of the rotavirus infection in foals with different age, race, management conditions and its molecular epidemiology for the deeper understanding of the epidemiology of rotavirus infections in horses, in Turkey.

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RESEARCH ARTICLE

Genetic Analysis of the Partial M RNA Segment of Crimean-Congo Hemorrhagic Fever Viruses in Turkey

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Summary

Crimean-Congo hemorrhagic fever (CCHF) is a fatal tick-borne zoonosis extensively common in Africa, Asia, Eastern Europe and the Balkan Peninsula. CCHF has been reported in Turkey with high frequency since 2002. Genetic diversity of CCHF virus (CCHFV) isolates circulating in Turkey were studied by two recent studies from 2006 to the end of 2010. Since CCHFV disease has been an important public health concern in Turkey, it is necessary to continue genetic analysis of CCHFV viruses for the assessment of future patterns of disease. The aim of the present study was to genetic analysis of CCHFV isolates derived from infected patients over a two-year period (2011 and 2012) in several provinces of Turkey. Serum samples (n=10) were selected from CCHFV RNA positive patients and subjected to sequence analysis of the gene region encoding partial M segment. The nucleotide sequence alignments of the 10 partial M segments of CCHFV isolates showed that the nucleic acid relatedness of CCHFV isolates ranged from 94.4% to 100%. Phylogenetic analysis of M segment sequences revealed that CCHFV isolates circulating in Turkey belonged to the European lineage I and were closely related to the viruses previously found in Turkey and in the Eastern European-Russian and Balkan Peninsula. The results of the present study indicated the genetic stability and the lack of the genetic diversity of CCHFV isolates circulating in Turkey.

Keywords: *Crimean-Congo hemorrhagic fever, Genetic diversity, M segment, Glycoprotein precursor, Reassortment, Recombination, Turkey*

Türkiye'deki Kırm Kongo Kanamalı Ateşi Virüslerinin Kısmi M-segmentlerinin Genetik Analizi

Özet

Kırım-Kongo kanamalı ateşi (KKKA), kene kaynaklı, ölümcül bir zoonotik hastalık olup, Doğu Avrupa, Asya, Afrika ve Balkan yarımadasında yaygın olarak görülmektedir. Türkiye'de 2002 yılından itibaren artan bir sıklıkta görülmektedir. KKKAV'leri genetik analizi, yakın zamanda yapılan iki çalışmada 2006 ile 2010 yıllarını kapsayan süreçte araştırılmıştır. Kırım Kongo hastalığının ülkemizde önemli sağlık sorunu oluşturması, gelecek için hastalık sürecinin değerlendirilmesi ihtiyacı; virüsün genetik yapısının sürekli izlenmesini gerekli kılmaktadır. Bu çalışmada 2011 ve 2012 yılları arasındaki iki yıllık süreçte hastalardan elde edilen KKKA virüs (KKKAV) izolatlarının genetik analizi amaçlanmıştır. KKKAV pozitif 10 hastanın serumlarından elde edilen RNA örnekleri kullanılarak kısmı M segment sekansları elde edilmiştir. Elde edilen sekansların eşleştirilmesi sonucu izolatların sekans benzerliği %94.4 ile %100 arasında bulunmuştur. Filogenetik analiz sonucu sekans analizi yapılan izolatların Avrupa I kümesinde yer aldığı, Türkiye, Doğu Avrupa-Rusya ve Balkan Yarımadasında daha önce analiz edilen virüslerle yakın ilişkili olduğu tesbit edilmiştir. Bu çalışmada Türkiye'de dolaşımda olan KKKAV'lerinin genetik olarak sabit ve çeşitlilik yönünden de kısıtlı olduğu sonucu gözlenmiştir.

Anahtar sözcükler: *Kırım-Kongo kanamalı ateşi, Genetik çeşitlilik, M segment, Glykoprotein precursor, Reassortment, Recombination, Türkiye*

INTRODUCTION

Crimean-Congo hemorrhagic fever virus (CCHFV) is a member of the genus *Nairovirus,* a tick borne RNA virus in the family *Bunyaviridae* [1,2]. In humans, CCHFV is highly contagious and causes a severe acute hemorrhagic disease

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known as Crimean-Congo hemorrhagic fever (CCHF) with mortality rates reaching 30%. Disease is transmitted either through tick bites (primarily of the genus *Hyalomma*) or direct contact with infected blood or tissues of viremic hosts [3,4]. Since human infections can lead to nosocomial outbreaks, CCHF cases are required to be reported to the public health authorities [5].

CCHF has now been reported in more than 30 countries in Africa, Asia, Eastern Europe, the Middle East and Balkan Peninsula. Disease distribution correlates well with the geographical distribution of the tick vector *Hyalomma marginatum marginatum* [4,6]. In addition, it is possible that migratory birds may also play a part in viral dissemination by carrying infected ticks over great distances [7].

In Turkey, the first case of CCHF disease was confirmed in the Tokat province in the Kelkit Valley located in northern Turkey in 2002. The majority of cases were reported from the middle and eastern part of Anatolia, particularly from the provinces of Tokat, Sivas, Corum, Yozgat and Erzurum [8-10]. Between 2002 and 2010, 5,317 CCHF confirmed cases, and 267 deaths (average fatality rate of 5%) were reported by the Turkish Ministry of Health (http://www.saglik.gov.tr). Potential reasons for the emergence and increase in the number of CCHF cases in Turkey include climate change that may have a significant impact on *Hyalomma* tick reproduction rates as well as anthropogenic factors such as changes in agricultural and hunting habits [5,11].

CCHFV possesses a negative sense, single stranded, three segmented RNA genome comprised of small (S), medium (M) and large (L) segments [12]. The S segment codes for the nucleoprotein (NP) [13], the M segment encodes for a glycoprotein precursor which gives rise to two structural glycoproteins Gn (37 kDa) and Gc (75 kDa) and also encodes for a non-structural protein (NS_m) [13-15]. The glycoproteins, Gn and Gc, encoded by M-RNA segment, are responsible for virus attachment and induction of virus neutralizing antibodies [16]. Therefore, M segment is considered critical to the elicitation of immunopathologic responses in humans [17,18]. The large L segment encodes the RNA-dependent RNA polymerase enzyme (L protein) [1].

Genetic analysis studies based on S, M and L RNA segment sequences of CCHFV isolates have been used to define genetic groups or lineages [2,7,17,19-22]. These studies have indicated the natural occurrence of recombination and reassortment events resulting in worldwide genetic diversity of CCHFV [2,21,23]. Several studies have showed that the majority of CCHFV isolates from infected humans and ticks in Turkey belonged to the European lineage 1 that includes south-western Russia and Balkan Peninsula [8,17,22,24-26]. In addition, some viruses isolated in Turkey were grouped within the European lineage II that originally included CCHFV strain AP92 isolated in Greece [22,27-29].

As a result of frequent reassortment event(s) associated with M RNA segments, phylogenetic analysis based on M segment RNA sequences differ from those based on S and L RNA segment analyses [2]. It is likely that reassortment events associated with M RNA segments may result in enhanced virulence [17,18,30]. In addition, M-segment variability may also result in affecting antigenic and immunogenic epitopes of CCHFV. Therefore, investigations of M segment variability are of a great importance to the identification of new isolates and a better understanding of virulence mechanisms associated with respective CCHFV isolates.

Genetic analysis studies of CCHFV isolates involved in disease seasons from 2006 to the end of 2010 were conducted by two recent previous studies [22,26]. The results of these studies indicated that CCHFV viruses circulating in Turkey were closely related viruses and belonged to European lineage I. Since CCHF associated disease is an important health problem in Turkey, and the possible risk that new CCHFV isolates from other countries could be introduced into this region, a continuous investigation designed to define the genetic analysis of the circulating CCHFV isolates needs to be carried out.

The aims of the present study were i) to carry out phylogenetic analyses of partial 10 M RNA segments sequences from CCHFV isolates obtained from selected human cases from 2011 through 2012, ii) to investigate M segment based CCHFV genetic heterogeneity between isolates in Turkey and to define their relationship to sequences isolated from neighbouring countries.

MATERIAL and METHODS

Study Samples: The present study examined CCHFV positive samples of patients (n=10) from the provinces of the Kelkit Valley (situated in the middle Black Sea region) and other provinces of Turkey during CCHFV disease season in 2011 and 2012. The presence of CCHFV RNA was confirmed using a TaqMan-based real time RT-PCR assay as previously described by Yapar et al.[31]. A total of 10 CCHFV RNA positive samples (5 from 2011 and 5 from 2012) were selected from the Virology Reference and Research Laboratory Turkish National Public Health Agency (TNPHA), Ankara, Turkey.

Viral RNA Extraction and RT-PCR: CCHFV RNA was extracted from respective samples using a viral RNAeasy Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. A one step RT-PCR kit was used to amplify a 890 bp partial M segment of 10 CCHFV isolates using primers F (5'- ACAGGCTTTAGGAAACTAC-3') and R (5'-CAC CTGCAATAGCTTTCT-3'). The one step RT-PCR was carried out in a 50 µl reaction volume containing 10 µl of template RNA, 10 µl 5x reaction buffer, 3 µl of each primer (10 pmol), 2 µl enzyme mix, 2 µl dNTP mix (10 nm each) and 20 µl nuclease free water. The reaction mixture was amplified in a thermocycler at the following conditions: 50°C for 30 min and an initial denaturation of 15 min at 95°C followed by 33 cycles at 94°C for 45 sec, 55°C for 45 sec and 72°C for 1 min followed by a final step at 72°C for10 min [26].

DNA Sequencing: Following amplification of the 890 bp M CCHFV segments, amplicons were purified using Agencourt Ampure (Beckman Coulter, Brea, CA) and sequencing reactions were set up. Briefly, sequence reaction mixtures consisted of 3.5-5 ml of purified amplicon, 5 pmol primer and 4 ml of Dye terminator cycle sequencing Quick Start Kit (Beckman Coulter, Brea, CA). The sequencing reaction was then carried out as follows: initial denaturation at 94°C for 3 min followed by 30 cycles at 96°C for 20 s, 55°C for 20 s and 60°C for 4 min. PCR products were purified using a DyeTerminator removal kit (Agencourt Cleanseq, Beckman Coulter) and 20 ml of purified product was sequenced using a CEQ 8000 Genetic Analyser (Beckman Coulter Brea, CA).

Phylogenetic Analysis: Clustal W was used to align viral nucleotide and amino acid sequences using representative M nucleotide and amino acid sequences downloaded from GenBank [32]. The CCHFV sequences used for comparison and phylogenetic analysis were given in the phylogenetic tree and their GenBank accession numbers were indicated in brackets.

The evolutionary history of the Turkish isolates based on regional sequence comparisons was inferred using the neighbour-joining (NJ) method. The evolutionary distances were computed using the Maximum Composite Likelihood method [33] and are reflected as units of the number of nucleotide substitutions per site. Phylogenetic analyses were conducted using MEGA4 [34].

RESULTS

Amplification of CCHFV Partial M Segments: Single PCR bands corresponding to the 890 bp of partial M segments (envelope glycoprotein precursor Gn) from the 10 selected CCHFV isolates were successfully amplified and sequenced. The assigned accession numbers for Turkish CCHFV isolates and their provincial origin are described in *Table 1*.

Phylogenetic Analysis of CCHFV M-RNA Segment Sequences: The nucleotide sequence alignments of the 10 M segments of CCHFV isolates derived from patients in 2011 and 2012 showed sequence similarities ranging from 94.4 to 100%. The M segment nucleotide sequence similarity of these isolates with those derived from patients in 2009 and 2010 analysed in the previous study [26] was ranged from 94.9 to 99.7%. In addition, the sequence similarity between isolates subjected for the present and the previous study and Bulgarian vaccine strain V24/81 was ranged from 94.9 to 95.9%. The NJ-based phylogenetic analysis of 10 partial M segment sequences revealed that the CCHFV isolates analysed in the present study belonged to the European lineage I. These isolates were closely related to viruses characterized from Eastern Europe, Russia and the Balkan Peninsula including Hoti, Kosovo, Kashmanov and Drosdov *(Fig. 1)*.

Based on the partial M segment based phylogenetic analysis, the 10 CCHFVs were classified in two groups (I and II) together with representative viruses from Eastern Europe and the Balkan Peninsula *(Fig. 1)*. Group I included eight isolates that were closely related to Turkish representative viruses identified previously (Turkey200310848 and Turkey-Kelkit06) and isolates derived from patients in 2009 and 2010 (KYSR1761/09, AYDN193/10 and KSTM229/10). Two isolates from 2011 (KYSR207/11 YZGT327/11) comprised group II along with Russian viruses ROS/HUVL-100 and Kashmanov *(Fig. 1)*.

Alignment of the amino acid sequences deduced from the translation of the partial M segment nucleotide sequences coding for the envelope glycoprotein precursor identified several amino acid variations in 10/10 isolates primarily located between amino acids 674 and 760 *(Fig. 2)*. Similarity indices of amino acid sequences of the 10 isolates varied between 95.4 and 100%. Compared to the

Table 1. *Provincial origin of CCHFV isolates and their assigned accession number by GeneBank*
Tablo 1. *KKHA virüslerinin illere gore dağılımı ve GeneBank tarafından verilen resmi erişim numaraları*

Province	Isolation Year of Viruses				Total
	2011		2012		
	Virus Code	Accession No.	Virus Code	Accession No.	
Kayseri	KYSR207/11	JX308613			1
Yozgat	YZGT327/11	KC150005			1
Erzurum	ERZRM633/11 ERZRM634/11	JX308615 JX308614			2
Çorum	CRM2020/11	JX308616			1
Kastamonu			KSTM169/12 KSTM241/12 KSTM170/12	KC150000 KC150001 KC150002	3
Edirne			EDRN622/12	KC150003	1
Kırklareli			KRKLAREL707/12	KC150004	1
Total	5		5		10

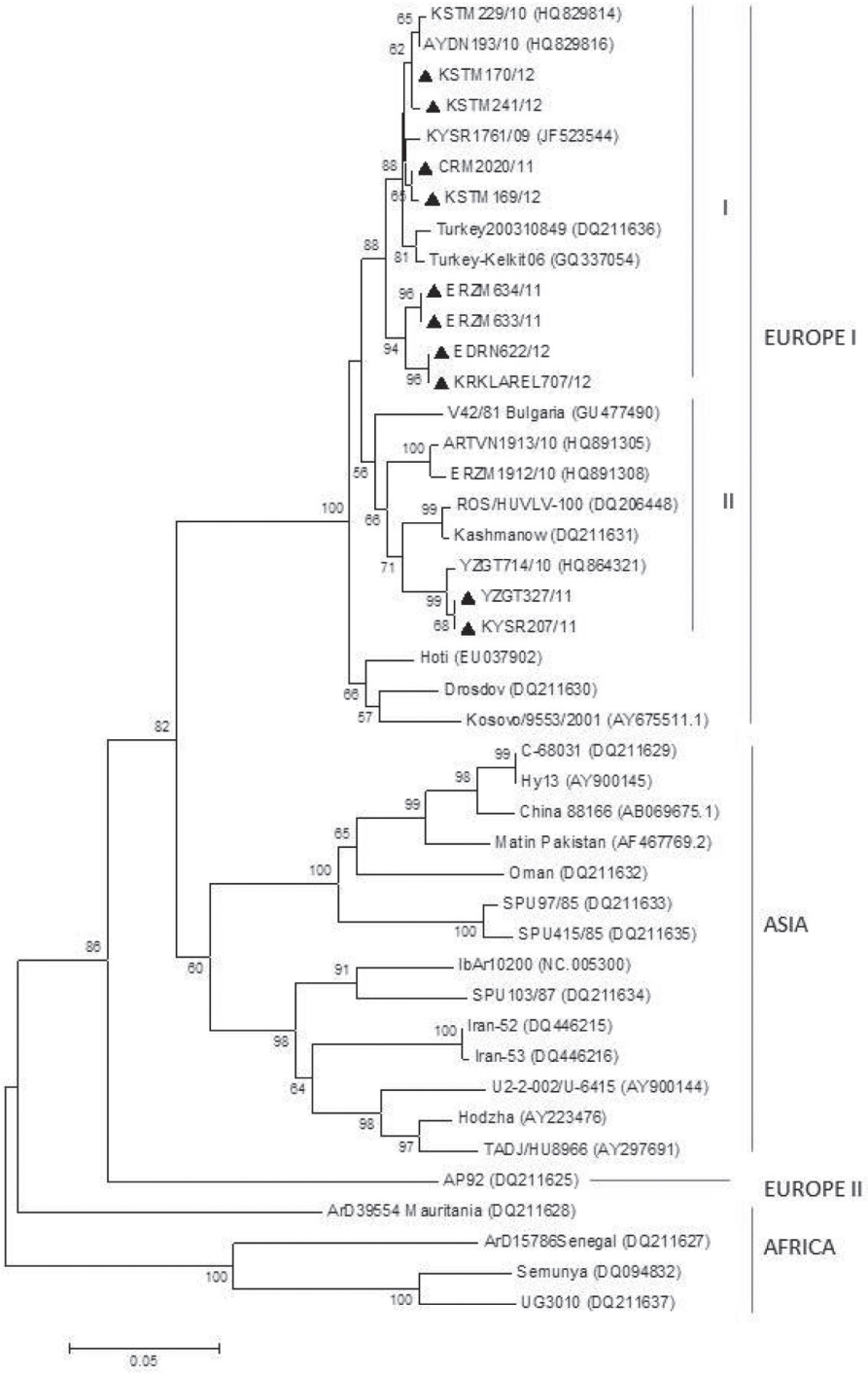

Fig 1. Phylogenetic analysis

A 890 bp of partial M segment amplified from 10 RNA samples obtained from CCHFV isolates between 2011 and 2012 were compared to representative viruses using the neighbour-joining method with Kimura two-parameter distances using MEGA 4 software. Isolates subjected for the present study are indicated by a triangle

Şekil 1. Filogenetik analiz

2011 ve 2012 yıllarına ait KKHAV virüs izolatlarından elde edilen 10 adet RNA örneklerinden 890 bç boyutunda M segmentler çoğaltılmıştır. M segment sekansları referans sekanslarla MEGA 4 yazılımı (neighbour-joining metot ve Kimura two-parameter distances) yöntemi kullanılarak karşılaştırılmıştır. Bu çalışmada kullanılan izolatlar üçgen ile belirtilmiştir

Turkish representative TR200310849 virus sequence, the most striking variation was observed in the replacement of an S to F at amino acid position 711 in all tested isolates in this study and in representative viruses, including Turkey-Kelkit06, Kashmanov, ROS/HULV-100, Drosdov, and Hoti. Isolates YZGT327/11 and KYSR207/11 that displayed identical nucleotide sequence and derived from patients with fatal outcome presented with four amino acid variations, including I674V, K679R, A681V, and I729F. An amino acid variation, A746T, was shared in the isolates of ERZM633/11 and ERZM634/11 that had identical nucleotide sequence and derived from patients with fatal outcome. Isolates EDRN622/12 and KRKLAREL707/12 that had also identical nucleotide sequence and derived from patients from fatal outcome displayed two amino acid variations, including K728R and A741T. An amino acid variation, I710L, was observed in isolate KSTM241/12 derived from another patient with fatal outcome. There was no amino acid variation in isolates CRM2020/11 and KSTM169/12 derived from recovered patients from the disease. Bulgarian vaccine strain V42/82 did not display any common amino acid variation with any of the tested isolates *(Fig. 2)*.

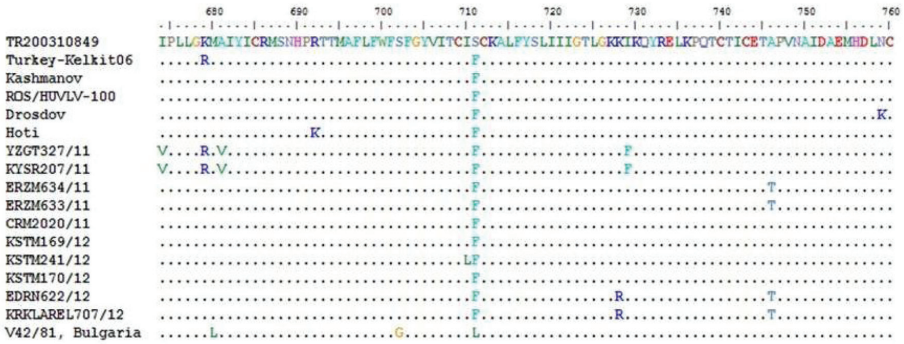

Fig 2. Alignment of the deduced partial CCHFV M segment amino acid sequence corresponding to the variable region between amino acids 674 and 760

Representative CCHFV isolates from Turkey (TR200310849, Turkey-Kelkit06) and from neighbouring countries (Kashmanov, ROS/HUVL-100, Drosdov, Hoti and V42/82 Bulgaria) were included in the alignment

Şekil 2. KKHAV kısmi M segmentinin 674 ve 760 amino asit dizisine karşılık gelen değişken bölgelerinin karşılaştırılması

Türkiye kökenli (TR0310849, Turkey-Kelkit06) ve komşu ülke kökenli izolatlar (Kashmanov, ROS/HUVL-100, Drosdov, Hoti ve V42/82 Bulgaria) karşılaştırmaya dahil edilmiştir

DISCUSSION

Since the first case of CCHF disease was emerged in 2002, an increasing number of CCHF disease cases resulting in fatal outcomes in humans have been reported in Turkey with majority of cases reported in the provinces of Kelkit Valley.

The recombination and reassortment events associated with the segmented RNA genome have inevitably resulted in worldwide genetic diversity of CCHFVs. In particular, more frequent reassortment event(s) occurring in M-RNA segment compared to reassortment events occurring in S and L segments make it more variable than S and L segments [2,21]. Thus, the genetic analysis of M segment is more beneficial for investigating CCHFV diversity.

In the present study, the genetic analysis of Turkish CCHFV isolates obtained from 10 confirmed human clinical cases from 2011 to 2012 was investigated by comparing the partial M segment sequences in phylogenetic analysis.

The NJ-based phylogenetic analysis of the M-RNA segments showed that all tested CCHFV isolates belonged to the European lineage I, including viruses from Eastern Europe-Russia (Drosdov, Kashmanov) and the Balkan Peninsula (Kosovo, Bulgaria) *(Fig. 1)*. The distribution of 10 CCHFVs in two groups on phylogenetic analysis was due to M-segment variability as expected.

The results obtained in our present study have been compared to two recent studies performed by Ozkaya et al.[22] and Kalaycioglu et al.[26] who described the molecular epidemiology of CCHFVs from patients between 2006 and 2008 and investigated genetic diversity of CCHFVs from patients between 2009 and 2010 in Turkey, respectively. The present study extended the phylogenetic analysis of viruses circulating in this region by including viruses collected from 2011 to 2012. In particular, comparison of the partial M-segment sequences involving in viruses studied between 2009 and 2010 in Kalaycioglu's previous study [26] and the present study confirmed the existence of a close relationship between the CCHFV isolates circulating in Turkey *(Fig. 1)*. In addition, a close relationship between the Turkish CCHFVs and Bulgarian vaccine strain V42/82 Bulgaria, the only available inactivated vaccine, may be beneficial for focusing protective efficacy of this vaccine or development of a novel vaccine *(Fig. 1)*.

Amino acid variations between amino acids 674 and 760 of the partial M segment glycoprotein precursor (Gn) protein region was showed the replacement of an S to F at amino acid position 71 in all tested isolates in the present study *(Fig. 2)*. Except for two isolates (CRM2020/11 and KSTM169/12) derived from patients recovered from the disease, the remaining viruses which isolated from individuals with fatal outcome displayed some amino acid variations *(Fig. 2)*. Whether or not these variations important for determining the virulence of viruses remain to be investigated. In addition, other parts and/or the full length M-RNA segment (s) of CCHFV isolated need to be sequenced in future studies for determining nucleotide and amino acid variability that may be critical for virulence mechanism of CCHFV.

The results of our study showed that CCHFV isolates circulating in Turkey were closely related and phylogenetically belonged to the European lineage I as described in previous studies. Our findings were in agreement with the Ozkaya's suggestion [22] that local topotype viruses were circulating and responsible for infections in Turkey. In addition, our results were also in agreement with the description of lack of the genetic diversity of Crimean-Congo haemorrhagic fever viruses in Turkey as described by Kalaycioglu et al.[26]

Since CCHFV is known to be a migrating pathogen, Turkey may not only serve as a 'donor' country for Europe as suggested by Mild et al.[7] but may also be a 'recipient' of

new CCHFV isolates from other parts of the world where the disease is endemic. Generally, CCHFV strains tended to be region or continent specific with certain lineages predominating and circulating in particular areas of the world, including Turkey unless new viruses are introduced either by viremic animals or animals carrying infected ticks. The indroduction of new viruses belonged to other lineages may result in reassortment event(s) between viruses that may lead to appearance of new isolates with enhanced virulence. Therefore, Turkish CCHFV cases need to be monitored for such incursions.

In conclusion, the results based on the genetic analysis of M segment of CCHFV isolates confirmed genetic similarity and stability between viruses circulating in Turkey. This information could be beneficial in future studies focusing on identifying the most potentially effective vaccine strain(s). It should also be noted that there is a potential risk for importing novel viruses from neighbouring and other countries. The more and continuous genetic analysis studies involving in full length M segments are essential for future studies in Turkey.

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RESEARCH ARTICLE

Risk Factors Associated with Passive Immunity, Health, Birth Weight and Growth Performance in Lambs: I. Effect of Parity, Dam's Health, Birth Weight, Gender, Type of Birth and Lambing Season on Morbidity and Mortality [1]

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Summary

This study was designed to examine the effect of birth weight, gender, birth type, lambing season, and dam parity and health status on lamb morbidity and mortality during the neonatal (first four weeks of life) and post-neonatal (first 5-12 weeks of life) periods in lambs born in two flocks in the 2009 lambing season in Kars, Turkey. EPI INFO 6 was used to determine differences in morbidity or mortality rates and risk according to risk factors including birth weights, genders, type of birth, lambing seasons, and parity and health of dams. The significance level was set at P<0.05 for all comparisons. The neonatal morbidity and mortality rates (48% and 33.3%, respectively) and risk of the lambs in the low birth weight group were significantly higher than those of the lambs in the medium birth weight (18.9%, χ^2=10.4, OR=3.9 for morbidity and 1.6%, χ^2=32.5, OR=30, for mortality) and high birth weight (9.8%, χ^2=26.9, OR=8.5 for morbidity and 1.2%, χ^2=46.5, OR=42.7 for mortality) groups (P<0.01 and P<0.001, respectively). The neonatal mortality rate and risk of lambs born in the winter season (9.2%) were significantly (P<0.01) higher than those of lambs born in the spring season (1.8%, χ^2=9.6 and OR=5.5). Lambs born to primiparous ewes had a neonatal morbidity rate (29.6%) and risk significantly higher than those of ewes at second parity (13.9%, χ^2=6.4, OR=2.6) and third parity (11.1%, χ^2=7.8, OR=3.3) (P<0.05 and P<0.01, respectively). Similarly, lambs born to primiparous ewes had a neonatal mortality rate (14.8%) and risk significantly higher than those of ewes at second parity (2.9%, χ^2=9.3, O=5.7), third parity (0%, χ^2=14.1) and ≥4 parity (2.4%, χ^2=4.2, OR=6.9) (P<0.001, P<0.001 and P<0.05, respectively). Lambs born to ill ewes had a neonatal morbidity rate (64.7%) and risk significantly higher (P<0.001) than those of lambs born to healthy ewes (13.8%, χ^2=30.38, OR=11.4). In the present study, it was concluded that the most significant risk factors for lamb morbidity and mortality were season of birth, parity, birth weight and health status of dams. Therefore, timely implemented health and management measures targeting these factors are required to reduce disease and death.

Keywords: *Lamb, Morbidity, Mortality, Risk factors*

Kuzularda Pasif İmmünite, Sağlık, Doğum Ağırlığı ve Büyüme Performansı ile İlişkili Risk Faktörleri: I. Anne Doğum Sayısı ve Sağlığı, Doğum Ağırlığı, Cinsiyet, Doğum Tipi ve Kuzulama Sezonunun Hastalık ve Ölümler Üzerine Etkisi

Özet

Bu çalışma Kars'ta iki sürüde 2009 kuzulama sezonunda doğan kuzularda doğum ağırlığı, cinsiyet, doğum tipi, kuzulama sezonu, anne doğum sayısı ve sağlığının neonatal (yaşamın ilk 4 haftası) ve sonraki (yaşamın ilk 5-12 haftalık kısmı) dönemlerde hastalık ve ölümler üzerine etkisinin incelenmesi amacıyla tasarlandı. Doğum ağırlığı, cinsiyet, doğum tipi ve kuzulama sezonu ve anne doğum sayısı ve sağlık durumu içeren risk faktörlerinin göre hastalık veya ölüm oran ve risk farklılıklarını belirlemek için EPI INFO 6 kullanıldı. Tüm karşılaştırmalarda önemlilik seviyesi P<0.05 olarak kabul edildi. Düşük doğum ağırlığı grubunda olan kuzuların hem neonatal hastalık ve ölüm oranları (sırasıyla %33.3 ve %48) hem de riskleri orta doğum ağırlığı (hastalık için %18.9, χ^2=10.4, OR=3.9 ve ölümler için %1.6, χ^2=32.5, OR=30) ve yüksek doğum ağırlığı (hastalıklar için %9.8, χ^2=26.9, OR=8.5 ve ölümler için %1.2, χ^2=46.5, OR=42.7) gruplarına göre önemli seviyede (sırasıyla P<0.01 ve P<0.001) yüksek bulundu. Kış sezonunda doğan kuzuların neonatal ölüm oran (%9.2) ve riski ilkbahar sezonunda doğanlara göre (%1.8, χ^2=9.6, ve OR=5.5) önemli seviyede (P<0.01) yüksek bulundu. İlk doğumunu yapan annelerden doğan kuzuların neonatal hastalık oran (%29.6) ve riski 2. (%13.9, χ^2=6.4, OR=2.6) ve 3. doğumunu (%11.1, χ^2=7.8, OR=3.3) yapan annelerden doğan kuzulara göre önemli seviyede (sırasıyla P<0.05 ve P<0.01) yüksek bulundu. Benzer şekilde, ilk doğumunu yapan annelerden doğan kuzularının neonatal ölme oranı (%14.8) ve riski 2. (%2.9, χ^2=9.3, O=5.7), 3. (0%, χ^2=14.1) ve 4. (%2.4, χ^2=4.2, OR=6.9) doğumunu yapan annelerden doğan kuzulara göre önemli seviyede (sırasıyla P<0.001, P<0.001 ve P<0.05) yüksek bulundu. Herhangi bir hastalığa maruz kalan annelerden doğan kuzuların neonatal hastalanma oranı (%64.7) ve riski sağlıklı olan annelerden doğan kuzulara göre (%13.8, χ^2=30.38, OR=11.4) önemli seviyede (P<0.001) yüksek bulundu. Bu çalışmada kuzu hastalık ve ölümleri için en önemli risk faktörlerinin doğum sezonu, anne doğum sayısı, doğum ağırlığı ve annenin sağlık durumunun olduğu sonucuna varılmıştır. Bu nedenle, bu faktörler üzerine odaklanarak zamanında sağlık ve sevk-idare önlemlerinin sağlanması kuzu hastalık ve ölüm oranlarını azaltmak için gereklidir.

Anahtar sözcükler: *Kuzu, Hastalık, Ölüm, Risk faktörleri*

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INTRODUCTION

High lamb morbidity and mortality during the first 12 weeks of life or the pre-weaning period cause the considerable economic loss, and greatly reduce the efficiency and profitability of lamb production. In many flocks worldwide, 6.9 to 37.5% of lambs die by three months of age [1-8] with neonatal lambs being at greater risk particularly during the first week of life [9-11]. In a study conducted in Kars region [6], the neonatal morbidity and mortality rates of lambs were determined as 48.6% and 20.8%, respectively, which is above economically acceptable rates. Some flocks experience severe losses, which may reduce the farmers' motivation for keeping sheep [1].

Although the majority of producers are aware that an efficient and profitable system would minimise lamb morbidity and mortality, currently applied management programmes may overlook the underlying factor, which would in return increase the risk of morbidity and mortality [2,4]. In a previous study [6] conducted on neonatal morbidity and mortality in this region, the majority of mortalities and morbidities in lambs were reported to have arisen from noninfectious causes such as birth stress, trauma, starvation due to hypothermia and mismothering, abdominal mass caused by trichobezoar, lameness, and congenital abnormality. It was indicated that the primary causes of neonatal diseases vary with environmental conditions and flock management. It has been suggested that lamb mortality could be reduced only if the specific causes of mortality on a given farm are identified and eliminated [2,12,13]. Nevertheless, as the main causes of lamb mortality in different production systems and countries display similarity, it would be more appropriate to identify all underlying factors associated with mortality and adapt farm and lambing management practices accordingly in predefined region or farms [2,13-16]. The main problems encountered in sheep production are diseases, malnutrition and poor management, which often result in reduced productivity and increased mortality. Management factors that have significant effect on lamb survival include the nutrition and immune status of the ewe, good hygiene practices and the colostrum intake of the lamb [2,15,17,18].

In order to prevent mortality, losses in production and profitability and high treatment costs, the adaptation and implementation of management practices could prove to be a useful alternative for the reduction and elimination of risk factors involved in disease development. Firstly, risk factors that predispose lambs to the development of disease must be identified [2]. In this respect, investigations are recommended to focus on the effect of multiple factors, including breed, age of dam, parity, gender, type of birth, birth weight, behaviour of the lamb and dam (ewe/lamb interaction) after birth, environmental factors such as lambing season, farm management (the feeding of dams particularly during gestation, good hygiene practices and colostrum intake), on mortalities [2,9,13,17,18]. However, to date, only a very limited number of studies have been conducted on the effect of environmental or flock management factors on morbidity. The impact of predisposition factors on morbidity and mortality varies from country to country in relation to varying conditions, such as the environment and the breeds raised. Nonetheless, it is interesting that very little research has been carried out in Turkey to determine the overall level of lamb mortality and morbidity, and to identify the underlying causes and other management factors that may have effect.

Despite the already significant contribution of sheep breeding to the national economy, it is imperative that the productive performance of the ovine population meets the increasing domestic demand for animal proteins. This can be achieved by increasing the number of lambs born per ewe in a given season. This target bears great significance as sheep breeding is a major economic activity in Anatolia, including Kars province. It plays a unique role in the utilisation of vast areas of natural grazing throughout Turkey [19,20]. The fat-tailed Akkaraman sheep, reared in Central and Eastern Anatolia, have adapted well to harsh environmental conditions and are resistant to unfavourable management conditions, poor feeding, and diseases. Approximately 87% of the sheep population in Turkey consist of the fat-tailed breeds mainly Akkaraman, which is considered valuable owing to its productivity [20,21]. In order to meet the increased domestic demand for this breed, it is important that its productive performance is increased and the spread of diseases is prevented. Although lamb morbidity and mortality rates have been reported to be high in this region [6], the risk factors predisposing lambs to morbidity or mortality have not been studied systematically. Therefore, the primary objective of this study was to determine the effect of certain risk factors such as lambing season, birth type, birth weight, gender of lamb, and parity and health status of dam on neonatal (birth to 28 days) and post-neonatal (from 28th day to 84th day) lamb mortality and morbidity in two crossbreed Akkaraman sheep flocks in Kars province, located in north-eastern Turkey. The study also identified the causes of lamb morbidity and mortality in the neonatal and post-neonatal periods. By the quantification of the risk factors, which affected mortality or morbidity, it was possible to identify the points at which changes in management might have decreased the morbidity and mortality rates of the lambs.

MATERIAL and METHODS

Animals, Data Collection and Farm Management

This study was carried out in two sheep farms located in the centre of Kars province in north-eastern Anatolia, Turkey, in 2009. All ewes and lambs were kept under identical feeding and management conditions. Management was typical of North-eastern Anatolian flocks with lambs being

born in winter (December to February) or spring (March to May), and being raised intensively. At birth, the lambs were ear-tagged and registered with an individual identification number, and gender, date of birth, parity of dam, and ear tag number and type of birth were recorded for each lamb. The lambs were weighed at birth (before colostrum intake) using a scale [CASIA DB2-150 kg (±30 g)]. After this procedure, lambs were allowed to naturally suckle their dams. The newborn lambs were kept with their dams during their first week of life. After this period, the lambs were transferred to a separate pen and allowed to suckle twice a day (in the morning and evening) for 3 months. Lambs had access to hay after the first week of neonatal life, and to straw and commercial growth feed (Bayramoglu AS, Turkey) as from the third week of life. This feeding regime lasted for three months. Subsequently, lambs were grazed on pasture and supplemented with hay and commercial feed when they were brought in for the night.

Clinical Examination

Clinical examination and case definition were performed as previously defined by the authors [6]. The health status of the lambs was monitored on farms by visits made on a daily basis during the neonatal period (first 4 weeks of life) and every two days in the post-neonatal period until the 12th week of life. Throughout the study period, ewes were determined to have disease (mastitis, pneumonia, enteritis, pregnancy toxaemia etc.) were categorized as ill and recorded with their ear tag number.

Statistical Analysis

The present study was conducted on 301 Akkaraman crossbreeds and 347 lambs born to these ewes. However, lambs, for which no data was able to be collected related to health status and the parameters investigated for their effects on morbidity and mortality, namely, birth weight, gender, birth type, lambing season, dam health status and parity, were not included in the study. Therefore, only 322 lambs, for which data on all the variables investigated in the study was obtained, were used. Data collected by a longitudinal survey were numerically coded and entered into a database (Microsoft Access) and analysed using EPI INFO 6. The lambs were categorized, based on their clinical examination results as healthy or ill. Clinical examination results were categorized for the neonatal (first four weeks of life) and post-neonatal (first 5 to 12 weeks of life) periods with a view to compare morbidity and mortality rates and their relations with variables. Birth weights were categorized as low (≤3 kg), medium (>3 to ≤4 kg) and high (>4 kg). Dam parity was categorized as 1, 2, 3 and ≥4. EPI INFO 6 [chi-squared (χ^2), odds ratios (OR) and relative risk (RR)] was used to determine the differences in morbidity and mortality rates and risk according to categorical risk factors [birth weight (low, medium, high), parity (1 2, 3, ≥4), gender (male versus female), type of birth (twin versus single), lambing season (winter versus spring), health status of dams (ill versus healthy)]. The significance level was set at $P<0.05$.

RESULTS

Health Status

The morbidity and mortality rates in the neonatal period were determined as 17.3% (60/347) and 3.8% (13/347), respectively. The majority of neonatal deaths occurred (84.6%, 11/13) in the first week of life. Diseases determined in the neonatal period were diarrhoea (n=32), suspected septicaemia (n=11), Fatigue-Anorexia Syndrome-FAS (n=11) and pneumonia (n=6). Twenty-five of the diseased lambs re-contracted disease in the period of 5-12 weeks, and 7 of these animals dead. The proportions of lambs that were ill and dead during the period of 5-12 weeks were 32.6% (109/225) and 4.8% (16/334), respectively. Lamb diseases encountered in this period were diarrhoea (n=62), pneumonia (n=25), pneumo-enteritis (n=12), and suspected septicaemia (n=4), while in some other animals the disease remained unclassified (n=6). *Table 1* presents the morbidities and mortalities during the neonatal and post-neonatal periods on the basis of gender, type of birth, birth weight, lambing season, dam health status and parity.

The strength of statistical associations (χ^2) and some epidemiological parameters (OR and RR) and their 95% confidence intervals for morbidities *(Table 2)* and mortalities *(Table 3)* according to gender, type of birth, birth weight, lambing season, dam health status and parity are presented.

Effect of Gender, Type of birth, Birth Weight, Lambing Season, Maternal Health Status and Parity on Morbidity and Mortality in the Neonatal and Post-neonatal Periods

With regard to birth weight, the neonatal morbidity and mortality rates of lambs in the low weight category were significantly ($P<0.001$) higher than those of medium ($\chi^2=10.4$ and $\chi^2=32.5$, respectively) and high ($\chi^2=26.9$ and $\chi^2=46.5$, respectively) birth weight. The neonatal morbidity rate of lambs with medium birth weight was significantly ($\chi^2=4.97$, $P<0.05$) higher than that of lambs with high birth weight. However, there was no significant difference between the neonatal mortality rates of lambs with medium and high birth weight ($\chi^2=0.1$ $P=0.7$). Additionally, there were no significant differences between the post-neonatal morbidity and mortality rates of lambs with different birth weights. With regard to lambing season, the neonatal mortality rate of lambs born in the winter season (9.2%) was significantly higher than those born in the spring season (1.8%, $\chi^2=9.6$ $P<0.01$). However, no significant difference was determined between lambs born in the winter and spring seasons for neonatal morbidity and post-neonatal morbidity and mortality rates Twin-born lambs displayed higher morbidity rates during both periods

(22.6% and 35.4%, respectively) compared to single-born lambs (14.3% and 31.3%, respectively), but the differences were statistically insignificant (χ^2=3.1 P=0.07 and χ^2=0.4, P=0.4 respectively). Furthermore, there were no significant differences in the neonatal or post-neonatal mortality rates between single- and twin-born lambs *(Table 2* and *Table 3)*.

Neonatal lamb morbidity and mortality rates were higher for the first parity. The neonatal morbidity rate of lambs born to primiparous ewes was significantly higher than that of lambs born to ewes at their second and third parity (χ^2=6.4 P<0.05 and χ^2=7.8 P<0.01, respectively), however, no significant difference was observed in comparison to lambs born to ewes with ≥4 parity (χ^2=1.26 P=0.2). Furthermore, the neonatal mortality rate of lambs born to primiparous ewes was significantly higher than that of lambs born to ewes at their second, third and ≥4 parity (χ^2=9.3 P<0.001, χ^2=14.1 P<0.001 and χ^2=4.2 P<0.05, respectively). However, no significant difference existed between the lambs included in the different parity groups for post-neonatal morbidity and mortality rates. Compared to lambs born to healthy dams, the neonatal morbidity rate of lambs born to ill dams was significantly higher (χ^2=30.38 P<0.001). However, the lambs born to ill and healthy dams did not significantly differ from each other for neonatal mortality and post-neonatal morbidity or mortality rates *(Table 2* and *Table 3)*.

Epidemiological Parameters for Lamb Morbidity and Mortality with Regard to Various Variables

Birth weight was a major risk factor for neonatal morbidity and mortality. In the neonatal period, lambs born with low birth weight when compared to those with medium and high birth weight had significantly (P<0.01 to P<0.001) higher risk of morbidity (OR=3.9 RR=2.5 and OR=8.5 RR=4.9, respectively) and mortality (OR=30 RR= 20.3 and OR=42.7 RR=28.8, respectively). Additionally, in comparison to high birth weight lambs, lambs born at medium birth weight had a higher risk of neonatal morbidity (OR=2.1 and RR=1.9) and mortality (OR/RR=1.4), the statistical difference was only evident for morbidity (P<0.05).) However, no significant differences were detected for mortality risk between lambs with medium and high birth weights in the neonatal period (OR/RR=1.4). Although, in general, it was ascertained that the post-neonatal morbidity and mortality risks of low birth weight lambs were higher than those with medium and high birth weights, and those of medium birth weight lambs were higher than those with high birth weight (generally OR>1), these results did not bear any statistical significance. Lambs born in the winter season had a significantly (P<0.01) higher risk of mortality than lambs born in the spring season (OR=5.5 and RR=5.1). However, there were no significant differences in the risk of neonatal morbidity or post-neonatal morbidity and mortality between lambs that were born in the winter and

Table 1. *Neonatal and post-neonatal morbidity and mortality rates in lambs according to birth weight, type of birth, gender, parity, lambing season, and dam health status*

Table 1. *Kuzularda cinsiyet, doğum ağırlığı, kuzulama sezonu, doğum tipi, annenin doğum sayısı ve sağlık durumuna göre neonatal ve post-neonatal morbidite ve mortalite oranları*

Factor	Group	N	Clinical Examination (%)								
			Period								
			Neonatal					Post-Neonatal			
			N1	Morbidity	N2	Mortality	N	N1	Morbidity	N2	Mortality
BW (kg)	Low	27	13	48.1	9	33.3	18	7	38.9	2	11.1
	Medium	122	23	18.9	2	1.6	120	42	35	5	4.2
	High	173	17	9.8	2	1.2	171	51	29.8	7	4.1
Type of Birth	Twin	84	19	22.6	2	2.4	82	29	35.4	5	6
	Single	238	34	14.3	11	4.6	227	71	31.3	9	4
Gender	Male	173	32	18.5	8	4.6	165	52	31.5	7	4.2
	Female	149	21	14.1	5	3.4	144	48	33.5	7	4.9
Parity	1	54	16	29.6	8	14.8	46	13	28.2	3	6.5
	2	137	19	13.9	4	2.9	133	52	39.1	6	4.5
	3	90	10	11.1	0	0	90	26	28.9	3	3.3
	≥4	41	8	19.5	1	2.4	40	9	22.5	2	5
Lambing Season	Winter	98	17	17.3	9	9.2	89	30	33.7	4	4
	Spring	224	36	16.1	4	1.8	220	70	31.8	10	4.5
Dam's Health	Healthy	17	11	64.7	2	11.8	15	5	33.3	0	0
	Ill	305	42	13.8	11	3.6	294	95	32.3	14	4.8

N: *Total number of animals,* ***N1:*** *Number of ill animals,* ***N2:*** *Number of animals that dead*

Table 2. *The effect of birth weight, type of birth, gender, parity, lambing season and dam health status on lamb morbidity*
Tablo 2. *Kuzu hastalıkları üzerine, cinsiyet, doğum ağırlığı, kuzulama sezonu, doğum tipi, annenin doğum sayısı ve sağlığının etkisi*

Factors	Comparisons	Period	χ^2	OR	95% CI	RR	95% CI
Birth Weight	Low vs. Medium	1	10.35**	3.99**	1.52-10.56	2.55**	1.35-4.32
		2	0.10	1.18	0.37-3.61	1.11	0.49-1.98
	Low vs High	1	26.90***	8.52***	3.12-23.28	4.90***	2.48-8.89
		2	0.63	1.49	0.49-4.48	1.30	0.58-2.29
	Medium vs. High	1	4.97*	2.13*	1.03-4.42	1.91*	1.02-3.61
		2	0.86	1.26	0.74-2.14	1.17	0.81-1.66
Type of Birth	Twin vs. Single	1	3.14	1.75	0.89-3.43	1.58	0.91-2.68
		2	0.46	1.20	0.68-2.12	1.13	0.76-1.62
Gender	Male vs. Female	1	1.13	1.38	0.73-2.63	1.31	0.76-2.27
		2	0.12	0.92	0.55-1.53	0.94	0.67-1.33
Parity	1 vs. 2 lambing	1	6.43*	2.62*	1.14-5.96	2.14*	1.12-3.99
		2	1.74	0.61	0.27-1.35	0.72	0.40-1.20
	1 vs. 3 lambing	1	7.82**	3.34**	1.29-8.91	2.67*	1.23-5.93
		2	0.006	0.97	0.41-2.28	0.97	0.51-1.76
	1 vs. ≥4 lambing	1	1.26	1.74	0.60-5.12	1.52	0.68-3.61
		2	0.37	1.36	0.46-4.05	1.26	0.56-2.93
	2 vs. 3 lambing	1	0.37	1.28	0.53-3.15	1.25	0.58-2.78
		2	2.46	1.58	0.86-2.92	1.35	0.90-2.07
	2 vs. ≥4 lambing	1	0.78	0.66	0.25-1.83	0.71	0.32-1.68
		2	3.71	2.21	0.91-5.46	1.74	0.94-3.57
	3 vs. ≥4 lambing	1	1.67	0.52	0.17-1.59	0.57	0.22-1.50
		2	0.57	1.39	0.54-3.67	1.28	0.65-2.77
Lambing Season	Winter vs. Spring	1	0.08	1.09	0.55-2.15	1.07	0.61-1.87
		2	0.10	1.09	0.62-1.89	1.05	0.72-1.51
Dam's Health	Ill vs. Healthy	1	30.38***	11.48***	3.67-37.17	4.69***	2.61-6.65
		2	0.007	1.04	0.31-3.45	1.03	0.39-1.96

Periods; 1: *Neonatal (First 4 weeks of life)* ***2:*** *Post-Neonatal (Period from the first 5th to 12th weeks of life), * P<0.05 ** P<0.01 *** P<0.001*

spring seasons. Twin-born lambs had a higher risk of morbidity in both periods (OR=1.8 RR=1.6 and OR=1.2 RR=1.1, respectively) than single-born lambs, but the differences were not significant (P=0.08 and P=0.4, respectively). Furthermore, there were no significant differences between twin- and single-born lambs for the risk of neonatal or post-neonatal mortality. The risk of neonatal and post-neonatal morbidity and morbidity did not differ between males and females *(Table 2* and *Table 3)*.

First parity was found to be an important risk factor for increased neonatal lamb morbidity and mortality. The risk of neonatal lamb morbidity for the first parity, compared to the second and third parity (OR=2.6 RR=2.1 and OR=3.3 RR=2.6, respectively), was significantly higher (P<0.05 and P<0.01). Similarly compared to lambs born to ewes with 2, 3, ≥4 parity (OR=5.7 RR=5.1, uncalculated and OR=6.9 RR=6.1, respectively), it was ascertained that the neonatal mortality risk of the first parity lambs was significantly higher (P<0.01, P<0.001 and P<0.05, respectively). It was observed that the neonatal and post-neonatal morbidity and mortality risks were higher for lambs born to ewes at their first parity compared to those born to ewes with 2, 3 and ≥4 parity, and it was also ascertained that the risks for the second parity were higher than those for 3 and ≥4 parity, while the risks for the third parity were greater than those for ≥4 parity (generally OR>1), yet, no statistical significance apart from that mentioned above was detected (P>0.05). Furthermore, it was determined that the risks neonatal morbidity and mortality for lambs born to ill ewes were greater than those born to healthy ewes (OR=11.4 RR=4.6 and OR=3.5 RR=3.2, respectively), but only the risk for morbidity was statistically significant (P<0.001) *(Table 2* and *Table 3)*.

Table 3. *The effect of birth weight, type of birth, gender, parity, lambing season and dam's health status on lamb mortality*
Tablo 3. *Kuzu ölümleri üzerine, cinsiyet, doğum ağırlığı, kuzulama sezonu, doğum tipi, anne doğum sayısı ve sağlığının etkisi*

Factors	Comparisons	Period	χ^2	OR	95% CI	RR	95% CI
Birth Weight	Low vs. Medium	1	32.47***	30.00***	5.35-220.9	20.33***	4.47-133.9
		2	1.57	2.88	0.35-19.09	2.67	0.37-14.29
	Low vs. High	1	46.52***	42.75***	7.66-313.1	28.83***	6.31-190.1
		2	1.77	2.93	0.38-17.59	2.71	0.39-12.83
	Medium vs. High	1	0.13	1.43	0.14-14.36	1.42	0.14-13.96
		2	0.001	1.01	0.27-3.68	1.01	0.28-3.49
Type of Birth	Twin vs. Single	1	0.81	0.51	0.08-2.48	0.52	0.08-2.37
		2	0.63	1.57	0.44-5.34	1.54	0.45-4.87
Gender	Male vs. Female	1	0.33	1.39	0.41-5.03	1.38	0.42-4.77
		2	0.07	0.87	0.26-2.83	0.87	0.28-2.71
Parity	1 vs. 2 lambing	1	9.31**	5.78**	1.48-24.14	5.07*	1.44-19.59
		2	0.28	1.47	0.27-7.05	1.45	0.29-6.24
	1 vs. 3 lambing	1	14.11***	NC***	2.76-NC	NC**	2.59-NC
		2	0.73	2.02	0.31-13.25	1.96	0.32-11.88
	1 vs. ≥4 lambing	1	4.16*	6.96*	0.82-154.7	6.07*	0.83-129.3
		2	0.09	1.33	0.16-12.01	1.30	0.18-10.98
	2 vs. 3 lambing	1	2.66	NC	0.43-NC	NC	0.44-NC
		2	0.19	1.37	0.29-7.12	1.35	0.31-6.75
	2 vs. ≥4 lambing	1	0.03	1.20	0.12-29.09	1.19	0.13-28.8
		2	0.02	0.89	0.15-6.73	0.90	0.17-6.40
	3 vs. ≥4 lambing	1	2.21	0.00	0.00-7.94	0.00	0.00-7.87
		2	0.21	0.65	0.08-5.88	0.66	0.09-5.63
Lambing Season	Winter vs. Spring	1	9.6 **	5.56**	1.51-22.08	5.14**	1.48-19.58
		2	0.00	0.98	0.25-3.55	0.99	0.26-3.32
Dam's Health	Ill vs. Healthy	1	2.76	3.56	0.49-19.59	3.26	0.51-13.49
		2	0.74	0.00	0.00-7.53	0.00	0.00-6.08

Periods; 1: *Neonatal (First 4 weeks of life)* ***2:*** *Post-Neonatal (First 5- 12 weeks of life)* ***NC:*** *Not calculated * P<0.05 ** P<0.01 ** P<0.001*

DISCUSSION

This study was carried out examine the effect of birth weight, gender, birth type, lambing season, and dam parity and health status on lamb morbidity and mortality during the first 12 weeks of life. This was the first detailed study for the determination of neonatal lamb morbidity and mortality and associated risk factors in a large number of lambs [6]. The mean morbidity and mortality rates ascertained in this study (7% and 10%, respectively) were lower than those reported by Gokce and Erdogan [6]. This difference might be attributed to different farm management programs. Farm management and particularly feeding programs, have a major effect on certain parameters including birth weight. The main causes of neonatal morbidity and mortality were found to be similar to those reported by Gokce and Erdogan [6].

In the present study, lamb birth weight was a significant risk factor, as lambs with low birth weight had a greater risk of mortality and morbidity, compared to those with medium or high birth weights. This, in general, is in agreement with previous reports [8,9,18,22-25]. It has previously been reported that lambs with a birth weight ≤3 kg have a significantly greater risk of mortality [16,26]. Khan et al.[16], hypothesized that lambs with low birth weight, being physically weak, were unable to suckle sufficient amounts of colostrum as a result the IgG concentration in their serum remained low, which might have led to an increased mortality and hypothermia in these lambs. Similarly, other researchers also argued that, lamb survival depends on good preferable weight and an adequate colostrum intake [2,27]. This was confirmed in the present study, in where the mortality rate of lambs, with birth weights less than 3 kg, was higher than those having birth weights greater than 3 kg. The authors of the present study also found that, the serum IgG level of lambs with low birth weights (≤3 kg) was lower than the lambs born at medium (>3 to ≤4 kg) or high (>4 kg) birth weights [28]. Apart from being a source of immunoglobulins, colostrum is also a major source of

energy. Lambs born at a low birth weight rapidly consume their body energy reserves and eventually, may die due to hypothermia if not able to suckle enough colostrum [18,29]. In this respect, the implementation of management programmes targeted at increasing lamb birth weight (i.e. sufficient concentrate feed intake by ewes in the last trimester of pregnancy), as well as the observation of animals during the lambing season so that the timely contact of lambs born at low birth weight is ensured with their dams (the housing of the lamb and dam in the same paddock, etc.) as strong lamb-dam interaction results in both colostrums production and colotrum intake in lambs and the control of body temperature with an aim to prevent hypothermia (adequate and early intake of colostrum by the lamb, treatment with glucose, enabling of drying of the animal etc.) are considered as important factors in the survival of lambs [18,22,26,29-31]. Factors that have effect on birth weight include birth type and gender of lambs, prenatal nutrition, health status, parity and placental size of dam as well as foetal genotype [18,26,28,30] therefore, measures aimed at increasing birth weight should address these factors especially at last trimester of gestation.

In the present study, a significantly higher proportion of lambs dead in the neonatal period during the winter season. Lambs born in the period from December to February were about 5 times more likely to die than were lambs born in the period from March to May. This may be linked to high stocking density or overcrowding, climate and weather conditions, as well as to the high infection potential in the winter season resulting from the accumulation of manure and contaminated bedding. This result is in line with some previous investigations [22,32,33]. Previous studies have pointed out to the fact that environ-mental conditions arising from harsh climate (cold, wind, rain), together with poor management, increase losses [6,22,33]. However, an insignificant effect of birth season on mortality rate was reported by Turkson and Sualisu [8], Mandal et al.[34], and Piwczynski et al.[35]. Severe cold weather can stress lambs, increasing their energy requirements by 500 percent, and the depletion of their energy reserves where in particularly lambs born at low birth weight susceptible to cold stress, starvation and disease exposure [2,4].

The odds of death or disease during the neonatal and post-neonatal periods did not differ significantly between the genders in this study. This finding is in line with previous reports obtained for the first three months of life [7,8,16,22,34,35]. As the birth weight of male lambs is expected higher, males are considered to be born physically stronger and to have greater advantage with regards to protection from diseases and the development of passive immunity. Thus, morbidity and mortality rates of male lambs are expected to be lower than those of female lambs. Aksakal et al.[36], having studied the Awassi breed, observed higher mortality rates in female lambs. However, some studies reported that, male lambs displayed higher mortality than females, particularly after the first three months of life [2,8,9,13,33,34]. This is attributed to dystocia caused by male lambs as they are usually born heavier and the advantage of female lambs to recognize their dams when compared to their male counterparts [15,22,26,30]. Similarly, Nash et al.[15], suggested that the gender variation observed in mortality, with females and castrated males having similar and lower risk, could reflect the effect of male hormones on immune function.

In the present study, an insignificant statistical relationship was observed for mortality rates between single- and twin-born lambs, which is in agreement with the results of Turkson and Sualisu [8], Turkson [37], Mandal et al.[34] and Yapi et al.[7]. However, the twin-born lambs were observed to be confronted with multiple disadvantages, including reduced low birth weight [18,28,35], increased exposure to mismothering, more difficult access to udders, competition for access to feed, inadequate suckling of colostrum or milk [1,2,9,18,29,35,36]. Nevertheless, in the present study, the association of birth type was marginal with only neonatal morbidity ($P = 0.07$). Similarly, mortality in twin-born lambs in the two periods were not significantly different from those in single-born lambs. Twinning slightly increased the risk of mortality in the post-neonatal period, but this difference was statistically insignificant. This result was similar to that reported by Turkson and Saualisu [8] and that reported by Nash et al.[2]. However, some studies [1,2] argued that multiple births increased the risk of neonatal or perinatal (first week of life) mortality.

Parity has a significant effect on neonatal lamb morbidity and mortality and this finding is in agreement with previous literature reports [9,15,16,25,30,34,36]. In the present study, the risk of neonatal mortality and morbidity tended to be higher for the first parity compared to ewes that had given birth before. This was attributed to primiparous dams producing less and low quality colostrum/milk and displaying poor mothering ability (mismothering). Primiparous ewes show impairments in the expression of maternal behaviour when compared to multiparous ewes [31]. Additionally, the first parity is characterized by a lower lamb birth weight and poor postnatal vigour compared to second and further births [15,18,28,30,31,34,38]. Furthermore, lambs born to primiparous ewes display slower neonatal behavioural progress (i.e. standing up and reaching the udder), compared to lambs born to more experienced multiparous ewes [30,31]. These factors increase either the risk of hypothermia and failure of passive immunity transfer or susceptibility to infection in lambs suffering from malnutrition born to primiparous ewes. The present study also observed that the poor health status of ewes increases both the rate and risk of neonatal morbidity and mortality in lambs. This could be explained with the insufficient production of colostrum or milk and the poor mothering ability observed in these ewes [25,29,39]. Consequently, the risk of morbidity and mortality increased in their lambs.

The present investigation revealed some important

environmental and animal-related factors that affect lamb morbidity in the Akkaraman crossbreed. The birth weight of lambs being lower than ≤3 kg and first parity significantly increased neonatal morbidity and mortality, while the poor health status of ewes significantly increased neonatal morbidity, and birth in the winter season increased the rate and risk of neonatal mortality. When developing programmes targeted at the prevention of potential mortality and morbidity, these results should be taken into consideration. However, further research is required to be conducted on a larger number of animals and farms as the number of lambs dead within the first 12 weeks of life was low in our study which may limit the interpretation of our results.

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RESEARCH ARTICLE

Comparison of the Effects of Bitter Melon *(Momordica charantia)* and Gotu Kola *(Centella asiatica)* Extracts on Healing of Open Wounds in Rabbits

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Summary

This study investigated the effects of topically applied oily homogenized and powder forms of bitter melon (*Momordica charantia*) (MC) and ointment formulation of gotu kola (*Centella asiatica*) (CA) extract and compared the results with untreated control and pure olive oil groups on wound healing in rabbits. A total of 30 New Zealand rabbits were divided into five equal groups (oily homogenized form of MC, powder form of MC, ointment of CA, control, pure olive oil). Full-thickness 5x5 cm skin wounds were created on the right mid-dorsum area and experimental groups were treated daily with the above mentioned extracts. Wounds were observed daily. Planimetry was performed for the unhealed wound area and the percentage of total wound healing on days 0, 7, 14, 21 and 28. Median time for the first observable granulation tissue was shorter in all experimental groups than in the control group ($P<0.05$). Filling of the open wound to skin level with granulation tissue was faster in the oily homogenized form of MC and ointment of titrated extract of CA groups ($P<0.05$). The average time for healing was shorter in the oily homogenized form of MC and ointment of titrated extract of CA groups than in other groups ($P<0.05$). The results demonstrate that topical application of the oily form of MC and ointment form of CA results in significant improvements on wound healing in rabbits.

Keywords: *Wound healing, Rabbit, Momordica charantia, Centella asiatica*

Tavşanlarda Kudret Narı *(Momordica charantia)* ve Gotu Kola *(Centella asiatica)* Ekstraktlarının Açık Yara İyileşmesi Üzerine Etkilerinin Karşılaştırılması

Özet

Bu çalışmanın amacı; tavşanlarda açık yara iyileşmesinde topikal *Momordica charantia*'nın (MC) yağlı homojenize formu ve toz formları ile *Centella asiatica* (CA) ekstratının pomat formunun etkilerini araştırmak, saf zeytinyağı uygulanan ve sağaltım uygulanmayan kontrol grubu ile karşılaştırmaktı. Bu çalışmada kullanılan otuz adet Yeni Zelanda tavşanı 5 gruba ayrıldı: MC'nın yağlı homojenize formu, MC'nın toz formu, CA'nın titre edilmiş ekstratının pomat formu (Madécassol® pomat), kontrol ve saf zeytinyağı. Her bir tavşanda dorsal orta hattın sağ tarafında tam kalınlıkta deriyi kapsayan birer yara (5x5 cm) oluşturuldu ve yukarıda belirtilen ekstratlarla tedavi uygulandı. Yaralar günlük olarak izlendi ve 0, 7, 14, 21 ve 28. günlerde iyileşmemiş yara alanı ve total yara iyileşme yüzdesini ölçmek için planimetri uygulandı. İlk gözlenebilir granulasyon dokusu için ortalama zaman; tüm deney gruplarında kontrol grubundan daha kısa bulundu ($P<0.05$). Yara yatağının granulasyon dokusu ile deri düzeyine kadar dolması; MC yağlı homojenize formu ve CA titre edilmiş ekstratının pomat formu uygulanan gruplarda diğer gruplardan daha hızlı idi ($P<0.05$). Ortalama iyileşme zamanı, MC yağlı homojenize formu ve CA titre edilmiş ekstratının pomad formu uygulanan gruplarda, diğer gruplardan daha kısa idi ($P<0.05$). Bu çalışmada elde edilen sonuçlar; MC yağlı homojenize formu ve CA ekstratının pomad formunun topikal uygulamasının, tavşanlarda açık yaraların iyileşme prosesinde önemli gelişmelere yol açtığını göstermiştir.

Anahtar sözcükler: *Yara iyileşmesi, Tavşan, Momordica charantia, Centella asiatica*

INTRODUCTION

In the past decade, research has been focused on the scientific evaluation of traditional herbal drugs. Bitter melon *(Momordica charantia)* is one such plant that has been frequently used as medicine [1-3]. *Momordica charantia* Linn., family Cucurbitaceae, also known as bitter pear melon, bitter gourd, balsam pear or balsam apple is a tropical

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annual plant that grows freely around dwelling places in uncultivated open spaces [4,5]. *M. charantia* has been reported to possess antilipolytic [6], analgesic [7], abortifacient [8], antiviral [9], cytotoxic [10], hypoglycemic [11] and antimutagenic [12], and also antidiabetic, antileukemic, antibacterial, anthelmintic, antimycobacterial, antioxidant, antiulcer, antiinflammatory, hypotensive, immunostimulant, and insecticidal properties [13-17]. Recently, *M. charantia* is cultivated in the fields at Marmara region (western Anatolia) [17]. The effects of aqueous extracts of *M. charantia*, which are widely used for various purposes in Turkey had been previously investigated [18,19]. The other common preparation type of *M. charantia* is "oily extract". This extract is used externally for the rapid healing of wounds and internally for the treatment of peptic ulcers [1,14,20].

Gotu kola (*Centella asiatica*), has been used as a traditional herbal medicine in Asiatic countries for hundreds of years [21]. A dermal product containing ingredients of Centella is reportedly useful in wound healing [21-23], and is reported to have antiulcerogenic [24], antimicrobial [21], sedative, anti-depressant, analgesic, and anticonvulsive properties [25] as well in Europe. *C. asiatica* contains three principal triterpenoid ingredients: Asiaticoside, asiatic acid, and madecassic acid which were found to contribute to wound healing [26,27]. Asiaticoside is the main active ingredient of *C. asiatica* and exhibits significant wound-healing activity in normal and delayed-healing models [28]. The wound healing property of *C. asiatica* extract has led to its commercial introduction under the trade name, Madécassol® [29].

The present work was undertaken to study the effects of topically applied oily homogenized and powder forms of *M. charantia* and ointment formulation of *C. asiatica* extract on wound healing in rabbits and to compare the results with untreated control wounds.

MATERIAL and METHODS

Plant Material

Fresh fruits of *M. charantia* were purchased from a herbalist in Bursa, Turkey and was authenticated at the Department of Pharmacognosie, Division of Biology, Faculty of Arts and Sciences, Uludag University, Bursa, Turkey with accession number *BULU32549B*.

Preparation of the Plant Material

The mature, fresh fruits of *M. charantia* were cut into small pieces. Hundred grams of chopped plant was immersed in 500 ml of olive oil and put inside a jar of pure olive oil and left under sunshine until the fruit and the seeds dissolved for approximately 20 days. Then they were homogenized by pressing with a spoon and were put into a refrigerator. The undiluted oily homogenized form of *M. charantia* was used in the study. Another 100 g portion of bitter melon was chopped into small pieces and put into a jar. The jar was placed in shadow and was covered with a semi-porous sheet allowing the evaporation of the liquid content of the plant, but limiting the entrance of dust or other contaminants. The dried fruit was then grinded to produce powder form.

Madécassol® (1% ointment, Bayer Co, Istanbul, Turkey), a formulation based on the titrated extract of *C. asiatica*, was obtained from the local pharmacy and used in this study. Madecassol® contains hydrocotyle (*Centella asiatica*; reconstituted titrated dry extract containing 40% asiaticoside and 60% madecassic and asiatic acids) and the other ingredients are essential oils of lavender and geranium, and purified water.

Study Population

A total of 30, six-month-old, New Zealand female rabbits (average weight: 2-2.5 kg) supplied by the Experimental Animals Unit at Uludag University, were used in the study. The rabbits were kept in standard cages, one in each, with 12 h light-12 h dark cycles. The room temperature and humidity were maintained at 19±1°C and 55±10%, respectively. All rabbits were fed 160 g pelleted rabbit diet (Ankara Feed-stuff Industry, Ankara, Turkey) daily and water was available *ad libitum*. The study protocol was approved by Uludag University Animal Care and Use Committee (Approval Number: 01-04/2010).

Rabbits were divided into five groups of six animals each: Oily homogenized form of *M. charantia* (MC), powder form of MC, ointment of titrated extract of *C. asiatica* (CA) (Madécassol® ointment, Bayer Co.), control and pure olive oil. A complete blood count was performed for each rabbit on days 0, 7, 14, 21 and at the end of the study.

Anesthesia

After premedication with xylazine HCl (Rompun®, Bayer Co.) (3 mg/kg, IM), anesthesia was maintained with ketamine HCl (Alfamine®, Alfasan International BV, Woerden, The Netherlands) (50 mg/kg, IM). Bacterial prophylaxis was achieved using intramuscular cephazolin sodium (Cefozin®, Bilim, Istanbul, Turkey) (30 mg/kg). All animals received routine pain control with subcutaneous carprofen (Rimadyl®, Pfizer Inc., Zaventem, Belgium) (4 mg/kg) for two days after wounding.

Operative Procedure

Each rabbit was positioned in sternal recumbency and the dorsal hair was clipped with an electric razor. The skin surface was surgically prepared with povidone-iodine (Betadine®, Kansuk, Istanbul, Turkey), and then draped. Full-thickness skin wounds (5 × 5 cm) were made with #11 scalpel blade on the right mid-dorsum area of each rabbit by excising the skin and the underlying cutaneous trunci muscle *(Fig. 1)*. Hemostasis was achieved by compressing sterile surgical sponges.

Treatment Protocol

Wounds were treated topically each day with the oily homogenized form of MC in group I, powder form of MC in group II, ointment of titrated extract of CA in group III, and pure olive oil (group V) from the first day after wound creation until complete healing occurred. Control group rabbits were left untreated (group IV). After applications, the wound areas were bandaged with sterile non-adherent pads and porous adhesive tapes.

Evaluation of Wound Healing

- Planimetry

Planimetry was performed on days 0, 7, 14, 21 and 28 on anesthetized animals with similar protocol, although measurements were made daily until day 28. The wound area of each lesion on each evaluation day was obtained by tracing the perimeter of the wound onto a sterile piece of clear acetate film with a special marking pen. The outlined area was defined as 'total wound area'. Thereafter, the examiner traced the margin at the leading edge of the advancing epithelium. This area was defined as 'unhealed wound area'. Wound tracings were digitized using digital scanning software (Sigma Scan® Pro 5.0, Systat Software Inc., San Jose, CA, USA) and the percentage of total wound healing was calculated by using a previously described two-step formula [30]. The unhealed wound area and the percentage of total wound healing were recorded at each day of measurement and used for statistical analysis.

Step 1

$$\text{Open wound day}_n \text{ as \% of original} = \frac{\text{Open wound area day}_n \times 100}{\text{Original wound area (day}_0\text{)}}$$

Step 2

$$\text{\% total wound healing day}_n = 100 - \text{Open wound day}_n \text{ as \% of original}$$

- Observations During Daily Wound Care

The bandages were changed daily and medication was applied at each bandage change. Each wound was evaluated for the presence of exudate or other abnormalities and wound appearance during the bandage changes. Information regarding the day that the first granulation tissue was observed, the day that the wound was covered, and the day the wound was completely filled with granulation tissue and epithelialized were recorded. The observations were performed in a non-blinded manner.

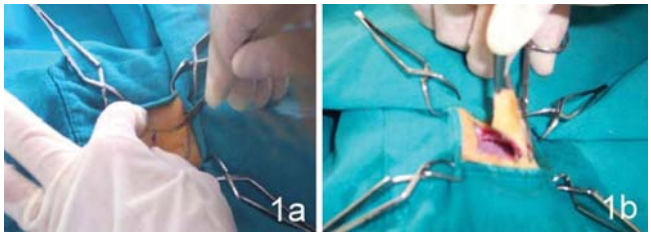

Fig 1. Excision of the skin and the underlying cutaneous trunci muscle with scalpel blade and scissors for creating identical full-thickness skin wounds (5 × 5 cm)

Şekil 1. İdentik ve tam kalınlıkta açık yara oluşturmak için deri ve altında bulunan *M. cutaneus trunci*'nin bistüri ve makasla eksizyonu (5 x 5 cm)

Statistical Analysis

All the data were calculated and the mean values were compared among the four groups using repeated measures model for analysis of variance (ANOVA). Where existed, the differences were determined by Duncan's multiple-range test. All analyses were performed using SPSS 13.0 (SPSS Science, Chicago, IL, USA). A *P*-value lower than 0.05 was considered significant.

RESULTS

Observations During Daily Wound Care

On the first day, all wounds appeared clean. In group II and IV, wounds had fresh view, while wounds in group I had initial signs of healing. All wounds were free of exudate throughout the study. On day 7, the wound size was reduced in all cases. In the group III, granulation tissue formation was easily noticeable at wound edges, especially it was more significant at the caudal region in three cases. In three cases of this group, black brown scab was noticed over the wounds. In the group I, granulation tissue formation was easily noticeable on the wound edges especially at caudal and ventral regions and black-grayish scabs were seen over some areas of the wound surface. In the group II, the powder accumulation was noticed as scab-like appearance over the wound surfaces with an increased amount of epithelial tissue at the wound edges. In the group IV and V, wounds exhibited thin granulation tissue formation at all wound edges on day 7 *(Fig. 2)*. On day 14, all wounds continued to reduce in size in parallel to wound healing. In the group III, a small elevation of granulation tissue in addition to gray-brownish scab was observed. In the group I, it had a crust-like appearance at wound rims. In the group II, when the hard crust over the wounds was removed, the underlying tissue was dark red and was considered as granulation tissue. Caudal and dorsal edges were still separate from the underlying tissues; adhesion was observed on cranial and ventral edges only. On day 21, coverage of the wound bottom and wound filling with granulation tissue were remarkable in the group I and III. In the group IV and V, wounds were usually flat with uncomplete epithelialization and no evidence of elevation with granulation tissue. On day 28, in the group I and III, all wounds had complete coverage of the wounds with granulation tissue and epithelialization, whereas wounds in the other three groups were not completely epithelialized *(Fig. 2)*.

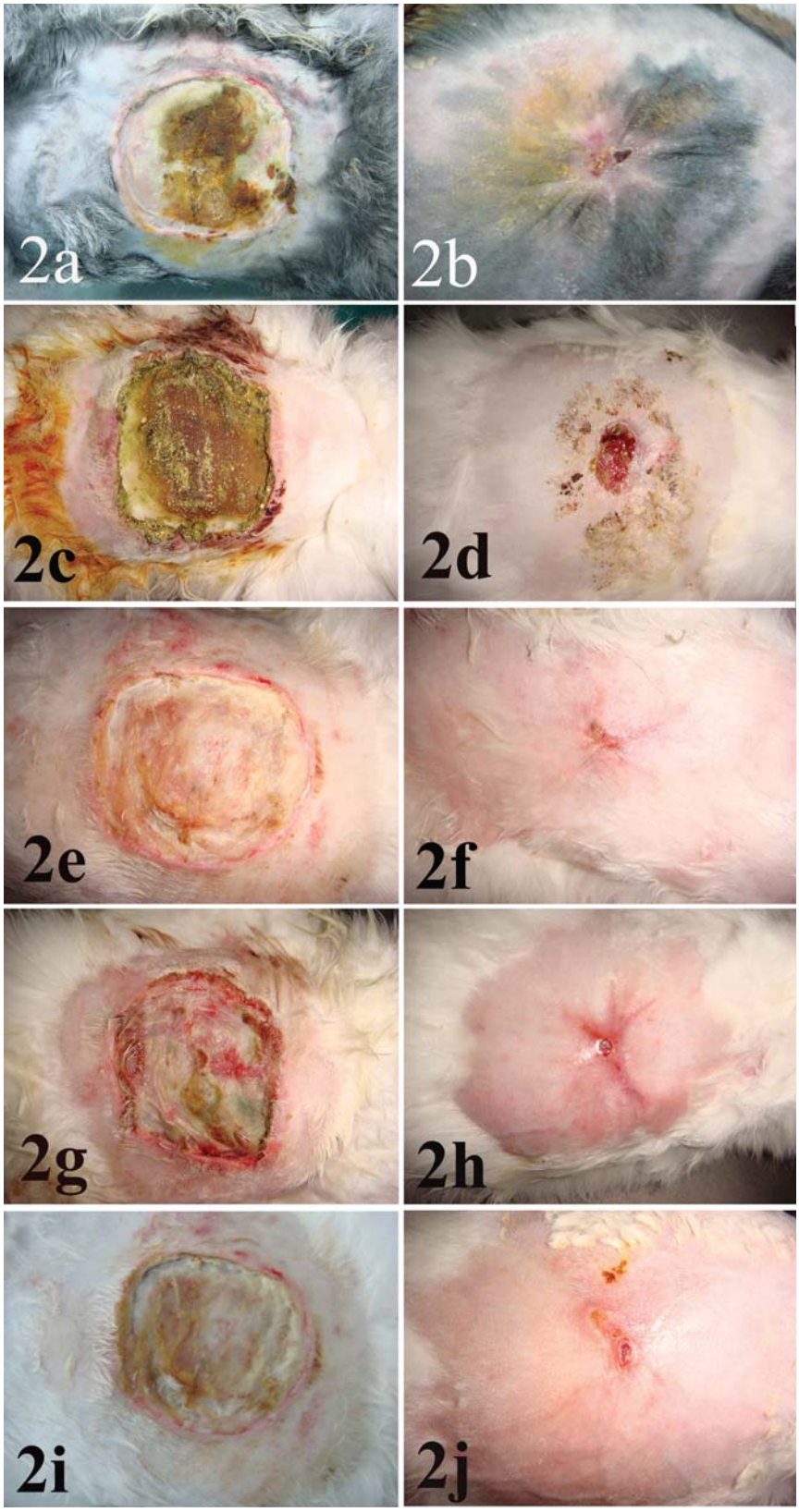

Fig 2. Progression of wound healing in the oily homogenized form-MC, powder form-MC, ointment form-CA, control and pure olive oil groups *(top to bottom, respectively)* on days 7 *(left column)* and 28 *(right column)*. Remarkable granulation tissue formation can be seen at the wound edges in the oily homogenized form-MC (a) and ointment form CA (e) treated animals on day 7. In the powder form of MC group, the wound size was reduced and scab-like powder accumulation was noticed over the wound surface (c). Thin granulation tissue formation is present at all wound edges in the control and pure olive oil group (g, i) on the same day. On day 28, all rabbits in the oily extract form of MC (b) and ointment of titrated extract of CA-treated animals (f) had complete coverage of the wounds with granulation tissue and epithelialization, whereas wounds in the other three groups were not completely epithelialized (d, h, j)

Şekil 2. Yukarıdan aşağıya sırasıyla MC- yağda hazırlanan homojenize formu, MC- toz formu, CA- pomadı ile tedavi edilen, kontrol ve saf zeytinyağı grubu tavşanlarda yara iyileşmesinin ilerlemesi (sol sütun 7. gün, sağ sütun 28. güne aittir). MC- yağda hazırlanan homojenize formu (a) ve CA- pomadı (e) uygulanan tavşanlarda 7. günde yara kenarlarında belirgin granulasyon dokusu gözükmekte. MC- toz formu grubunda, yara boyutu küçülmüş ve yara üzerinde kabuk benzeri toz birikimi gözlenmekte (c). Aynı günde kontrol ve saf zeytinyağı grubunda, tüm yara kenarlarında ince bir granulasyon dokusu gelişimi mevcut (g, i). MC- yağda hazırlanan homojenize formu (b) ve CA- pomadı (f) ile tedavi edilen tüm tavşanlarda 28. günde yaraların tümü granulasyon dokusu ve epitelizasyon ile kapanmışken, diğer üç gruptaki yaraların tam olarak epitelize olmadığı gözlenmekte (d, h, j)

The median time for the first observable granulation tissue was shorter in the group I, II and III than in the control and pure olive oil group (2, 2, 2 vs. 3.5, 3.3 days, respectively) (P<0.05), but was not different among these groups (2, 2 vs. 2 days) (P>0.05). Filling of the open wound to skin level with granulation tissue was faster in the group I and III than in the group II, IV and V (14, 16 vs. 23, 25, 24 days, respectively) (P<0.05), but was not significantly different between the group II, IV and V (23, 25 vs. 24 days, respectively) (P>0.05).

The average time for healing was shorter (P<0.05) in the group I and III than in the group II, IV and V (27.42, 27.66 vs. 30.66, 32.66, 31.58 days, respectively), but was not different between the group II, IV and V (30.66, 32.66 vs. 31.58 days, respectively) (P>0.05). Granulation tissue did not become excessive at any of the wounds either in the treated or control groups during this study. Complete blood count values were within normal limits on days 7, 14, 21 and 28 (data not shown).

Planimetry

A significant decrease in wound area was measured in group III when compared with the other four groups on day 7 (P<0.05, *Table 1*). The mean unhealed wound area in the group I and III was significantly smaller than in the other three groups on days 14 and 21 (P<0.05, *Table 1*). The mean percentage of total wound healing in the group III was significantly higher than in the other groups on day 7 (P<0.05). On days 14 and 21, the mean percentage of total wound healing in the group I and III was significantly higher than in the other groups (P<0.05), but no significant differences were observed between these two groups (P>0.05, *Table 1*). At the end of the study, all wounds in the group I and III had fully recovered, whereas wounds in the other three groups were not completely epithelialized.

DISCUSSION

Wound healing is a complex biological process, including inflammation, cell migration, angiogenesis, extracellular matrix synthesis, collagen deposition, and re-epithelialization [31]. Plant products are potential agents for wound healing and largely preferred because of their widespread availability, non-toxicity, ease of administration, absence of unwanted side effects and their effectiveness as crude preparations [32,33]. These findings prompted us to further investigate other tropical plants which had been reported to have medicinal values for *in vivo* wound healing.

M. charantia is a plant effectively used for the rapid healing of wounds in folk medicine [4]. Prasad *et al.*[34] researched wound-healing property of *M. charantia* and showed a statistically significant response (P<0.01) in terms of wound contracting ability, wound closure time, period of epithelialization when compared with the control group in an excision, incision and dead space wound model in rats. Sharma et al.[35] observed significant wound healing activity in animals treated with Momordica extract compared with other groups in rats. In our excision wound model, animals treated by oily homogenized form of *M. charantia* showed a significant reduction in wound area and time for epithelialization and these animals showed faster epithelialization of wounds than the powder form of *M. charantia,* the pure olive oil and control groups. Healing after *M. charantia* application was similar to that observed after *C. asiatica* application. We believe that the constituents present in the oily homogenized form of *M. charantia* may be responsible for promoting the wound healing activity. Our findings are in agreement with those demonstrated previously by Teoh *et al.*[36] and Ono *et al.*[37].

Madécassol®, a formulation based on the titrated extract of *C. asiatica* is a well-known commercial ointment for promoting dermal wound healing. This extract significantly shortens the wound-healing time, acting more specifically on the immediate process of healing [28]. Our findings are in agreement with those demonstrated previously by Shukla *et al.*[28], and Poizet and Dumez [29].

A dry, desiccated wound will not heal as good as a moist wound. Winter [38] proposed his classic hypothesis that the optimum environment for epithelialization is a moist environment. Topical ointments and gels provide such an environment and aid wound healing. In our study,

Table 1. *Comparison of the mean unhealed wound area and the percentage of total wound healing on days 7, 14, 21 and 28 among groups of rabbits treated with oily extract form-MC, powder form-MC, ointment form-CA, untreated controls and pure olive*

Tablo 1. *MC- yağda hazırlanan ekstratı, MC- toz formu, CA- pomadı ile tedavi edilen, kontrol grubu ve saf zeytinyağı grubu tavşanlarda ortalama iyileşmemiş yara alanı ve total yara iyileşme yüzdelerinin 7,14, 21 ve 28. günlerde karşılaştırılması*

Group	Day 0	Day 7		Day 14		Day 21		Day 28	
	Wound Area (cm^2)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)	Unhealed Wound Area (mm^2 ± SE)	Total Wound Healing (%)
Group I (oily homogenized form-MC)	25.0	19.64±4.81[a]	26.70±14.74[a]	4.85±2.17[b]	80.60±5.67[b]	0.74±0.25[b]	97.18±1.02[b]	0	100
Group II (powder form-MC)	25.0	19.95±4.52[a]	26.27±15.24[a]	11.39±3.12[a]	54.44±9.02[a]	5.69±3.30[a]	91.06±9.48[a]	1.73±0.52	99.23±1.87
Group III (ointment form-CA)	25.0	15.68±1.54[b]	30.17±10.69[b]	5.59±3.29[b]	77.64±28.67[b]	0.94±0.78[b]	95.13±4.41[b]	0	100
Group IV (Control)	25.0	19.59±4.17[a]	27.08±11.26[a]	12.59±4.82[a]	49.64±8.87[a]	6.92±2.03[a]	90.43±6.56[a]	1.78±1.12	98.23±1.59
Group V (Pure olive oil)	25.0	19.18±2.95[a]	27.26±11.80[a]	12.33±.3.00[a]	50.67±12.01[a]	6.37±2.26[a]	90.78±5.59[a]	1.74±1.63	98.96±2.24

[a,b] *Different superscripts within the same column indicate significant difference among groups (P<0.05)*

the rate of wound contraction in treated rabbits by the oily homogenized form of *M. charantia* and the ointment of titrated extract of *C. asiatica* was significantly higher. Furthermore, the period of epithelialization was shorter in treated wounds. These results further support the effectiveness of *M. charantia* and *C. asiatica* in wound healing.

We did not observe any abnormal findings in the complete blood count throughout the study and all rabbits were clinically healthy throughout the study which suggest that topical applications of *M. charantia* or *C. asiatica* do not result in systemic abnormalities.

The results obtained in the present study demonstrate that topical application of the oily homogenized form of *M. charantia* and ointment form of *C. asiatica* showed significant increase on the healing process of open wounds in rabbits and encourage us to carry out a wider and more profund study on these plants to obtain better knowledge about their therapeutic potentials.

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RESEARCH ARTICLE

Abomazum Deplasmanlı Sütçü Sığırlarda D (-) ve L (+) Laktik Asit ile Bazı Biyokimyasal ve Hematolojik Parametrelerin Diagnostik ve Prognostik Açıdan Öneminin Belirlenmesi [1]

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Özet

Bu araştırmanın amacı, abomazum deplasmanlı ineklerde D(-) ve L(+) laktik asit ile bazı biyokimyasal ve hematolojik parametrelerin tanı ve prognozdaki önemini değerlendirmektir. Çalışmada, 10 sağa, 10 sola abomazum deplasmanlı ve 10 sağlıklı (kontrol) olmak üzere 3 farklı grupta toplam 30 süt ineği kullanıldı. Çalışmada kan pH, HCO_3^- ve SO_2, ortalama değerleri P<0.01, BE ortalama değerleri P<0.001, pvCO2 ve Cl^- ortalama değerleri ise P<0.05 düzeyinde önemli bulundu. Sağa abomazum deplasmanlı (RDA) hayvanlarda D (-) ve L (+) laktik asit, sola abomazum deplasmanlı (LDA) hayvanlarda ise L (+) laktik asit düzeyi sağlıklı hayvanlardan daha yüksek olup, gruplar arası ortalama değerler istatistiksel anlamda önemli (P<0.001) bulundu. Gruplar arası GGT, ALP, LDH ve AST ortalama değerleri istatistiksel anlamda farklı (P<0.05) bulundu. Total lökosit, hematokrit ve hemoglobin ortalama değerlerinin gruplar arası farkı önemli bulundu (P<0.05). Sonuç olarak, LDA'lı ineklerde karaciğer yağlanması ve laktat metabolizmasının bozulması nedeniyle kanda L+LA miktarının, RDA'lı ineklerde ise, abomazumdaki işemi, nekroz ve bağırsak savunmasının bozulması sonucu D ve L+LA miktarının yükseldiği ve üç ineğin öldüğü saptanmıştır. Bu nedenle, RDA'lı ineklerin prognozunu değerlendirmede, operasyon öncesi D ve L+LA miktarı önemlidir.

Anahtar sözcükler: *Abomazum deplasmanı, D(-)laktik asit, L(+) laktik asit*

Determination of Diagnostic and Prognostic Importance of the D (-) and L (+) Lactic Acid and Some Biochemical and Hematological Parameters in Holstein Dairy Cows with Abomasal Displacement

Summary

The purpsose of the present study was to evaluate of diagnostic and prognostic importance of the D (-) and L (+) lactic acid and some biochemical and hematological parameters of cattle with abomasal displacement. In this study, 10 cattle with right abomasal displacement, 10 cattle with left abomasal displacement and 10 healthy (control) cattle, totally 30 dairy cattle in different groups were used. Mean blood pH, HCO_3^- and SO_2, values (P<0.01), mean value of BE (P<0.001); mean value of $pvCO_2$ and Cl^- (P<0.05) were found statistically significant. Higher D (-) and L (+) lactic acid levels in cattle with right abomasal displacement, and higher L (+) lactic acid levels in cattle with left abomasal displacement were observed and statistically significant differences were found than healthy cattle (P<0.001). Statistically significant differences were observed between groups at mean values of GGT, ALP, LDH and AST levels (P<0.05). Mean values of total leukocytes, hematocrit and hemoglobin levels between groups were statistically significant (P<0.05). As a result, L+LA levels in blood was increased, because of the fatty liver and impaired lactate metabolism in cattle with left abomasal displacement and both D and L+LA levels increased in cattle with right abomasal displacement due to abomasal ischemia, necrosis and impaired intestinal defense, and 3 cattle died. For that reason, D (-) and (L+) lactic acid levels may be prognostic criterion in cattle with right abomasal displacement at preoperative period.

Keywords: *Abomasum displacement, D(-)lactic acid, L(+) lactic acid*

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GİRİŞ

Süt ineklerinde gebeliğin son dönemi ile laktasyonun ilk haftaları arasında metabolik değişimler meydana gelmektedir. Diğer metabolik hastalıklar gibi, ekonomik açıdan büyük önem taşıyan abomazum deplasmanları da (AD) bu dönemde sıkça görülmektedir [1,2]. Hastalığın predispoze faktörleri arasında; beslenme, ırk, genetik yatkınlık, ikiz veya güç doğum, laktasyon, ketozis, karaciğer yağlanması, insülin direnci, hiperglisemi, hipokalsemi, retensiyo sekundinarum, endometritis, mastitis ve ayak hastalıkları yer almaktadır [1,3].

Bazı araştırmalarda abomazumun sola deplasmanlarında (LDA) anorexi sonucu kan pH'sı ve bikarbonat (HCO_3^-) miktarında artış olduğu bildirilirken [1,4], pH, HCO_3^-, Na^+ ve Cl^-'un fizyolojik sınırlar içerisinde kaldığını belirten araştırmalarda bulunmaktadır [5]. LDA prognozunda K^+'un değerlendirilmesi gerektiği bildirilirken [6], düşük K^+ ve Cl^- miktarına sahip RDA'lı sığırların ya iyileşemediği ya da eski verimine kavuşamadığı iddia edilmektedir [7].

AD görülen sütçü sığırlarda doğum sonrası serum gamaglutamil transpeptidaz (GGT) seviyesinin yüksek olduğu ve bunun karaciğer tahribatına işaret ettiği bildirilmiştir [8]. Serum aspartat aminotransferaz (AST) seviyesindeki artışın her iki deplasman tanısında önemli, ancak tek başına yeterli olmadığı bildirilirken [9], AST miktarı yüksek LDA'lı ineklerin tedavisinde başarısız olunduğu da ifade edilmiştir [10]. Bunun yanında, LDA ve RDA'da AST seviyesinin yükseldiği [8,11] ancak, bunun prognoz için bir ölçü olmayacağı savunulmuştur [8]. El-Attar ve ark.[12] da, alanin aminotranferaz (ALT), AST, laktat dehidrojenaz (LDH) ve kreatin kinaz (CK) seviyelerinin AD'da arttığını saptamıştır.

Fizyolojik laktik asit olarak bilinen ve iki stereo izomerden biri olan L (+) laktik asid, hayvanlarda eksersiz ve normal metabolizma sırasında fermentasyon ürünü olarak laktat dehidrojenaz enzimi (LDH) aracılığıyla pürivattan sentezlenir. Daha sonra bir kısmı CO_2 ve H_2O'ya parçalanarak enerji oluşumunda, bir kısmı da glikojen sentezi için glikojenez metabolizmasında kullanılır [1]. D (-) laktik asit (D-LA), başlıca rumen ya da bağırsaklardaki sindirim sistemi mikroflorası ve karaciğer tarafından trioz-fosfattan sentezlenmektedir. Metabolizması ve eliminasyonu çok yavaş olan D-LA'nın, konsantrasyonu da L+laktik aside (L+LA) göre çok düşüktür. Bunun nedeni, memelilerde D laktat dehidrojenaz'ın bulunmaması ve bu enzimin yerini aldığı bildirilen D-2-hidroksi asid dehidrojenazın da sınırlı bir etkiye sahip olmasıdır. D-LA'nın kan-beyin bariyerini aşarak beyinde biriktiği ve merkezi sinir sistemi üzerinde toksik etki gösterdiği bilinmektedir [13]. Kandaki laktat miktarı, abomazum volvulusunda oluşan hemodinamik ve metabolik bozuklukların derecesi ile hastanın prognozu hakkında da bilgi vermektedir [14-17]. Araştırmalar ≥10 mM/L'nin üzerindeki kan laktat konsantrasyonlarının ölümcül olduğunu bildirmektedir [14,17]. Deplasman operasyonundan hemen sonra ölen sığırlardaki kan laktat seviyesi iyileşenlerden daha yüksek bulunmuştur [4].

Deplasmanlarda anyonik gapın arttığı [4,15], bunun abomazumda oluşan nekroz [4], laktat ve üremiden kaynaklandığı, ayrıca volvuluslu (AV) sığırlarda anyonik gap (AG) ile serum L+LA arasında önemli bir bağlantının olduğu vurgulanmıştır [15]. AV'li ineklerde baz açığı (BE) konsantrasyonu azaldığında hayatta kalma oranının da azaldığı ve BE ≤ -0.1 mEq/L olanlarda hayatta kalma oranının en düşük seviyede olduğu saptanmıştır.

RDA'da LDA'ya göre daha yüksek bir lökosit (WBC), hemoglobin (hb) ve hematokrit (PCV) düzeyi saptanmış, lökositozun sekunder endotoksemi ve abomasitisten kaynaklandığı bildirilmiştir [7,12,18].

Bu çalışmada, LDA ve RDA'lı Holstein ırkı sütçü sığırlarda D (-) ve L (+) laktik asit ile bazı biyokimyasal ve hematolojik parametrelerin diagnostik ve prognostik açıdan öneminin belirlenmesi amaçlanmıştır.

MATERYAL ve METOT

Çalışmada hayvan materyali olarak Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi kliniklerine getirilen ve abomazum deplasmanı şüphesi olan 20, Burdur ili ve çevresinden temin edilen 10 sağlıklı olmak üzere toplamda 30 adet, 4-6 yaş arası, 500-600 kg canlı ağırlığa sahip, laktasyon döneminde bulunan Holstein ırkı sütçü sığır kullanıldı (HADYEK 10.03.2009/37).

Hayvanlarda sağ ve sol tarafta açlık çukurluğu ve 11-13. kostalar düzeyinde perküsyon-oskültasyon yöntemi kullanılarak yapılan klinik muayenede sol tarafta pink sesi alınanlar sola, sağ tarafta pink sesi alınanlar ise sağa abomazum deplasmanlı olarak kabul edildi. Operasyon sonrası tanı kesinleştirildi. Sola abomazum deplasmanı tanısı konulanlar I. Gurup (n=10), sağa abomazum deplasmanı tanısı konulanlar II. Gurup (n=10) ve sağlıklı olanlar ise kontrol Gurubu (n=10) olarak guruplandırıldı.

Hastaların cerrahi tedavisinde LDA'lı ineklerde (I.Gurup) tek aşamalı laparoskopik abomasopeksi, RDA'lı ineklerde (II. Gurup) ise sağ fossa-paralumbar abomasopeksi yöntemi kullanıldı.

I. ve II. Guruptaki hayvanlardan operasyon öncesi, operasyondan 24 ve 72 saat sonra olmak üzere 3; kontrol gurubundaki hayvanlardan ise 1 defa K_3EDTA'lı tüplere (2 ml'lik vacuette), jelli tüplere (8 ml'lik SGS) ve heparinli enjektörlere kan örnekleri alındı. Jelli tüplere alınan kan örnekleri 5.000x10 dak. santrifüj edildikten sonra elde edilen serum örneklerinde, ELISA yöntemi kullanılarak D (-) (Biovision Catalog no: K667-100) ve L (+) laktik asit (katalog no: K607-100) analizleri yapıldı. Aynı örneklerde biyokimya otoanalizatörü (IDEXX) kullanılarak AST, GGT, ALP, LDH ve CPK enzim düzeyleri belirlendi. K_3EDTA'lı tüplere alınan kan örneklerinde total lökosit, hemoglobin, eritrosit ve mikrohematokrit analizler yapıldı (Vet Scan HM II Abasis, ABX pentra 120). Heparinli enjektöre alınan ve kanüllü macun

bloğuna saplanarak havayla teması engellenen kan örnekleri, buz içinde 3 saat veya soğuk zincir olmadan 15 dak. içinde Opti CCA kan gazları cihazında analiz edildi [19,20].

Tüm istatistiksel analizler SAS (2000) bilgisayar paket programı kullanılarak yapılmıştır [21].

BULGULAR

I. ve II. gurupta bulunan hayvanlarda operasyon öncesi bakılan kan gazları ve elektrolitler (pH, $PvCO_2$, SO_2, BE, HCO_3^-, Cl^-, Na^+, K^+), biyokimyasal (D (-) laktat, L(+) laktat, GGT, AST, CK, ALP, LDH) ve hematolojik (Hb, PCV, RBC, WBC) parametrelerin gurup ortalamaları ve istatistiksel önemleri *Tablo 1*'de gösterildi.

I. gurupta bulunan hayvanlarda operasyon öncesi, operasyon sonrası 24 ve 72. saatlerde bakılan kan gazları ve elektrolitler (pH, P_vCO_2, SO_2, BE, HCO_3^-, Cl^-, Na^+, K^+), biyokimyasal (D (-) laktat, L(+) laktat, GGT, AST, CK, ALP, LDH) ve hematolojik (Hb, PCV, RBC, WBC) parametrelerin gurup ortalamaları ve istatistiksel önemleri *Tablo 2*'de gösterildi.

II. gurupta bulunan hayvanlarda operasyon öncesi, operasyon sonrası 24 ve 72. saatlerde bakılan kan gazları ve elektrolitler (pH, $PvCO_2$, SO_2, BE, HCO_3^-, Cl^-, Na^+, K^+), biyokimyasal (D (-) laktat, L (+) laktat, GGT, AST, CK, ALP, LDH) ve hematolojik (Hb, PCV, RBC, WBC) parametrelerin gurup ortalamaları ve istatistiksel önemleri *Tablo3*'te gösterildi.

Guruplar arası D (-) ve L (+) laktik asit konsantrasyonlarının karşılaştırmaları ise *Şekil 1*'de gösterildi. Bu çalışmada sağa abomazum deplasmanlı 3 inek tedavi sonrası ölmüştür.

TARTIŞMA ve SONUÇ

Süt ineklerinde ekonomik önem taşıyan AD'nın insidansı gittikçe artmaktadır [1,2].

RDA'lı hayvanlardaki $pvCO_2$ değerleri kontrollerden yüksek olup (P<0.05), literatürdeki [22] bulgularla uyumludur. Bu artışın solunum kompenzasyonu [20] ve dolaşım bozuk-

Tablo 1. *Sağlıklı inekler ile sağa ve sola abomazum deplasmanlı ineklerin operasyon öncesi bazı biyokimyasal ve hematolojik parametrelerinin ortalama değerleri ve istatitiksel değerlendirmeleri*

Table 1. *Statistical evaluation of the mean values of some biochemical and hematological paremeters of the cattle with right and left abomasal displacement and healthy before operation*

Parametreler	LDA	RDA	Kontrol	P
Kan Gazları ve Elektrolitler				
pH	7.46±0.03	7.47±0.03	7.51±0.02	-
$pvCO_2$ (mmHg)	$43.44±3.59^{ab}$	$47.13±3.39^{a}$	$37.75±3.33^{b}$	*
pvO_2 (mmHg)	34.56±2.28	35.00±3.79	41.75±7.86	-
SO_2 (mmHg)	$68.12±2.57^{b}$	$66.87±3.65^{b}$	$89.75±4.39^{a}$	**
BE (mmol/L)	6.41±2.80	8.84±2.40	6.00±1.25	-
HCO_3^- (mEq/L)	31.08±2.94	33.73±2.60	29.35±1.69	-
Cl^- (mEq/L)	103.67±1.73	105.86±3.08	107.75±0.85	-
Na^+ (mEq/L)	141.56 ±2.04	141.50±1.60	144.00±2.00	-
K^+ (mEq/L)	3.57±0.29	3.45±0.28	3.75±0.16	-
Biyokimya				
D (-) laktat (nmol/µL)	$0.214±0.05^{b}$	$0.372±0.08^{a}$	$0.157±0.04^{b}$	***
L (+) laktat (nmol/µL)	$0.162±0.01^{a}$	$0.108±0.01^{b}$	$0.015±0.01^{c}$	***
GGT (U/L)	66.00±14.63	83.00±19.76	46.25±2.66	-
AST (U/L)	166.38±33.44	216.60±36.08	90.75±3.25	-
CK (U/L)	275.9±74.06	394.8±84.52	179.8±66.12	-
ALP (U/L)	$64.67±8.50^{ab}$	$77.38±7.82^{a}$	$55.75±5.36^{b}$	*
LDH (U/L)	$320.9±32.14^{a}$	$401.8±48.12^{a}$	$109.8±53.01^{b}$	*
Hematoloji				
Hb (g/dl)	$11.91±0.47^{a}$	$12.03±0.53^{a}$	$9.97±0.68^{b}$	*
PCV (%)	$35.87±3.79^{a}$	$36.12±4.73^{a}$	$30.00±3.91^{b}$	*
RBC (10^{12}/L)	6.28±0.90	6.35±0.80	6.80±1.00	-
WBC (10^{9}/L)	$6.11±0.80^{ab}$	$8.85±0.38^{a}$	$5.37±1.09^{b}$	*

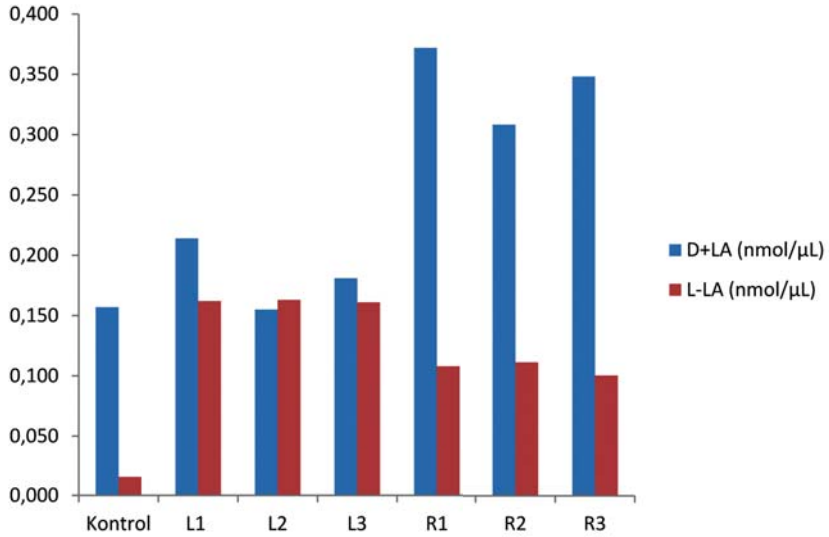
*- : önemsiz, * P<0.05, ** P<0.01, *** P<0.001,* ***a,b,c:*** *Aynı satırda farklı harfler içeren grup ortalamaları arası farklar önemlidir,* ***LDA:*** *Sola Abomasum Deplasmanı,* ***RDA:*** *Sağa Abomasum Deplasmanı*

Tablo 2. *Sola abomazum deplasmanlı ineklerin tedavi öncesi ve sonrası bazı biyokimyasal ve hematolojik parametrelerinin ortalama değerleri ve istatitiksel değerlendirmeleri*

Table 2. *Statistical evaluation of the mean values of some biochemical and hematological paremeters of the cattle with left abomasal displacement before and after treatment*

Parametreler	LDA Pre-operatif	LDA Post-operatif 24. saat	LDA Post-operatif 72. saat	Kontrol	P
Kan Gazları ve Elektrolitler					
pH	7.46±0.03^{ab}	7.37±0.01^{c}	7.41±0.03^{bc}	7.51±0.02^{a}	**
$pvCO_2$ (mmHg)	43.44±3.59^{ab}	36.00±2.30^{c}	37.75±1.42^{bc}	37.75±3.33^{bc}	*
pvO_2 (mmHg)	34.56±2.28	38.89±2.45	36.75±1.79	41.75±7.86	-
SO_2 (mmHg)	68.12±2.57^{b}	68.33±2.10^{b}	69.12±2.44^{b}	89.75±4.39^{a}	**
BE (mmol/L)	6.41±2.80^{ab}	-4.21±1.24^{c}	-0.16±2.00^{bc}	6.00±1.25^{ab}	***
HCO_3^- (mEq/L)	31.08±2.94^{ab}	20.43±1.29^{c}	24.21±1.83^{bc}	29.35±1.69^{ab}	**
Cl^- (mEq/L)	103.67±1.73^{b}	109.22±1.06^{ab}	110.98±2.04^{a}	107.75±0.85^{ab}	*
Na^+ (mEq/L)	141.56 ±2.04	140.89±1.45	143.75±2.45	144.00±2.00	-
K^+ (mEq/L)	3.57±0.29	3.57±0.23	3.66±0.23	3.75±0.16	-
Biyokimya					
D (-) laktat (nmol/µL)	0.214±0.05	0.155±0.07	0.181±0.07	0.157±0.04	-
L (+) laktat (nmol/µL)	0.162±0.01^{a}	0.163±0.01^{a}	0.161±0.00^{a}	0.015±0.01^{b}	***
GGT (U/L)	66.00±14.63	70.67±11.41	67.14±12.31	46.25±2.66	-
AST (U/L)	166.38±33.44	196.63±39.92	188.43±43.38	90.75±3.25	-
CK (U/L)	275.9±74.06	317.1±46.05	225.2±61.56	179.8±66.12	-
ALP (U/L)	64.67±8.50	54.33±5.33	55.43± 3.25	55.75±5.36	-
LDH (U/L)	320.9±32.14^{a}	300.1±34.21^{a}	315.2±41.61^{a}	109.8±53.01^{b}	*
Hematoloji					
Hb (g/dl)	11.91±0.47^{a}	10.48±0.59^{ab}	9.80±0.56^{b}	9.97±0.68^{b}	*
PCV (%)	35.87±3.79^{a}	31.55±5.31^{ab}	29.50±4.75^{b}	30.00±3.91^{b}	*
RBC (10^{12}/L)	6.28±0.90	6.12±0.12	6.07±0.92	6.80±1.00	-
WBC (10^{9}/L)	6.11±0.80	6.20±0.72	6.04±0.42	5.37±1.09	-

- : *önemsiz, * P<0.05, ** P<0.01, *** P<0.001,* ***a,b,c:*** *Aynı satırda farklı harfler içeren grup ortalamaları arası farklar önemlidir*



Şekil 1. L1: Sola Abomasum Deplasmanı Operasyon Öncesi 0. saat; L2: Sola Abomasum Deplasmanı Operasyon Sonrası 24. saat; L3: Sola Abomasum Deplasmanı Operasyon Sonrası 72. saat; R1: Sağa Abomasum Deplasmanı Operasyon Öncesi 0. saat; R2: Sağa Abomasum Deplasmanı Operasyon Sonrası 24. saat; R3: Sağa Abomasum Deplasmanı Operasyon Sonrası 72. saat

Fig 1. L1: left abomasal displacement 0. h pre-operation; L2: left abomasal displacement 24. h post-operation; L3: left abomasal displacement 72. h post-operation; R1: right abomasal displacement 0. h pre-operation; R2: right abomasal displacement 24. h post-operation; R3: right abomasal displacement 72. h post-operation

luğu [19] ile bağlantılı olduğu sanılmaktadır. AD'li ineklerin SO_2 ortalama değerleri kontrol grubundan daha düşük (P<0.01) bulunmuştur.

Bu çalışmadaki LDA'lı sığırların post-operatif 24. saat BE (P<0.001), pH ve HCO_3^- (P<0.01) ortalama değerleri kontrollerden daha düşüktür. Bu sonuç, metabolik asidozisi

Tablo 3. *Sağa abomazum deplasmanlı ineklerin tedavi öncesi ve sonrası bazı biyokimyasal ve hematolojik parametrelerinin ortalama değerleri ve istatitiksel değerlendirmeleri*

Table 3. *Statistical evaluation of the mean values of some biochemical and hematological paremeters of the cattle with right abomasal displacement before and after treatment*

Parametreler	RDA Pre-operatif	RDA Post-operatif 24. saat	RDA Post-operatif 72. saat	Kontrol	P
Kan Gazları ve Elektrolitler					
pH	7.47±0.03	7.46±0.01	7.49±0.02	7.51±0.02	-
$pvCO_2$ (mmHg)	47.13±3.39^{a}	44.14±2.71^{ab}	46.33± 3.75^{ab}	37.75±3.33^{bc}	*
pvO_2 (mmHg)	35.00±3.79	39.71±4.80	39.00±4.28	41.75±7.86	-
SO_2 (mmHg)	66.87±3.65^{b}	71.42±4.35^{b}	75.33±5.43^{b}	89.75±4.39^{a}	**
BE (mmol/L)	8.84±2.40	7.11±3.39	10.33±2.94	6.00±1.25	-
HCO_3^- (mEq/L)	33.73±2.60	31.68±3.50	34.75±3.24	29.35±1.69	-
Cl^- (mEq/L)	105.86±3.08	108.00±1.37	107.83±1.75	107.75±0.85	-
Na^+ (mEq/L)	141.50±1.60	141.14±1.83	142.83±2.66	144.00±2.00	-
K^+ (mEq/L)	3.45±0.28	3.44±0.28	3.91±0.21	3.75±0.16	-
Biyokimya					
D (-) laktat (nmol/µL)	0.372±0.08^{a}	0.308±0.06^{a}	0.348±0.07^{a}	0.157±0.04^{b}	***
L (+) laktat (nmol/µL)	0.108±0.01^{b}	0.111±0.01^{b}	0.100±0.01^{b}	0.015±0.01^{a}	***
GGT (U/L)	83.00±19.76^{ab}	97.40±14.41^{a}	86.50±16.76^{ab}	46.25±2.66^{b}	*
AST (U/L)	216.60±36.08^{ab}	287.8±84.69^{a}	155.8± 25.15^{ab}	90.75±3.25^{b}	*
CK (U/L)	394.8±84.52	375.0±77.35	176.0± 24.50	179.8±66.12	-
ALP (U/L)	77.38±7.82^{a}	53.40±5.50^{b}	60.25±1.11^{ab}	55.75±5.36^{b}	*
LDH (U/L)	401.8±48.12^{a}	387.0±45.04^{a}	352.0±28.18^{a}	109.8±53.01^{b}	*
Hematoloji					
Hb (g/dl)	12.03±0.53^{a}	11.24±0.62^{ab}	10.45±0.72^{ab}	9.97±0.68^{b}	*
PCV (%)	36.12±4.73^{a}	33.87±4.94^{ab}	31.33±5.24^{ab}	30.00±3.91^{b}	*
RBC (10^{12}/L)	6.35±0.80	6.24±0.89	6.43±1.00	6.80±1.00	-
WBC (10^{9}/L)	8.85±0.38^{a}	7.19±1.79^{a}	6.59±1.70^{ab}	5.37±1.09^{b}	*

- : *önemsiz, * P<0.05, ** P<0.01, *** P<0.001,* ***a,b,c:*** *Aynı satırda farklı harfler içeren grup ortalamaları arası farklar önemlidir*

akla getirmekte ve Schotman'ın [23] LDA'da %15-20 oranında asidoz şekillendiği fikrini doğrulamaktadır. Şahal ve ark.[22] ise, RDA ve LDA arasında ortalama pH,BE ve HCO_3^- değerleri bakımından fark olmadığını bildirmişlerdir. LDA'da oluşan asideminin laktat ve pirüvat gibi organik asitlerin üretiminden kaynaklandığı [16,19,20,24,25] sanılmaktadır. LDA'lı ineklerde operasyon öncesi L+LA konsantrasyonunun RDA ve kontrol grubundan daha yüksek (P<0.001) bulunması bu görüşümüzü destekler niteliktedir. AV'unun en son döneminde dehidrasyon, aneorobik metabolizma ve yetersiz perfüzyon nedeniyle sığırlarda kan laktat konsantrasyonun yükseldiği [26], bu nedenle laktat miktarının RDA'nın prognozu için önemli olduğu bildirilmektedir [27]. Ayrıca kaynaklar [14,17,26] ≥10 mM/L'nin üstündeki kan laktat miktarını ölümcül olarak ifade etmekte, ölen sığırlardaki kan laktat seviyesinin ölmeyenlerden daha yüksek olduğunu bildirmektedir. Bu çalışmada ölen RDA'lı ineklerin L+LA değerleri 0.080-0.130 nmol/µL aralığında saptanmış ve LDA'lı ineklerin ortalama değerlerinden (0.161±0.00-0.162±0.01 nmol/µL) daha düşük olduğu görülmüştür. Ancak sağlıklı ineklerle karşılaştırıldığı zaman RDA'lı ineklerin LLA ortalama değerleri oldukça yüksek (P<0.001) bulunmuştur. Bu çalışmada, LDA'lı ineklerin L+LA ortalama değerleri diğer gruplardan farklıdır (P<0.001). İneklerde şirurjikal stresin etkisiyle laktat seviyesinin operasyonu takiben pik yaptığı belirtilse de [25], RDA grubunda operasyon sonrası önemli bir artış bulunmamıştır. LDA'da kronik iştahsızlık sonucu hepatik lipidozisin daha çok görüldüğü ve bunun da karaciğer fonksiyonlarını olumsuz etkilediği belirtilmektedir [1,19]. Bu nedenle, LDA'lı ineklerde artan L+LA miktarının, karaciğer yağlanması, karaciğerde laktat metabolizmasının aksaması, metabolik asidozis ve dehidrasyon sonucu kas dokuda artan laktat oluşumu ile ilişkili olduğu sanılmaktadır [16,19,20].

RDA'lı ineklerin operasyon öncesi D-LA düzeyleri LDA ve kontrol grubundan daha yüksektir (P<0.001). İshalli buzağılarda HCO_3^- kaybı, metabolik asidozis sonucu laktik asit metabolizmasındaki enzimlerin inaktivasyonu [13,19], ruminal asidozis, bağırsaklardaki patojenlerin yol açtığı villus atrofisi ve malabsorbsiyon nedeniyle [28] D hiperlaktatemi oluştuğu bildirilmektedir. Bu çalışma ise, D hiperlaktatemi görülen RDA'lı ineklerin HCO_3^- ve kan pH'sı

ortalama değerleri normal sınırlarda bulunmaktadır. Ayrıca, ölen RDA'lı ineklerin tedavi öncesi ve sonrası HCO_3^-, BE ve pH değerleri sırasıyla, 23.1-39.7 mEq/L, -1.6-11.9 mmol/L ve 7.37-7.55 aralığında saptanmıştır. Bu bulgulara bakıldığında, RDA'da ölen ineklerde metabolik asidozisin bulunmadığı görülmektedir. Yine, LDA'lı ineklerde post-operatif 24. saat HCO_3^-, BE ve pH ortalamaları en düşük olmasına karşın, bu grupta D-LA seviyesi bildirimlerin [16,19] aksine en düşük düzeyde kalmıştır. Çalışmada ölen RDA'lı 3 ineğin operasyondan önce, 24 ve 72 saat sonraki D-LA değerleri sırasıyla 0.430-0.560, 0.230-0.420 ve 0.280-0.500 nmol/µL arasında saptanmıştır. Bu sonuçlar, RDA'da görülen D-LA artışının ve üç ineğin ölüm sebebinin doku hipoksisi ve abomazumdaki nekroz olabileceğini akla getirmektedir. Başoğlu ve ark.[29] kontrol ve ince bağırsak obstrüksiyonlu hastalarla karşılaştırıldığında akut mesenterik işemili hastalarda D-LA düzeylerinin önemli oranda yükseldiğini dolayısıyla D-LA'nın akut mesenterik işeminin erken tanısında yararlı olabileceği sonucuna varmışlardır. Bu artışın gastrointestinal kanalda bakteriler ve ürettikleri D-LA'nın işemideki mukoza tahribatı nedeniyle dolaşıma geçmesi, bakterilere karşı normal savunma mekanizmasının zayıflaması ve D-LA'yı inaktive eden enzim sisteminin olmamasından kaynaklandığını ifade etmişlerdir. RDA'da abomazumda ülser, abomazum mesenterinde işemi, konjesyon, ödem ve nekroz geliştiği kaynaklarca da [1,20,22] bildirilmektedir. AV'li sığırlarda başarısız postoperatif sonuçların dolaşım şokundan ziyade abomazum, omazum, ve duodenumdaki lokal tahribattan kaynaklandığı bildirilmektedir [26]. Ayrıca, RDA'da görülen iştahsızlık, durgunluk ve inkoordinasyonun yüksek D-LA ile ilişkili olduğu düşünülmekte, kaynakta da [13] D-LA'nın, ataksi, ilerleyen uyuşukluk ve koma gibi bozukluklara yol açtığı ifade edilmektedir. Bu sonuçlar, özellikle operasyon öncesi D-LA miktarının prognozda önemli olduğunu ve yüksek D-LA konsantrasyonuna sahip olan ineklerin yaşama şansının azaldığına işaret olabilir. Araştırmacıların [26,27] ölen hayvanlarda D-LA düzeyleri konusunda bir çalışmaları olmadığı ve bu hayvanlardaki laktat artışının D veya L laktik asitten mi kaynaklandığı bilinmemektedir.

Bu araştırmada LDA grubunun tedavi sonrası Cl^- ortalama değerlerinde gözlenen artış ($P<0.05$), araştırmacıların [11,12,30] bulgularına benzemektedir. RDA'lı hastalarda prerenal azotemi ve dehidrasyon nedeniyle hipokloreminin şiddetlendiği savunulsa da [1,19] bu araştırmada benzer bir sonuç saptanamamıştır.

Araştırmada RDA grubunun post-operatif 24. saat serum GGT ortalama değerleri kontrol grubuna göre yüksek ($P<0.05$) olup, araştırmacının [18] bildirimine uyumludur. Oysa, Güzelbektaş ve ark.[31] LDA'da daha yüksek ortalama saptamışlardır. Kanda GGT miktarı artışı karaciğer tahribatı, endotoksemi ve karaciğer yağlanmasıyla [8,18] ilişkilidir. Bir araştırmada [11] tedavi sonrası GGT seviyesinin azaldığı bildirilmiş, ancak bu çalışmada benzer bir sonuç bulunamamıştır. Çalışmada RDA'lı ineklerin kontrol grubuna göre daha yüksek ($P<0.05$) bir AST düzeyine sahip olduğu ve araştırmacıların [18,32] bulgularına benzediği görülmektedir. Ancak, bazıları da [31] serum AST değerlerinin LDA'da daha yüksek olduğunu iddia etmektedirler. AD'da yüksek AST değerinin prognoz için bir ölçü olamayacağı bildirilse de [8], Staufenbiel ve ark.[10] çalışmasında, AST düzeyleri yüksek olan AD'lı ineklerin tedavisinde başarısız olunduğunu vurgulamıştır. Çalışmada RDA'lı ineklerin pre-operatif serum ALP ortalama değerleri en yüksek ($P<0.05$) olup, kontrol grubundan farklıdır. Bu yüksek değerlerin hipokalsemi, karaciğerdeki ödematöz lezyonlar, hepatositlerdeki permeabilite artışı, şiddetli açlık ve endotoksemiden de [1,19,20] kaynaklandığı bildirilmektedir. Hastalarda saptanan LDH ortalama değerlerinin kontrol grubundan yüksek ($P<0.05$) olduğu görülmüş ve araştırmacıların [12,18] bulgularıyla uyumlu bulunmuştur. AD'li ineklerde yüksek LDH miktarı, hepatik lipidozis ve hepatositlerdeki hücresel bozukluğa işaret etmektedir [3,19].

Araştırmada en yüksek hb ortalama değerleri AD'lı hayvanların pre-operatif değerleri olup, kontrol grubundan farklıdır ($P<0.05$). Çalışmalarda [12,22] da benzer sonuçlar bildirilmiş, ancak bulgularımızdan farklı olarak RDA'da post-operatif hb ortalama değerlerinde azalma olduğu ileri sürülmüştür [22]. Ayrıca, dehidrasyon [19,33] nedeniyle RDA'daki hb ortalamalarının LDA'dan daha düşük olduğu ileri sürülmüş [7], bunun bulgularımızla uyuşmadığı dikkati çekmiştir. Aynı şekilde AD'li ineklerin pre-operatif PCV ortalama değerleri kontrol grubundan yüksek ($P<0.05$) ve araştırmacının [22] bulgularına benzerdir. Bunun, kan sıvısının ön midelere geçişinden kaynaklandığı sanılmaktadır [19]. Kaynaklarda [7,34] ise, PCV miktarında bir değişim olmadığı bildirilmiştir. RDA grubunun operasyon öncesi WBC ortalama değerleri, kontrol grubu değerlerinden yüksektir ($P<0.05$). Araştırmacılar [7,12,18,34] da benzer bulguları bildirmekte, görülen lökositozisin cerrahi stres [27], sekunder endotoksemi ve abomasitise immun cevap olarak meydana geldiği [12,18] düşünülmektedir.

Sonuç olarak, LDA'lı ineklerde karaciğer yağlanması ve laktat metabolizmasının bozulması nedeniyle kanda L+LA miktarının, RDA'lı ineklerde ise, abomasumdaki işemi, nekroz ve bağırsak geçirgenliğinin bozulması sonucu D ve L+LA miktarının yükseldiği ve 3 ineğin öldüğü saptanmıştır. Bu nedenle, RDA'lı ineklerin prognozunu değerlendirmede, operasyon öncesi D ve L+LA miktarının önem taşıdığı, ayrıca bu konuda yeni araştırmaların yapılmasının faydalı olacağı kanaatine varılmıştır.

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RESEARCH ARTICLE

Die Therapeutische Wirksamkeit von Tylosin bei der Kälberkryptosporidiose

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Zusammenfassung

In der vorliegenden Arbeit wurde auf 23 (18 Kälber Therapiegruppe, 5 Kälber Kontrollgruppe) durchfällige, in deren Kot *Cryptosporidium parvum* oozysten nachgewiesene Kälbern die therapeutische Wirksamkeit des Tylosins (Tylan® 200, Elanco) geprüft. Die Kälber der Therapiegruppe wurden einmal täglich, 5 Tage lang, in einer Dosierung von 20 mg/kg/Tag, Intramuskulär, mit Tylosin behandelt. Im Kot von 4 Kälbern konnten ab 4. Tag, bei 8 Kälbern ab 6. Tag und bei 6 Kälbern ab 7. Tag nach Behandlungsbeginn keine Oozysten mehr nachgewiesen werden. Bei der Kontrollgruppe wurde die Oozysten im Kot bis 10 Tagen untersucht und wurde keine wichtige verminderung bei der Oozystenzahlen beobachtet. In dieser Arbeit unter Berücksichtigung der Oozystzahlen im Kot wurde Tylosin in einer Dosierung von 20 mg/kg, einmal täglich, für die Dauer von 5 Tagen Intramuskulär beim Bekämpfung der Kälberkryptosporidiose wirkungsvoll gefunden.

Schlüsselworte: *Cryptosporidium spp., Kalb, Durchfall, Therapie, Tylosin*

Buzağı Kriptosporidiozunda Tylosin'in Terapötik Etkinliği

Özet

Çalışmada dışkılarında *Cryptosporidium parvum* ookistleri tespit edilen 23 (18 buzağı tedavi grubu, 5 buzağı kontrol grubu) ishalli buzağıda tylosine'nin (Tylan® 200, Elanco) terapötik etkinliği incelenmiştir. Tedavi grubundaki buzağılara günde bir kere 20 mg/kg dozunda tylosin 5 gün süreyle intramuskuler olarak uygulandı. Dört buzağıda tedavinin dördüncü, 8 buzağıda altıncı, 6 buzağıda yedinci gününden itibaren dışkıda ookiste rastlanmadı. Kontrol grubunda ise 10 gün süresince incelenen dışkılarında ookist sayısında önemli bir azalma görülmedi. Çalışmada dışkıdaki ookist sayılarına bakılarak tylosine'nin günde bir kere 20 mg/kg dozunda 5 gün süreyle intramuskuler olarak uygulanması buzağı kriptosporidiozunun tedavisinde etkili bulundu.

Anahtar sözcükler: *Cryptosporidium spp., Buzağı, İshal, Tedavi, Tylosin*

EINLEITUNG

Die Durchfallerkrankungen der Kälber darunter besonders Kälberkryptosporidiose zählen zu den häufigsten und wirtschaftlich bedeutungsvollen Erkrankungen in der Türkei. Man schätzt die Aufzuchtverluste bei Kälbern in der Türkei heute auf mindestens 20%, wobei der größte Teil der verendeten Kälber mit Diarrhöe einhergehende Magen-Darm-Erkrankungen zeigt [1,2]. Bei fast allen Durchfallerkrankungen von neonatalen Kälbern liegen infektiöse Ursachen wie *bovine Rotaviren*, *bovine Coronaviren*, darmpathogene *E. coli* und *Cryptosporidium parvum* zugrunde [3,4]. *Cryptosporidium parvum* war als primär pathogener Erreger bis vor einigen Jahren noch nicht bekannt [5]. In den letzten Jahren gibt viele Berichte über Durchfälle bei Kälbern, die primär auf Kryptosporidien zurückzuführen sind [6-8].

Kryptosporidiose stellt eine bedeutende, subklinisch oder klinisch verlaufende Jungtiererkrankung dar [9], da der Krankheit bei erwachsenen Tieren symptomlos verläuft.

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Die Erkrankung tritt meistens in den ersten vier Lebenswochen auf und die Symptome sind häufig vom fünften bis 14. Lebenstag sichtbar [10]. 24 Stunden nach Auftreten der ersten klinischen Symptome kann eine Oozystenausscheidung festgestellt werden [11]. Der profuse, 2 bis 14 Tage anhaltene Durchfall verläuft mit Exsikkose, Gewichts- und Flüssigkeitsverlust [12-15].

Bei Kryptosporidium infizierten Kälbern, bei den andere Tieren und Menschen wurden auf ihre Wirksamkeit etwa 200 Wirkstoffe geprüft. Nur einige, wie Decoquinate, Halofuginon, Sulfadimidin, Paramomycin, Lacalocid und Sulfadiazin-Trimetoprim sind als mehr oder weniger gegen *Cryptosporidium parvum* Infektionen wirksam gefunden [1,2,16-18]. Die Antibiotika aus der Makrolid gruppe wie Spiramycin, Azithromycin, Clarithromycin, Roxithromycin wurden beim Menschen gegen Kriptosporidiose getestet [19]. Auch im Veterinärbereich Arbeiten über die Kriptosporidiose wurde mit Azithromycin, Tilmicosin, Spiramycin, Erythromycin und Tylosin durchgeführt. Hierbei die Untersuchungen über die Wirkung von Tylosin bei der Kälberkryptosporidiose sind unzureichend.

In der vorliegenden Arbeit wurden bei dem Kälberkryptosporidiose die therapeutische Wirksamkeit des Macrolid-Antibiotikums Tylosin geprüft und die klinischen und hämatologischen Veränderungen untersucht.

MATERIAL und METHODEN

Die vorliegende Arbeit wurde bei den 23 durchfälligen Kälbern durchgeführt. Achtzehn Kälber wurde als Therapiegruppe und 5 Kälber als Kontrollgruppe eingeteilt. Die Kälber waren im Alter von 1 bis 15 Tagen. Die vorgestellten Kälber wurden am Aufnahmetag nach den Methoden von Dirksen [20] klinisch untersucht und wurde die Kotproben in 24 stündigen Abständen bis zur Entlassung auf *C. parvum* Oozysten untersucht. Die Diagnosestellung erfolgte anhand Karbolfuchsinfärbung: 0.2 g Kälberkot wurden mit 2 µl Karbolfuchsin (Merck, Nr. 9215) auf einem Objektträger vermischt und dünn ausgestrichen. Unmittelbar nach der Trocknung wurden 20 zufällig eingestellte Blickfelder bei 1000-facher Vergrößerung untersucht und durchschnitt der gezählten oozysten nach dem in *Tabelle 1* dargestellten Schema bewertet [21].

Zur Überprüfung der Wirksamkeit beim therapeutischen Einsatz wurden insgesamt 18, die an *C. parvum* erkrankte Kälber mit Tylosin (Tylan® 200, Elanco Animal Health, a Division of Eli Lilly and Company, Indianapolis, USA) in einer Dosierung von 20 mg/kg, ein mal täglich, für die Dauer von 5 Tagen Intramuskulär behandelt. Die an *C. parvum* erkrankten fünf Kälber wurden als Kontrollgruppe eingestuft. Den 12 Kälbern der Therapie Gruppe und 5 Kälber der Kontrolgruppe wurde unter Berücksichtigung der Laborbefunde Hämatokrit (Hkt) und Basenüberschuß (BE) eine intravenöse symptomatische Infusionstherapie eingeleitet [22]. Gegen Elektrolyt- und Wasserverluste wurden Elektrovet®, (Zusammensetzung in mEq/L: Na^+ 140, Cl^- 103, Azetat 47, K^+ 10, Citrat 8, Ca^{++} 5, Mg^{++} 3; Vilsan), Natriumchlorid-lösung (Natriumchlorid 9 g, aqua dest. Ad 1.000 ml) und gegen Basendefizide 1.3% ige Natriumbikarbonat-Lösung verwendet. Nach Wiederkehr der Sauglust wurde den Kälbern zweimal täglich je 2.000 ml Elektrolytpulver (Lectade®, Pfizer) verabreicht.

Tabelle 1. *Bewertung von Oozystenbefunden bei 20 zufällig ausgewählten Blickfeldern bei 400-facher Vergrößerung*

Tablo 1. *20 rasgele seçilmiş mikroskop sahasında, 400 büyütme ile tespit edilmiş ookist bulgularının değerlendirmesi*

Nachgewiesene Oozysten	Bewertungsindex
Keine Oozysten	0 (negativ)
1- 5 Oozysten	1 (geringgradig)
6-10 Oozysten	2 (mittelgradig)
>10 Oozysten	3 (hochgradig)

Säure-Basen-Haushalt im venösen Blut pH-, aktuelles Bikarbonat- (HCO_3^-), Kohlendioxidpartialdruck- (pCO_2), Basenüberschuß- (BE), Natrium- (mmol/l), Kalium-(mmol/l), Hämatokrit- (%) und Hämoglobin- (g/dL) Werte wurden mittels tragbare Blutgasanalysator Gastat-mini (Technomedica co, Ltd., Yokohama-Japan) mit der Sensor card 983, bei 37°C untersucht.

Mit Hilfe der elektronischen Datenverarbeitung (SPSS for Windows, Release 15.0, SPSS Inc. USA) wurde für jeden der genannten Parameter Mittelwert (x), Standardfehler des Mittelwertes (±SEM) und Schwankungsbreite ($X_{min} - X_{max}$) errechnet. Vergleichbare Blutparametern wurden im Man Whitney U und Wilcoxon tests auf statistisch erfassbare Unterschiede geprüft.

ERGEBNISSE

Die 12 männlichen und 11 weiblichen erkrankten Kälber dieser Arbeit gehörten zu den verschiedenen Rassen (Deutsch-Schwarzbunte 15, Montofon 4, Simenthal 3, Kreuzungstier 1). Acht Kälber aus der Therapiegruppe, 1 Kalb der Kontrollgruppe waren 6 bis 10 Tage alt, da 8 Kalb aus der Therapie gruppe 1 bis 5 Tage alt war und 2 Kälber der Therapie gruppe, 4 Kälber der Kontrollgruppe im alter von 11 bis15 Tagen waren.

Die Ergebnisse der klinischen Erstuntersuchungen von Kälbern beider Gruppe sind in der *Tabelle 2* weitergegeben. 3 Kälber waren gering-17 Kälber mittel-, und 3 Kälber waren hochgradig erkrankt. Bei allen Kälbern wurde dünn-pastöse bis profuse Diarrhöen festgestellt. Bei 17 Kälbern war der Kot hellgelb-grün, bei 3 Kälber rötlich braun gefärbt. 13 Kälber waren apathisch, 12 Kälber lagten viel. Sauflust verminderte sich beim 10 Tiere, da 9 Kälber keine Sauflust zeigten. Die Körpertemperatur lag beim 14 Tier zwischen 39.6- und 40.0°C. Die Augäpfel waren bei 17 Tieren eingesunken und bei 22 Tieren war die

Hautelastizität reduziert. In 2 bis 4 Tage nach Behandlungsbeginn verschwanden die Durchfallsymptome bei behandelten Kälbern. Obwohl bei der Kontrollgruppe die Durchfallsymptome sich verbessern, verschwanden sie nicht und wurde im Kot der Kälber noch Oozysten nachgewiesen.

Anhand der Oozystenzahlen vor der Behandlung war die Bewertungsindex von 7 Kotproben der Therapie Gruppe als ein, eine Kotprobe als zwei, zehn Kotproben als drei eingestuft *(Tabelle 1)*. Bei der Kontrollgruppe war die Bewertungsindex von 2 kotproben 2 und von 3 kotproben 3. Wie auf der *Tabelle 3* ersichtlich ist hatte sich die Oozystenzahlen beim 15 Kälber am ersten Tag nach der Behandlung bei der Therapiegruppe sich vermehrt, aber ab dem 2. Tag der Behandlung sankten die Oozystenzahlen allmählich ab. Im Kot von 4 Kälbern konnten ab 4. Tag nach Behandlungsbeginn, bei 8 Kälbern ab 6. Tag und bei sech Kälbern ab 7. Tag nach Behandlungsbeginn keine Oozysten mehr nachgewiesen werden. Bei der Kontrollgruppe wurde die Oozysten im Kot bis 10 Tagen untersucht und

Tabelle 2. *Die Ergebnisse der klinischen Erstuntersuchungen von den Kälbern der Kontroll-und Therapiegruppe*
Tablo 2. *Kontrol ve Tedavi grubu buzağıların ilk klinik muayene bulguları*

Parameter	Beurteilung der Parameter	Kälber zahl
Haltung	Physiologisch	3
	Aufgekrümmt	8
	Liegt viel	12
Verhalten	Ruhig	10
	Apathisch	13
Körpertemperatur	38°C -39.5°C	5
	39.6°C -40.0°C	14
	38.0°C > , 41.0 °C<	4
Hautturgor	Gut	1
	Mäßig	8
	Schlecht	6
	Sehr schlecht	8
Augäpfel	Nicht eingefallen	6
	Bleistiftstark eingefallen	14
	Fingerstark eingefallen	3
Kotkonsistent	Auseinander laufent	14
	Flüssig	9
Kotfarbe	Ockerfarben	3
	Hellgelb grünlich-braun	17
	Rötlich- braun	3
Kotgeruch	Babygeruch	5
	Übelriechend	12
	Stinkend	6
Freßlust	Säuft langsam	4
	Säuft wenig	10
	Säuft nicht	9

schwankungen (Vermehrungen und Absinkungen) bei der Oozystenzahlen beobachtet. Außerdem wurde auch am 10. Tag der Arbeit Oozystenausscheidung im Kot der Kontrollkälber nachgewiesen. Trotz dass der Kälber in der Kontrolgruppe Oozysten ausschieden, haben sich die klinische Erscheinungen verbessert.

Die Ergebnisse der Blutgasanalysen und des Säure-Basen-Status vor und nach der Behandlung der Therapigruppe und Kontrollgruppe sind in *Tabelle 4* und *5* dargestellt. Die Werte zeigen innerhalb der Gruppen vor und nach der Behandlung signifikante Unterschiede, zwischen die Gruppen vor und nach der Behandlung im Gegenteil keine signifikante Unterschiede.

Bei der Therapiegruppe war der pH-Wert des venösen Blutes erwartungsgemäβ erniedrigt: Als tiefster Wert wurde ein pH von 7.121, ein Bikarbonatgehalt (HCO_3^-) von 14.7 mmol/L und ein Basenüberschuβ (BE) von –13.8 mmol/L gemessen. Bei der Kontrolgruppe wurde tiefster Wert von pH als 6.99, von HCO_3^- 12.1 mmol/L und von BE -17.0 mmol/L gemessen. Nach der Behandlung erreichten die pH, HCO_3^- und BE Werte des venösen Blutes der beiden Gruppe teilweise wieder physiologische Werte.

Die Hämatokrit- und Hämoglobinwerte zeigten bei den erkrankten Kälbern der Therapiegruppe vor der Behandlung signifikant höhere Werte als danach. Als höchste Werte wurden ein Hämatokritwert von 50.20 L/L und ein Hämoglobin von 16.4 (g/dL) gemessen. Der höchste Hämatokritwert der Kontrolgruppe war 48.3 L/L und der Hämoglobinwert 16.1 (g/dlL). Diese Werte sankten innerhalb der Behandlung deutlich ab und erreichten dabei teilweise wieder physiologische Werte (*Tabelle 6* und *7*). Der Mittelwerte der Natriumgehalt beider Gruppe lag vor der Behandlung an der unteren Grenze der Normalbereiche und die Werte haben sich nach der Behandlung normalisiert. Bei den Kälbern konnte teilweise ein hochgradiger Anstieg des Kaliums, die nach der Behandlung sich verminderte, nachgewiesen werden.

DISKUSSION

Die vorliegende Untersuchung zeigte auf, daß die Befallhäufigkeit bei 6 bis 10 Tage und 1 bis 5 Tage alten Kälber fast gleich aber höher als 11 bis 15 Tage alten Kälber ist. Henriksen and Krogh [15] berichteten daß Kälber im Alter von 8 bis 14 Tagen mehr von der *C. parvum*- Infektion betroffen sind, da andere Autoren [2,23] eine Häufigkeit im Alter von 11 bis 15 Tage berichten.

Die klinischen Auswirkungen der Kryptosporidiose unterscheiden sich sehr im Berichten. Bei den kranken wird am häufigsten ein wässrig-gelblicher, schleimfetzen enthaltene und stechend-faulig riechende Durchfall, eine Dehydration und eine bis 40.4°C erhöhte Körpertemperatur beobachtet [12,24].

Tabelle 3. *Oozystenzahlen von den Kälbern der Kontroll- und Therapiegruppe*
Tablo 3. *Kontrol ve tedavi grubu buzağıların ookist sayıları*

Kälber		Oozystenzahlen in Tagen										
		0	1	2	3	4	5	6	7	8	9	10
Therapiegruppe	1	1	10.18	9.15	0.2	0						
	2	15.1	12.5	3.15	0.7	0						
	3	9.9	11.6	3.8	0.3	0						
	4	24.6	18.05	3.5	1.8	0						
	5	49.1	77.4	38.5	20	3.3	0.35	0				
	6	4.35	6	7.2	4.2	0.8	0.2	0				
	7	16	20.5	9.75	3.4	0.65	0.15	0				
	8	33.65	40.7	17.35	5.7	2.4	0.85	0				
	9	62.7	50.7	34.25	5.35	2.45	0.55	0				
	10	1.5	4.15	2.65	2.05	2.0	0.25	0				
	11	2.6	4.15	2.2	1.2	0.55	0.3	0				
	12	1.5	6.6	2.75	2.03	1.3	0.7	0				
	13	30.85	29.15	22.35	10.8	9.95	2.05	0.3	0			
	14	28	37.5	25.5	11.85	2.13	4.1	0.95	0			
	15	1.65	41.9	25.75	17.31	6.35	3.24	1.6	0			
	16	12.95	22.85	10.5	9.6	6.8	1.45	0.2	0			
	17	10.95	20.4	14.5	11.5	8.7	3.15	2.7	0			
	18	2.1	4.75	5.7	7.9	4.6	1.2	0.8	0			
Kontroll	1	45	53.6	143.75	230	135.3	77.25	37.1	8.4	2.25	2.1	1.8
	2	7.65	20.75	216	138.45	177.65	95	139.5	93	59	91.75	48.75
	3	21	54.3	184.95	81.55	162	243.6	86.9	48.1	41.2	32.9	29.4
	4	8.4	17.7	77.35	171.05	117.3	54.9	98.2	94.85	67.75	49.1	33.8
	5	27.3	36.05	127.05	242.65	137.45	93.7	95.45	48.25	56.2	39.25	38.55

Tabelle 4. *Übersicht von Blut-pH, Bikarbonatgehalt (HCO_3^-), Basenüberschuß (BE), pO_2 und pCO_2 in venösem Blut der erkrankten Kälbern der Therapiegruppe vor sowie 5. Tag der Behandlung*
Tablo 4. *Tedavi grubundaki buzağıların tedavi öncesi ve tedavinin 5. günündeki venöz kandaki kan pH, HCO_3^-, BE, pO_2 ve pCO_2 değerleri*

Zeitpunkt der Probeentnahme	Mittelwert ± Standardabweichung, min-max Werte	pH ($-logH^+$)	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3^- (mmol/L)	BE (mmol/L)
Vor der Behandlung n=12	X ± SEM	7.256 ±0.08	51.40±10.81	34.90±15.5	22.25±4.88	-5.05±5.97
	xmin-xmax	7.121-7.371	33.0-72.30	17.40-78.10	14.7-30	-13.80-4.20
Nach der Behandlung n=12	X ± SEM	7.396±0.04	46.25±10.35	40.90±8.2	28.31±3.79	3.93±3.61
	xmin-xmax	7.313-7.427	35.40-69.5	25.10-53.0	24.1-35.1	0.40-10.90
Statistische Auswertung		P=0.002	P=0.272	P=0.077	P=0.01	P=0.004

Tabelle 5. *Übersicht von Blut-pH, Bikarbonatgehalt (HCO_3^-), Basenüberschuß (BE), pO_2 und pCO_2 in venösem Blut der erkrankten Kälbern der Kontrollgruppe vor sowie 5. Tag der Behandlung*
Tablo 5. *Kontrol grubundaki buzağıların tedavi öncesi ve tedavinin 5. günündeki venöz kandaki kan pH, HCO_3^-, BE, pO_2 ve pCO_2 değerleri*

Zeitpunkt der Probeentnahme	Mittelwert ± Standardabweichung, min-max Werte	pH ($-logH^+$)	pCO_2 (mmHg)	pO_2 (mmHg)	HCO_3^- (mmol/L)	BE (mmol/L)
Vor der Behandlung n=5	X ± SEM	7.149±0.12	54.9±6.84	30.22±8.07	16.46±3.2	-9.48±5.02
	xmin-xmax	6.998-7.324	46.2-63.4	23.9-43.80	12.1-19.6	-17.0-3.10
Nach der Behandlung n=5	X ± SEM	7.403±0.01	47.70±6.7	41.78±0.94	27.7±3.2	3.8±0.9
	xmin-xmax	7.382-7.418	36.0-52.0	41.10-43.10	23.2-32.2	3.00-5.20
Statistische Auswertung		P=0.043	P=0.08	P=0.08	P=0.043	P=0.043

Tabelle 6. *Übersicht von Hämatokrit (PVC%), Hämoglobingehalt (g/dl), Na (mmol/L) und K (mmol/L) Werte, bei erkrankten Kälbern der Therapiegruppe vor, sowie 5. Tag der Behandlung*

Tablo 6. *Tedavi grubundaki buzağıların tedavi öncesi ve tedavinin 5. günündeki hematokrit (PVC%), hemoglobin (g/dl), Na (mmol/L) ve K (mmol/L) değerleri*

Zeitpunkt der Probeentnahme	Mittelwert ± Standardbweichung, min-max Werte	Na (mmol/L)	K (mmol/L)	Hct (PVC%)	Hb (g/dl)
Vor der Behandlung n=12	X ± SEM	139.02±5.62	5.67±1.36	32.42±8.25	9.68±2.85
	xmin-xmax	128.9-146.1	4.40-7.90	21.2-50.20	6.9-16.40
Nach der Behandlung n=12	X ± SEM	143.50±4.55	4.58±0.53	29.55±3.82	9.11±1.17
	xmin-xmax	132.4-149.6	3.90-5.70	22.70-34.20	7.8-11.0
Statistische Auswertung		P=0.01	P=0.005	P=0.583	P=0.582

Tabelle 7. *Übersicht von Hämatokrit (PVC%), Hämoglobingehalt (g/dl), Na (mmol/L) und K (mmol/L) Werte bei erkrankten Kälbern der Kontrollgruppe vor, sowie 5. Tag der Behandlung*

Tablo 7. *Kontrol grubundaki buzağıların tedavi öncesi ve tedavinin 5. günündeki hematokrit (PVC%), hemoglobin (g/dl), Na (mmol/L) ve K (mmol/L) değerleri*

Zeitpunkt der Probeentnahme	Mittelwert ± Standardabweichung, min-max Werte	Na (mmol/L)	K (mmol/L)	Hct (PVC%)	Hb (g/dl)
Vor der Behandlung n=5	X ± SEM	133.86±6.17	5.98±1.03	33.16±9.39	12.1±2.39
	xmin-xmax	125.9-140.3	4.90-7.20	26.4-48.3	9.7-16.1
Nach der Behandlung n=5	X ± SEM	143.76±2.03	5.06±0.89	26.14±4.24	10.72±1.66
	xmin-xmax	140.2-145.0	3.9-5.9	22.20-32.00	8.8-13.4
Statistische Auswertung		P=0.043	P=0.176	P=0.043	P=0.042

In der vorliegenden Arbeit wiesen Kälber beider Gruppe wie in der Beobachtungen von anderen Autoren gering- bis hochgradige metabolische Azidose auf. Die Ursache der auch den Saugreflex beeinflussende metabolischen Azidose bei neugeborenen Kälbern mit Durchfall ist die Bikarbonatverluste über den Darm [25,26]. Wie aus *Tabellen 4* und *5* ersichtlich, sanken vor der Behandlung wegen der Bikarbonat verlust über den Durchfallkot die pH-, HCO_3^- und Basenüberschuss im Blut geringgradig ab. Nach der symptomatischen Infusionstherapie mit Pufferbasen stiegen pH-Wert und Bikarbonatspiegel im Blut deutlich an, während sich das Basendefizit verringerte *(Tabelle 4-5)*. Wegen die hohen enteralen Flüssigkeitsverluste bei durchfälligen Kälbern dieser Arbeit traten höhere Hämoglobingehalte und Hämatokritwerte vor der Behandlung auf *(Tabelle 6-7)*. Nach der symptomatischen Infusionstherapie Normalisierten sich dieser Parameter.

Beim durchfälligen Kälbern treten neben die Flüssigkeitverluste auch Natrium und Chlorid Verluste auf [27,28]. Die in *Tabelle 6* und *7* zusammengestellten Ergebnisse Des Natriums zeigen ein fast einheitliches Bild. Bei durchfallkranken Kälbern mit einer Azidose beschrieben Hartmann et al.[25] eine Hyperkaliämie mit Werten von >6 mmol/L im Plasma. In unserer Arbeit wurde teilweise ein hochgradiger Anstieg des Kaliums nachgewiesen werden. Beim Durchfall gehen erhebliche Mengen an Kalium über den Kot verloren trotzdem kommt es im Laufe des Durchfalls zu einer Hyperkaliämie. Bei der metabolischen Azidose wird Kalium in der Zelle gegen Wasserstoffionen im Blut ausgetauscht, außerdem kommt es aufgrund des osmotischen Gradienten zur Verschiebung von Kalium aus dem Intrazellularraum in den Extrazellularraum [29]. Zusammen mit Tylosin durchgeführte, zusätzliche symptomatische Infusionstherapie in der vorliegenden Arbeit verminderte die Kaliumgehalt der Kälber und beinflusste den Heilungsverlauf positiv.

Bei Kälbern wurden gegen Kriptosporidiose verschiedener therapeutische Behandlungsversuche durchgeführt aber konnte bisher keine effektive, spezifische Therapie zur vollständigen Eliminierung von *C. parvum* gefunden werden. Ionophore wie Lasalocid, Monensin, Narasin und Salinomycin zeigten sich wirkungslos oder wirkten in toxischen Dosen [30,31]. Es konnte mit Sulfonamiden wie Sulfadimethoxin, Trimethoprin und Sulfadiazin, als auch mit Diclazuril und Toltrazuril, außerdem mit Paramomycinsulfat, Halofuginon Laktat, Decoquinat keine ausreichende Verminderung der Oozystenausscheidung erzielen [11,18,31]. Ulutaş et al.[32] berichten, daß Spiramisin bei der 5 tägigen Behandlung in einer Dosierung von 30 mg/kg, 2 mal täglich wirkungsvoll bei der Kälberkryptosporidiose ist. Paraud et al.[33] behandelten Ziegenlämmer mit Tilmicosin ohne Erfolg.

Das nur in der Veterinärmedizin eingesetzte Tylosin gehört auch zur Gruppe der Makrolid-Antibiotika und wurde bei Rindern zur Behandlung von Pneumonien, Fußräude, Metritis und Mastitis eingesetzt. Außerdem findet auch zur Behandlung von Arthritiden beim Kalb, gegen Campylobacter-Infektionen beim Schaf und gegen Mykoplasma-Pneumonie der Ziegen eine Verwendung [34,35]. Temizel et al.[36] setzten Tylosin erfolgreich bei der Kryptosporidiose der Ziegenlämmer ein. Bollam [37] schlußfolgerte, dass bei mit Azithromycin behandelter Gruppe die klinische Verbesserung und die Verminderung der Oozysten

Ausscheidung früher stattfindet als mit Tylosin behandelte Gruppe. In unserer Arbeit wurde Tylosin (Tylan) in einer Dosierung von 20 mg/kg, einmal täglich, für die Dauer von 5 Tagen Intramuskulär verwendet. Ab 6. Tag nach Behandlungsbeginn 5 Kälber und ab 7. Tag nach Behandlungsbeginn 3 Kälber schieden keine Oozysten aus. Dieser Befund wurde auf die postantibiotischen Efekt von Tylosin hindeutend gefunden.

Da es unzureichende Arbeiten mit Tylosin beim Kälberkryptosporidiose gibt, trotzt die Dosierung unserer Arbeit klinisch als auch parasitologisch erfolgreich ist, ist weitere Untersuchungen, mit verschiedenen Dosierungen und Verwendungen über die Wirkung von Tylosin bei der Kälberkryptosporidiose zu empfehlen.

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RESEARCH ARTICLE

Effectiveness of Different Progesterone Analogues and GnRH on Reproductive Parameters in Nulliparous Saanen Goats at the End of the Transition Period

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Summary

The objective of this study was to compare the efficacy of different estrus synchronization protocols on reproductive parameters in nulliparous Saanen goats at the end of the transition period. In this experiment, 71 nulliparous Saanen does were used and divided into four treatment groups as norgestomet implant plus GnRH (Imp-G), norgestomet implant (Imp), FGA sponge plus GnRH (Spo-G), and FGA sponge (Spo). The progestagen treatments were administered for 11 days. At progestagen withdrawal, all does were injected with 360 IU of eCG and 125 µg of PGF2α. At the end of the treatments, all the does were joined with fertile bucks. There were no significant differences (P>0.05) in reproductive parameters among the treatments groups. Does were in estrus within 29.2±0.36 h for all the treatments combined. It was concluded that, the use of norgestomet implants and FGA sponges in combination with eCG and PGF2α were effective for estrus response, onset of estrus, fecundity, prolificacy, and fertility in nulliparous Saanen does at an age of 7-9 months under local conditions at the end of the transition period also GnRH was found to be ineffective in increasing the reproductive parameters.

Keywords: *Estrus synchronization, GnRH, Nulliparous Saanen goat, Progestagens, Transition period*

Geçiş Döneminin Sonundaki Nullipar Saanen Keçilerinde Farklı Progesteron Analogları ve GnRH Uygulamalarının Reprodüktif Parametreler Üzerine Etkinliği

Özet

Çalışmada, geçiş döneminin sonundaki nullipar Saanen keçilerine uygulanan farklı östrus senkronizasyon protokollerinin reprodüktif parametreler üzerine etkilerinin karşılaştırılması amaçlandı. Çalışmada 71 adet nullipar Saanen keçisi kullanıldı ve keçiler rastgele 4 gruba ayrıldı: norgestomet implant + GnRH (Imp-G), norgestomet implant (Imp), FGA sünger + GnRH (Spo-G) ve FGA sünger (Spo). Progesteron tedavileri 11 gün süreyle uygulandı. Progesteron tedavilerinin son günü tüm keçilere 360 IU eCG ve 125 µg PGF2α yapıldı. Tedavilerin sonunda tüm keçiler fertil tekeler ile çiftleştirildi. Tedavi grupları arasında reprodüktif parametreler açısından istatistiksel bir farkın olmadığı belirlendi (P > 0.05). Tüm tedaviler değerlendirildiğinde keçilerin 29.2±0.36 saat içinde östrus gösterdiği saptandı. Sonuç olarak, yerel çevre şartlarında yetiştirilen ve geçiş sezonunun sonunda bulunan 7-9 aylık nullipar Saanen keçilerinde norgestomet implant ve FGA süngerin eCG ve PGF2α ile birlikte kullanılmasının östrus oranı, östrus başlangıcı, fekundite, prolifikasyon ve fertilite oranları bakımından etkili olduğu; GnRH kullanımının ise fertilite parametrelerinde artış sağlamadığı belirlendi.

Anahtar sözcükler: *Östrus senkronizasyonu, GnRH, Nullipar Saanen keçisi, progestagenler, Geçiş dönemi*

INTRODUCTION

Goats are bred in a wide range of production systems [1]. In temperate regions, they are bred mainly for dairy production, but also for meat and fiber [2]. Goat breeding is, economically and socially, important in Turkey. Milk goats are raised mainly in the Aegean, Marmara, and Thrace regions in Turkey. Saanen goats were first brought to Turkey in

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1959, and this breed is still being raised as purebred and as crosses [3-5]. In the female goat, the age of puberty is highly variable and is dependent upon the genetic make-up of the animals, kidding season, body energy reserves, nutrition, stress, and management system [6,7]. Several studies have reported that the average age of puberty in Saanen goats varies between 6 and 12 months in Turkey [5,8,9], 5 months in Brazil [6], 7.5 months in Mexico [10,11], and 8 months in France [12].

Goats are known to exhibit seasonality in breeding activity and the onset and length of the breeding season is dependent on factors such as latitude, climate, breed, physiological stage, presence of the male, breeding system, and photoperiod [2]. The breeding season usually begins in summer or early autumn, in response to shortening day-length, and ends in late winter or early spring. The anestrous period includes the late winter/early spring to early- or mid- summer period, while the transition period expands from late spring to the onset of the ovulatory period [13-15]. A study conducted to determine the breeding season of small ruminants in Afyonkarahisar, Turkey, found that the breeding season occurred between the mid August and November [16].

Techniques used to control reproduction in goats allow for greater distribution of milk and meat production throughout the year and synchronization of kidding over a limited period, and facilitate supplementary feeding to meet the demands of lactation [2,17]. The most widely practiced methods of estrous synchronization involve progesterone or progestagen-based protocols [18]. Synchronization of estrus in female goats has been achieved through administration of progesterone or its synthetic analogues, with a vaginal device or a subcutaneous auricular implant [19-22]. The combination of equine chorionic gonadotropin (eCG), prostaglandin F2α (PGF2α) and progestagen treatments eliminates variability in goats' ovulatory response, increases production of ovulatory follicles and ovulation rate, and improves fertility [19,22-24]. İnce and Köker [25] reported that treatment with progestagen in conjunction with PGF2α and eCG was convenient for estrous synchronization of Turkish Saanen goats during the breeding season in Turkey. Follicular wave synchronization protocols using gonadotropin releasing hormone (GnRH) analogues have been designed on the principle of controlling the induction of the emergence of a new follicular wave by removing the suppressive effect of the existing dominant follicles [26]. Intramuscular administration of GnRH at the time of insertion of a progestagen device induces intermediate ovulation or turnover of the dominant follicles in cows. When comparing estrous synchronization via insertion of intravaginal progestagen devices alone and synchronization via a combination of intravaginal progestagen devices and GnRH in dairy cows, several researchers have found the combination to be favorable for conception rates [27,28]. In small ruminants, synchronization protocols consisting of progestagen, GnRH and PGF2α increase estrous response and pregnancy rates [29]. However, there are only a few studies that have reported the effects of different synchronization methods and hormones on nulliparous goats [22,30-32]. Therefore, the objective of this study was to compare the effectiveness of an intravaginal sponge (FGA) and ear implant (norgestomet) with or without GnRH in combination with eCG and PGF2α, on estrus response, onset of induced estrus, fecundity, prolificacy, fertility, and sex of kids in nulliparous Saanen goats under local environmental conditions at the end of the transition period.

MATERIAL and METHODS

This study was conducted on nulliparous Saanen does kept on a goat farm situated close to the province of Afyonkarahisar, Turkey (latitude, 38°45´2´´N; longitude, 30°32´3´´E). This region, at an altitude of 1015 m, is characterized by a continental climate, with an average annual temperature of 11.2°C. The experiment was initiated at the end of July under a natural photoperiod environment and lasted to the end of the gestation period.

Animals

A total of 71 nulliparous Saanen does, 7-9 months of age and weighing between 29 and 38 kg, were used. Body condition scores ranged from 2.5 to 3 (on a1-5 scale where 1 = emaciated and 5 = obese). The animals were fed dry hay supplemented with a commercial mixture and had free access to shade, water, and mineral blocks. The goats were housed in indoor shelters at night and the entire flock was maintained under natural lighting conditions. Adult, intact, and fertile (according to farm records) bucks (n = 6) were housed separately from the does.

Experimental Design

Does were randomly divided into four treatment groups: norgestomet implant plus GnRH (Imp-G), norgestomet implant (Imp), FGA sponge plus GnRH (Spo-G), and FGA sponge (Spo) *(Fig. 1)*. During does care, clinical evaluation and performing the study we obeyed the statement of Helsinki Declaration.

Females in the Imp-G group (n = 18) received a subcutaneous auricular implant (3.3 mg norgestomet, Crestar SO, Intervet, Turkey) and were injected intramuscularly with GnRH (4 µg of busereline acetate, Receptal, Intervet, Turkey) at the time of implant insertion. In the Imp group (n = 17), animals received a subcutaneous auricular implant (3.3 mg norgestomet, Crestar SO, Intervet, Turkey) without any injection of GnRH. Females in the Spo-G group (n = 19) were treated with intravaginal sponges (20 mg of FGA, Chronogest CR, Intervet, Turkey), and injected intramuscularly with GnRH (4 µg of busereline acetate, Receptal, Intervet, Turkey) at the time of sponge insertion. In the Spo group (n = 17), animals were treated with intravaginal sponges (20 mg of FGA, Chronogest CR, Intervet, Turkey) without any injection of GnRH. The progestagen treatments were administered for 11 days. At progestagen withdrawal,

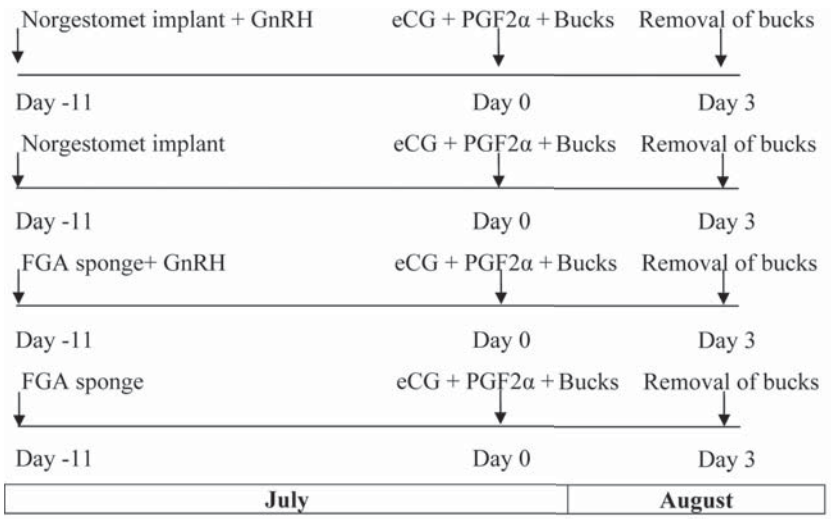

Fig 1. Schematic diagram of the overall experimental design

Şekil 1. Tüm deney tasarımının şeması

all the does were injected intramuscularly with 360 IU of eCG (Chronogest/PMSG, Intervet, Turkey) and 125 µg of PGF2α (cloprostenol, Estrumate, Intervet, Turkey).

At the end of the treatments, all the does were joined with fertile bucks (n = 6). Searching for the male, restlessness, vocalization, frequent urination, tailing, contraction, hyperemia and edema of the vulva, vaginal mucous discharge and immobility on mounting were accepted as the onset of estrus, according to Fonseca and Torres [30]. All the does were observed continuously between 20 and 37 h after treatment for onset of estrus and mating. When signs of estrus were observed, the goats were subjected to controlled mating. Estrus was monitored 12-h intervals by visual observation, for 3 days after the introduction of the bucks. Estrous response, onset of induced estrus, fecundity, prolificacy, fertility, and sex of kids were compared between the treatments. Fertility, prolificacy, and fecundity were recorded after parturition. These parameters were defined as follows:

Estrous response: Number of does in estrus/number of total does x 100

Onset of induced estrus: Time from implant-sponge removal and first mounting acceptance

Pregnancy rate: Number of does kidded + number of does aborted/number of total does x 100

Parturition rate: Number of does kidded/number of does pregnant x 100

Twinning rate: Number of does having twin kids/number of does kidded x 100

Fecundity: Number of kids born per doe of the mated

Prolificacy: Number of kids born per doe kidded

Fertility: Number of does kidded

Statistical Analyses

Data on estrus response, pregnancy rate, parturition rate, twinning rate, fecundity, prolificacy, fertility, and sex of kids were analyzed using chi-square tests. Onset of induced estrus data were analyzed using the Kruskal Wallis test. All the results were analyzed at a statistical confidence level of $P<0.05$ by using PASW statistical software (Version 18.0, PASW Inc, Chicago, IL, USA).

RESULTS

Estrus response, onset of estrus, pregnancy rate, parturition rate, twinning rate, and sex of kids did not differ ($P>0.05$) among the treatment groups *(Table 1)*. No norgestomet implants were lost during the experimental period in the implant groups. Sponges were lost in three does in the sponge groups. There were no site reactions near the auricular implants at the time of implant withdrawal in does in the Imp-G and Imp groups. There were no vaginal abnormalities at sponge insertion in the does. However, when the sponges were removed after 11 days, all the does in the Spo-G and Spo groups excibited clinical signs of vaginitis, including abnormal hemorrhagic or purulent vaginal discharge with unpleasant odor. Two abortions occurred in the Imp-G group, and one abortion occurred in the Spo-G group in late pregnancy.

Estrus responses were 83.3%, 82.4%, 73.7, and 82.4% in the Imp-G, Imp, Spo-G, and Spo groups, respectively. The mean onset of estrus was 29.2±0.36 h. Parturition rates were 84.6%, 100%, 92.3% and 100% in the Imp-G, Imp, Spo-G, and Spo groups, respectively.

The results for fecundity, prolificacy, and fertility rate are presented in *Table 2*. Fecundity rates were 0.93, 1.14, 0.92 and 1.00 in the Imp-G, Imp, Spo-G, and Spo groups, respectively. Prolificacy rates were 1.27, 1.23, 1.08, and 1.16 in the Imp-G, Imp, Spo-G and Spo groups, respectively. The fecundity, prolificacy and fertility rates did not differ ($P>0.05$) among the treatments.

DISCUSSION

The timing of puberty is clearly not a simple function

Table 1. *Estrus response, pregnancy, parturition and twinning rate, and onset of estrus (mean ± SEM) of nulliparous Saanen goats following induction of estrous using different synchronization methods.*

Tablo 1. *Farklı senkronizasyon metotları ile östrus indüksiyonu yapılan nullipar Saanen keçilerinde östrus, gebelik, doğum ve ikizlik oranları ile östrus başlama zamanı (ortalama±SEM) değerleri*

Parameters		Imp-G (n=18)	Imp (n=17)	Spo-G (n=19)	Spo (n=17)	Total (n=71)	P-values
Estrus response (%)		83.3 (15/18)	82.4 (14/17)	73.7 (14/19)	82.4 (14/17)	80.3 (54/71)	0.868*
Onset of estrus (h)		29.6 ± 0.74	29.8 ± 0.85	29.0 ± 0.76	28.3 ± 0.55	29.2 ± 0.36	0.489**
Pregnancy rate (%)		72.2 (13/18)	76.5 (13/17)	68.4 (13/19)	70.6 (12/17)	71.8 (51/71)	0.959*
Parturition rate (%)		84.6 (11/13)	100 (13/13)	92.3 (12/13)	100 (12/12)	94.1 (48/51)	0.289*
Twinning rate (%)		23.1 (3/13)	23.1 (3/13)	7.7 (1/13)	16.7 (2/12)	17.6 (9/51)	0.700*
Sex of kid (%)	Male	57.1 (8/14)	37.5 (6/16)	53.8 (7/13)	57.1 (8/14)	50.9(29/57)	0.652*
	Female	42.9 (6/14)	62.5(10/16)	46.2 (6/13)	42.9 (6/14)	49.1(28/57)	

** Variables were analyzed by the use of chi-square test, ** Variables were analyzed by the use of Kruskal-Wallis test*

Table 2. *Fecundity, prolificacy and fertility values of nulliparous Saanen goats following induction of estrus using different synchronization methods*

Tablo 2. *Farklı senkronizasyon metotları ile östrus indüksiyonu yapılan nullipar Saanen keçilerinde fekundite, prolifikasi ve fertilite değerleri*

Parameters	Imp-G (n=18)	Imp (n=17)	Spo-G (n=19)	Spo (n=17)
Fecundity	0.93	1.14	0.92	1.00
Prolificacy	1.27	1.23	1.08	1.16
Fertility (%)	61.1	76.5	63.1	70.6

of chronological age. Several factors, such as photoperiod, growth, body fat/composition, diet, stress, gonadal steroids, energy metabolism, and olfactory cues affect puberty [7,33]. The onset of puberty is more closely related to body weight and size than to age [34]. In general, breeding in goats should be delayed until the animal has attained 60-75% of its mature body weight [35]. A study by Freitas et al.[6] reported that 92.9% of Saanen kids reached puberty and showed estrus at 50% of adult body weight. The mean body weight of mature Saanen does varies between 38.1 and 64 kg [19,25,36,37]. The mean body weight of peripubertal Saanen does varies between 22.5 and 34.5 kg [6,8,9,11,38]. The estrus synchronization protocols used in the study resulted in greater synchrony (83.3, 82.4, 73.7, and 82.4% in the Imp-G, Imp, Spo-G and Spo groups, respectively) in the nulliparous Saanen goats those of which are at an age of 7-9 months and with body weights varying between 29 and 38 kg.

Intravaginal sponges impregnated with progestagens have been widely used for estrous synchronization in ewes and goats [39]. Nevertheless, they are a predisposing factor for vaginal infections, leading to vaginitis, typically characterized by erythema, a purulent vaginal discharge, and abundant vaginal leucocytes [40,41]. These changes in the vagina may be attributed to the physical action and/or constant absorption and retention of the vaginal secretions by the intravaginal sponge during insertion, which stimulates bacterial growth [42]. In the present study, there were no site reactions near the auricular implants at the time of implant withdrawal in does in the Imp-G and Imp groups. However, when the sponges were removed, all the does in the Spo-G and Spo groups excibited clinical signs of vaginitis, including abnormal hemorrhagic or purulent vaginal discharge with unpleasant odor. Therefore, it was thought that subcutaneous auricular implants (norgestomet) are more practical than intravaginal sponges (FGA) for nulliparou goats because of the side effects of intravaginal sponges, including vaginitis and abnormal hemorrhagic or purulent vaginal discharge with unpleasant odor.

The total estrous response was 80.3%, and the mean interval to onset of estrus was 29.2±0.36 h in the present study. These results showed that both norgestomet and FGA were effective in inducing estrus in nulliparous Saanen goats, which was consistent with the findings of Fonseca and Torres [30] and Freitas et al.[43]. Our estrous response results differ from those obtained by Alaçam et al.[44] who found only a 54.5% response from treatment with intravaginal MAP sponges and 40% estrous response from treatment with two injections of PGF2α 10 days apart in nulliparous Saanen goats. In addition, the overall mean interval to onset of estrus was shorter in our study than that reported by Simoes et al.[45] and Bukar et al.[22] and longer than that reported by Dogan et al.[46]. Prostaglandin administration reduces the interval to estrus and also promotes greater synchrony [47]. By comparing the present results with those reported by other authors [22,30,43], it is possible to propose that the synchronization obtained for females treated with eCG is due to the action of this gonadotrophin on follicular development. The efficiency of estrous synchronization treatments is known to be influenced by factors such as nature, dose and route of administration, synchronization protocols, nutrition, and season [48,49]. Furthermore, differences in breed or defective luteal function in individual does might influence the response of does to different estrous synchronization protocols [22].

Pregnancy and parturition rates were not affected by treatment in this study. The overall pregnancy and parturition rates were 71.8% and 94.1%, respectively. Progestagens, when associated with gonadotropins and luteolytic agents, generally produce good results in estrous induction and favor higher fertility rates at delivery [50]. These findings were confirmed in the present study, where pregnancy and parturition rates were satisfactory after treatment with

norgestomet implants and FGA sponges in combination with eCG and PGF2α. Our results are in agreement with the findings of Cetin et al.[20] and Koker et al.[37]. However, the pregnancy rates observed in our study were higher than those reported by Waldron et al.[51], Oliveira et al.[19], and Uslu et al.[52]. Pregnancy and parturition rates may vary depending on breed, season, nutrition, and overall conditions of animal care [53]. The percentage of multiple births in multiparous Saanen goats is estimated to be approximately 60% [37]. However, Simoes et al.[45] reported that single ovulation was observed in 76% of estrous periods in nulliparous goats. Ince [5] reported that single births were 77.3% in 2 year-old Saanen goats and 46.2% in goats that were more than 5 years of age. The overall twinning rate in our study was 17.6%, and this result agrees with the findings of Bolacalı and Küçük [54], Simoes et al.[45] and Ince [5].

The fecundity, prolificacy, and fertility rates were similar in all the treatment groups, which agree with the findings of Fonseca and Torres [30] and Cetin et al.[20]. However, our results for these parameters were lower than those reported by Freitas et al.[43], Oliveira et al.[19], and Titi et al.[55]. These differences may be attributable to the dose, route, and duration of progestagen treatment, or to the dose of eCG. All major measures of reproductive performance (prolificacy, fertility, and fecundity) are affected by genetics and by a variety of environmental factors. With respect to prolificacy, there is considerable variation in the incidence of twin ovulations and twin births among and within breeds. The component of the diet which is probably the most important with respect to ovarian function is energy [56]. Furthermore, there is a linear relationship between the dose of eCG and ovulation number [57] In our study, 360 IU of eCG was used in all the treatment groups to stimulate ovulation without inducing a high incidence of multiple ovulations, since the goats were nulliparous.

GnRH and its agonists are widely used to overcome reduced fertility due to ovarian dysfunction, for synchronization of the estrous cycle, to induce ovulation, as stimulation for embryo transfer, and to improve the conception rate [58]. Titi et al.[55] reported that a combination of GnRH, progestagen sponges, and PGF2α was effective in synchronizing estrus and improving fecundity in goats. Similar studies have also shown that GnRH analogues used for estrous synchronization increased estrous response and improved pregnancy rates in treated goats [29,59]. On the other hand, Sarıbay et al.[15] found that GnRH addition to progesterone sponges was not improved reproductive parameters. The results for estrous response, pregnancy rates, and fertility were similar among the groups treated with or without GnRH in the present study, and our results were in agreement with the results of Sarıbay et al.[15]. This finding indicates that the use of GnRH in combination with 11-day norgestomet or FGA did not influence estrous synchronization or fertility in nulliparous Saanen goats under local environmental conditions.

We conclude that the use of norgestomet and FGA in combination with eCG and PGF2α is very effective for estrous response, onset of estrus, fecundity, prolificacy, and fertility in nulliparous Saanen does under local environmental conditions. The results of this study indicate that estrus can be induced in nulliparous does as early as 7-9 months of age. This study shows that it is not always necessary to include GnRH in estrous synchronization protocols in nulliparous Saanen does. However, further studies with different breeds are required.

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RESEARCH ARTICLE

Kars Yöresindeki Sığırlarda *Anaplasma marginale* Seroprevalansı [1]

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[1] *Bu makale Kafkas Üniversitesi Bilimsel Araştırma Projeleri Komisyon Başkanlığı tarafınca desteklenen 2011-VF-04 kodlu projeden hazırlanmıştır*

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Özet

Bu çalışma Türkiye'nin Kars yöresindeki sığırlarda *Anaplasma marginale* seroprevalansının araştırılması amacıyla yapıldı. Bu amaçla Kars yöresindeki 5 farklı odaktan 188 sığırdan kan serumu örnekleri alındı. *Anaplasma marginale*'ye karşı oluşan antikorların saptanması için ticari Kompetetive-ELISA (C-ELISA) testi kullanıldı. C-ELISA sonuçları serum örneklerinin %52.1'inde *Anaplasma marginale*'ye karşı pozitif antikor bulunduğunu göstermiştir. Bu çalışmanın sonuçları Türkiye'nin Kars yöresindeki sığırlarda *Anaplasma marginale* enfeksiyonunun yaygın olduğunu göstermektedir.

Anahtar sözcükler: *Anaplasma marginale, Sığır, Seroprevalans, Kars, Türkiye*

Seroprevalance of *Anaplasma marginale* in Cattle in Kars Region

Summary

This study was conducted to investigate the seroprevalance of *Anaplasma marginale* in cattle in Kars region of Turkey. For this purpose, blood serum samples were collected from 188 cattle from 5 different districts of Kars region. A commerically available Competitive enzyme-linked immunosorbent assay (C-ELISA) were used for determine antibodies to *Anaplasma marginale*. The C-ELISA results showed that 52.1% of serum samples were positive for antibodies to *Anaplasma marginale*. The results of this study show that *Anaplasma marginale* infection in cattle is common in Kars region of Turkey.

Keywords: *Anaplasma marginale, Cattle, Seroprevalance, Kars, Turkey*

GİRİŞ

Sığırlarda anaplazmozis, intraeritrositik riketsiyal bir etken olan *Anaplasma marginale* tarafından oluşturulan ve başlıca anemi ile seyreden enfeksiyöz bir hastalıktır. Anaplazmozis sığırlarda şiddetli anemi, verim kaybı, abort ve mortaliteye yol açtığından dünyanın birçok yerinde ciddi ekonomik kayıplara neden olmaktadır. *Anaplasma marginale,* sığırlardan başka koyun, keçi ve yabani ruminantlarda da intraeritrositik enfeksiyona yol açmaktadır [1,2]. Hastalık etkeni, mekanik olarak sivrisinekler, kontamine iğne veya cerrahi malzemelerle bulaşır. Etken biyolojik olarak da kenelerle nakledilir [2]. *A. marginale* transplasental yolla da bulaşmaktadır [3,4]. Biyolojik nakilde çeşitli kene türleri görev yapmaktadır. Bunlar: *Argas persicus, Ornithodoroslahorensis, Boophilus annulatus, B. calcaratus, B. decoloratus, B. microplus, Dermacentor albipictus, D. andersoni, D. occidentalis, D. variabilis, Hyalomma excavatum, H. Rufipes, Ixodes ricinus, Rhipicephalus bursa, R. sanguineus, R. evertsi, R. sanguineus, R. simus*'dir [5,6]. Arslan ve ark.[7], bu kene türlerinden bazılarının Kars yöresinde de bulunduğunu saptamışlardır.

Anaplazmozis özellikle tropikal ve subtropikal bölgelerde olmak üzere, dünyanın değişik bölgelerindeki sığırlarda hastalık yapmaktadır [8]. Avrupa'da ise Akdeniz çevresindeki ülkelerde yaygındır [9,10]. Sığır anaplazmozisi Asya ve Afrika'da endemiktir [2,11]. Ülkemizde de anaplazmozisin bulunduğuna dair çalışmalar mevcuttur [12-14]. Ancak Kars yöresinde sığır anaplazmozisi ile ilgili bir çalışmaya rastlayamadık.

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Anaplazma etkenleri eritrositler içinde 0.3-1 µm çapında noktalar şeklinde görülen mikroorganizmalardır. Hastalığın şiddetine göre bir eritrosit içinde 6-7 etken bulunabilir [15]. Enfektif doza göre değişmek üzere, hastalığın inkubasyon periyodu 21-45 gün arasında değişir [16]. Etkenler eritrositler içerisinde çoğalır, ekstravasküler hemolizis ve bundan dolayı ilerleyici bir anemiye neden olurlar [17].

Klinik anaplazmozis genellikle 1 yaşından büyük sığırlarda görülür [18]. Hastalık perakut, akut ve subklinik formlarda seyreder. Perakut anaplazmozisde klinik semptomlar birkaç saat içinde oluşur ve ölümle sonuçlanır. Perakut seyir genellikle yüksek süt verimli ineklerde görülür [2,15]. Anaplazmoziste ilk semptom yüksek ateştir. Bu durum eritrositlerin %1'inden azının enfekte olmasından önce başlar. Parazitemi dönemi boyunca ateş 40°C'nin üzerindedir. Ateş ölümden önce normalin altına düşer. Hastalıkta güçsüzlük, zayıflama ve ikterus, süt veriminde azalma, kalp ve solunum frekansında artış ve depresyon oluşur. Hastalığın ilerlemesiyle gastrointestinal atoni, ruminal stazis, konstipasyon ve dehidrasyon gelişir [2,3]. Hastalık sığırlarda serebral hipoksiye bağlı nörolojik yetersizlikler, dişilerde abort ve infertilite bozukluklarına neden olabilir [2,15,16]. Hayvanlar yıllarca klinik semptom göstermeden etkeni taşıyabilirler [3].

Akut dönemde hastalığın tanısı mikroskobik olarak etkenlerin eritrositlerde görülmesiyle konulabilir. Bu safhadaki laboratuvar değerlerine bakıldığında ciddi hemolitik anemi bulgularını yansıtan hematokrit, eritrosit ve hemoglobin düşüklüğü saptanır [3,15]. Hastalıkta klinik tanı anamnez, semptomlar, hematolojik analizler, kan frotisi muayeneleri ve nekropsi bulguları ile gerçekleştirilir. Fakat subklinik olgularda her zaman mikroskobik yöntemle tanı konulamayabilir. Bu nedenle kronik olgularda çeşitli seroloji yöntemleriyle tanı gerçekleştirilir [2,19]. Ayrıca tanı için moleküler diagnostik yöntemlerle de kullanılmaktadır [2,20]. Son yıllarda kullanılmaya başlanan kompetitif ELİSA yöntemiyle daha güvenilir teşhis konulmaktadır [19,20].

Bu çalışmanın amacı Kars ve ilçelerindeki sığırlarda *A. marginale*'ye karşı oluşan antikorların varlığını araştırmaktır. Anaplazmozis, dünyanın birçok bölgesindeki sığırcılıkta büyük ekonomik kayıplara neden olmaktadır. Bu nedenle yüksek sığırcılık potansiyelinin olduğu Kars bölgesindeki anaplazmozis varlığının araştırılması bilimsel ve ekonomik anlamda önem taşımaktadır.

MATERYAL ve METOT

Çalışma Alanı

Bu çalışma Kars merkez, Arpaçay, Sarıkamış, Akyaka ve Kağızman ilçelerinden sağlanan sığır kan materyali ile yapıldı. Kars Türkiye'nin kuzeydoğusunda 43:05 E enlem ve 40:36 N boylamları arasında yer alır. Kars'ta rakım 1750 m'dir. Kağızman ortalama rakım 1078 m, Akyaka 1490 m, Arpaçay1920 m, Sarıkamış 1668 m'dir. Kars ilinde yaz ayları sıcaklık ortalaması 15.9-19.3ºC arasında değişmektedir.

Çalışma Materyali

Çalışmanın hayvan materyalini 1-6 yaşta, farklı ırk ve cinsiyette 188 baş sığır oluşturdu. Bu sığırların 160'ı her odaktan 40'ar olmak üzere Kars merkez, Akyaka, Kağızman ve Sarıkamış'tan, 28'i ise Arpaçay ilçesinden sağlandı. Sığırların vena jugularis'lerinden 10 ml kan örneği alındı. Alınan bu kan örnekleri 3.000 devirde 10 dak. santrifüj edildikten sonra serumları ayrılarak analiz gününe kadar -20ºC'de saklandı.

Competitive- ELISA Testi

Çalışma için ticari kompetetif ELISA (C-ELISA) kiti kullanıldı. C-ELISA testi, üretici firmanın test prosedürüne göre yapıldı (Anaplasma antibody test kit, C-ELISA, catalog number: 282-2VMRD-USA).

ELISA Sonuçlarının Değerlendirilmesi

Test sonucu, spektrofotometrik olarak 650 nm filtre absorbanslarında okunmak suretiyle belirlendi. Bu aşamada negatif kontrol optik dansite (OD) 0.40-2.10 aralığında alındı. Pozitif kontrol ve örneklerin değerlendirilmesinde test prosedüründe belirtilen hesaplama yöntemi kullanıldı. Pozitif kontrol hesaplamalar sonucunda %30'a eşit ve büyük olarak kabul edildi. Örneklerin değerlendirilmesi ise test prosedüründe belirtilen yöntemle hesaplandı ve %30'dan küçük olanlar negatif, bu değerden büyük veya eşit olanlar ise pozitif olarak değerlendirildi.

İstatistiksel Analizler

Bölgeler arası farklılıkların istatistiksel analizi Ki-Kare (X^2) testi kullanılarak yapıldı.

BULGULAR

Bu çalışmada Kars ve 5 ilçesinden alınan 188 baş sığır serumu *Anaplasma marginale* antikorları yönünden araştırıldı. Toplam olarak bu serumların %52.1'inde (98/188) antikor pozitif bulundu. Akyaka'daki sığırların %52.5'i (21/40), Kars merkezdeki sığırların %50'si (20/40), Kağızman'daki sığırların %57.5'i (23/40), Sarıkamış'taki sığırların %52.5'i (21/40) ve Arpaçay'daki sığırların %46.4'ünde (13/28) antikor pozitif bulundu *(Tablo 1)*.

TARTIŞMA ve SONUÇ

Bu çalışmada Kars merkez ve bazı ilçelerindeki sığırlarda *A. marginale*'ye karşı oluşan antikorların varlığı araştırıldı. Bu çalışmada *A. marginale* enfeksiyonlarının tanısı için C-ELISA testi kullanıldı. Persistent enfeksiyonlarda, kan frotilerinde enfekte eritositlerde hastalık etkeni her zaman saptanamadığından, tanı genellikle çeşitli serolojik

Tablo1. *Anaplazma marginale antikorları yönünden pozitif hayvanların bölgelere göre dağılımı*

Table1. *Regional distribution of frequency of animals positive for anti-Anaplasma marginale antibodies*

Bölgeler	Hayvan Sayısı	Pozitif Hayvan Sayısı (%)
Akyaka	40	21 (%52.5)
Merkez	40	20 (%50.0)
Kağızman	40	23 (%57.5)
Sarıkamış	40	21 (%52.5)
Arpaçay	28	13 (%46.4)
Total	**188**	**98 (%52.1)**

Bölgeler arasında istatistiksel açıdan önemli bir fark bulunmamıştır (X^2: 0.904; P>0.05)

testlerle yapılmaktadır [5,12]. Bu testlerin içinde C-ELISA'nın yüksek bir sensitivite ve spesifiteye sahip olduğu ortaya konmuştur [19,22].

Birdane ve ark.[12] çalıştıkları 357 hayvanın 147'sinde eritrositlerin içinde anaplazma etkeni bulamadıklarını, fakat bu hayvanların *A. marginale* antikorları yönünde C- ELISA ile pozitif olduklarını, C-ELISA'nın akut ve kronik anaplazmozisin tanısında etkili bir test olduğunu saptamışlardır.

Bu çalışmanın sonuçları Kars ve ilçelerindeki sığırların yüksek oranda *A. marginale* ile karşılaşmış olduklarını göstermektedir. Total olarak Kars ve ilçelerindeki sığırlarda *A. marginale* antikorlarının varlığı %52.1 olarak bulundu. Merkez ve ilçeler arasında istatistiksel olarak önemli bir fark saptanmamasına karşın, en yüksek oran %57.5 ile Kağızman ilçesinde, en düşük oran %46.4 ile Arpaçay ilçesinde saptandı. Bölgeler arası oransal farklılığın nedeni agro-ekolojik özellikler, toprağın özellikleri ve iklim koşullarından kaynaklanmaktadır [11]. Nitekim Kağızman bölgesi Kars'ın en sıcak ilçesidir. Bölgenin rakımı ortalama Kars rakımından daha düşüktür. Dolayısıyla kene ve sinek aktivitesinin daha yüksek olduğu söylenebilir. Ancak bu konuda kesin bir sonucun ortaya konulması için Kağızman yöresinde daha çok sayıda hayvan üzerinde moleküler ve serolojik araştırmaya, akut klinik olguların araştırılmasına, froti incelemelerine ve kene türleriyle diğer bulaşma yollarının araştırılmasına ihtiyaç vardır.

Birdane ve ark.'nın [12] İç Ege bölgesindeki sığırlarda yaptıkları bir çalışmada; *A. marginale* seroprevalansı 3-4 yaşlı sığırlarda %58.21; >4 yaşlı sığırlarda %82.07 olarak bulunmuştur. Yaptığımız bu çalışmada ise Kars yöresindeki 1-6 yaşlı sığırların %52.1'inde *A. marginale* seropozitif olarak bulundu. Bu sonuçlara bakıldığında İç Ege deki oranın Kars bölgesinde yüksek olduğu ortaya çıkmaktadır. Bu durum bölgesel iklim ve kene türleri farklılıklarından kaynaklanabilir. Bu çalışmada kan örneği alınan sığırların hiçbirinde klinik anaplazmozis bulguları saptanamasına karşın, yüksek oranda *A. marginale* seropozitifliği saptandı. Bu sonuç sığırların kronik olarak enfekte veya taşıyıcı olduklarını göstermektedir [6].

Çalışmamızda Kars ve çevresindeki ilçelerde 1-6 yaşlı sığırlarda *A. marginale* enfeksiyonunun yüksek bir yaygınlık gösterdiği ve bölge için önemli bir sorun olduğu ortaya konulmuştur. Çiftlik sahipleriyle yaptığımız görüşmelerde kenelerle yeterli ve düzenli bir mücadelenin yapılmadığını saptadık. Yetersiz insektisit kullanımının bu yüksek oranda etkili olduğu söylenebilir. Ayrıca diğer hastalıklara karşı yapılan aşılamalar sırasında birçok hayvanda aynı enjektörün kullanımının da bulaşmada etkili olduğu bilinmektedir [21]. Bölgedeki seroprevalans yüksekliğinde bu uygulamaların da rolünün olabileceğini düşünmekteyiz. Ayrıca bölgede mera hayvancılığının yaygın olması nedeniyle yabani ruminatlarında hastalığın yayılmasında önemli olabileceğini düşünmekteyiz [1].

Sorunun çözümü için özellikle meraya çıkış döneminde yeterli kene ve sinek mücadelesinin yapılması, aşılama ve diğer uygulamalar sırasında bir hayvanda kullanılan enjektörün diğer hayvanlarda kullanılmaması yönünde duyarlı davranılması ve gerekli hijyen kurallarına önem verilmesi gerekmektedir. Ayrıca anaplazmozise karşı aşılama çalışmaların yapılması gerektiğini düşünmekteyiz.

Sonuç olarak bu araştırma, Kars yöresindeki sığırlarda *A. marginale* seroprevalansını ortaya koyan bir ön çalışma niteliğindedir. Bölgedeki bu hastalığı gerçek boyutunun tam olarak ortaya konulması için daha kapsamlı epidemiyolojik, moleküler ve klinik çalışmalara ihtiyaç vardır. Ayrıca bölgede ciddi ekonomik kayıplara neden olan, tüm nedenleri ortaya konulamayan sığırlardaki abortların etiyolojisinde *A. marginale*'nin de göz önünde bulundurulmasını düşünmekteyiz.

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RESEARCH ARTICLE

Effectiveness of the Local Application of 1% Tioconazole in the Treatment of Bovine Dermatophytosis

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Summary

This study consisted of twenty-five dermatophytotic cattle, aged 1.5-11 months obtained from Kars province and its surrounding, diagnosed as dermatophytosis following clinical and microbiologically. All animals were divided into two groups; trial group (n=15) and control group (n=10). The skin lesions of the trial animals were treated using a pomade containing 1% tioconazole once daily for a period of 5 days. In 12 of the trial animals the amount of keratinized tissue found in the dermatophytosis lesions decreased significantly following the 3rd application and had disappeared completely after the 5th application. On the other hand, the remaining 3 animals in the trial group were applied the medicament seven times due to the persistence of keratinized tissue in the lesions. In all of the animals administered with tioconazole, new hair growth in the site of the lesions resumed in the 3rd-4th weeks and complete recovery occurred within 7-8 weeks. In conclusion, 1% tioconazole, used for the first time for the treatment of bovine dermatophytosis in this study was found to be rather effective owing to its ease of application and strong therapeutic effect.

Keywords: *Cattle, Dermatophytosis, Tioconazole*

Sığır Dermatofitozisinin Tedavisinde %1'lik Tiokonazol'ün Lokal Kullanımının Etkinliği

Özet

Çalışma materyalini, Kars ve çevre köylerinden sağlanan, 1,5-11 aylık yaşta, klinik ve mikrobiyolojik olarak dermatofitozis tanısı konulan 15 deneme ve 10 kontrol olmak üzere, toplam 25 sığır oluşturdu. Deneme grubundaki hayvanların derilerindeki lezyonların üzerine %1 tiokonazol içeren kremden 5 gün boyunca günde 1 kez sürüldü. Deneme grubundaki hayvanlardan 12'sinde 3. uygulamayı takiben, dermatofitoz lezyonlarındaki keratinize dokuların büyük oranda azaldığı, 5. uygulama sonunda ise tamamen kaybolduğu görülürken 3 hayvanda keratinize doku dökülmediği için 7. uygulamaya gidildi. İlaç uygulanan tüm hayvanlarda lezyonlu bölgelerde 3-4. haftalarda kıllanmanın başladığı, 7-8. haftalarda ise tamamen iyileştiği görüldü. Sonuç olarak, sığırlarda dermatofitozis olgularının tedavisinde ilk olarak denenen %1'lik tiokonazol'ün, kullanımının kolay olması ve tedavi edici etkisinin yüksek olması nedeniyle, sığırlarda dermatofitozis olgularının sağaltımında oldukça etkili bulunmuştur.

Anahtar sözcükler: *Sığır, Dermatofitozis, Tiokonazole*

INTRODUCTION

Dermatophytosis, otherwise known as ringworm, is a fungal disease of keratinized tissues, including the skin, hair and nails, caused by dermatophytes. These ubiquitous agents cause infections of varying severity in both humans and animals [1,2]. The dermatophyte most frequently encountered in cattle and sheep is *Trichophyton verrucosum*, but dermatophytosis cases arising from infection with pathogenic agents, including *T. equinum*, *Microsporum gypseum*, *M. nanum*, *M. canis* and *Epidermophyton* sp. have also been reported [3-8].

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Dermatophytes cause alopecia, scurf and crust formation on the skin, down grading of the hide and skin, and growth retardation. As an enzootic disease of zoonotic nature, dermatophytosis leads to high treatment costs, which together with the restriction of the trade of infected animals, results in major economic loss [3,4,6].

Predisposing factors in the development of the disease include poor lighting and high humidity of animal shelters, high stocking density in animal production, and prolonged confinement of animals. The infectious agents may remain active for several years in contaminated animal shelters and on equipment. The occurrence of the disease is also closely related to the susceptibility and immunocompetence of animals [4,9]. Compared to adults, dermatophytosis is observed more frequently in young animals. As the development of the immune system advances, the possibility of the occurrence of the disease decreases. Weather conditions are also influential on the occurrence of this illness. The number of dermatophytosis outbreaks tend to increase during winter as well as during the humid spring and autumn seasons, whilst animals infected with trichophytes recover spontaneously during summer [2].

Tioconazole is a synthetic antifungal preparation that contains imidazole as the active substance. It is a topical preparation known to be safe and strongly effective against certain opportunistic yeasts and dermatophytes [10,11]. It shows fungicidal effect by causing direct damage to the membrane of fungi [12].

Various pharmaceutical products and vaccines are used for the treatment of bovine dermatophytosis [3,6,13-15]. In the present study, the objective was to investigate the effectiveness of the local application of 1% tioconazole in the treatment of bovine dermatophytosis.

MATERIAL and METHODS

Twenty-five cattle of varying sex and breed, which were 1.5-11 months of age, raised in Kars province and its surrounding villages and diagnosed with dermatophytosis, constituted the material of the present study. This study was conducted between december 2011 and march in 2012. Of these animals, 15 were allocated to the trial group, and 10 were maintained for control purposes and not subjected to any treatment.

In the trial and control groups, the site of lesions, which were sampled, were first cleansed with 70% ethyl alcohol. Subsequently, skin scrapings and hair specimens were taken from the periphery of the keratinized regions into sterile petri dishes [16]. After treated with 10% potassium hydroxide, the samples were observed under the microscope. Furthermore, inoculations were performed onto Sabouraud Dextrose Agar and petri dishes were incubated at 32°C for a period of 2-6 weeks. At the end of the incubation period, the slides prepared from the grown cultures were examined microscopically for the presence of hyphae, mycelia, spores, Chlamydia spores, and macro- and microconidia [17,18].

The skin lesions of 12 of the animals included in the trial group were applied a pomade containing 1% tioconazole (Dermo-Trosyd krem, 1%i Pfizer®) for 5 days, in accordance with the instructions for use. On the other hand, the remaining 3 animals of the trial group were administered with the pomade for a period of 7 days due to the persistence of keratinized tissue in the skin lesions. The control animals were not subjected to any kind of treatment throughout the study period. Following the cease of treatment, the recovery of the animals included in the trial group was monitored at one-week intervals during two months.

RESULTS

Clinical Results

The clinical examination of the animals included in the study demonstrated that the localization site of the dermatophytosis lesions was the head in 10 *(Fig. 1a)*, the head and neck region in 8, the neck in 5, and various body parts in 2 of the cattle. Although the general condition of the animals appeared normal, the body condition scores of 8 cattle were poor.

In 12 of the animals included in the treatment group, keratinized tissue in the skin lesions was observed to have reduced significantly after 3 applications and to have disappeared completely after the 5th application. In the remaining 3 animals included in the trial group, the persistence of keratinized tissue in the skin lesions required 7 applications to be made.

Post-treatment controls performed at 1-week intervals in the animals administered with tioconazole revealed that new hair growth resumed as from weeks 3-4 and that complete recovery was achieved by the 7th-8th weeks *(Fig. 1b)*. On the other hand, no change was observed in the dermatophytosis lesions of the control animals, which were not subjected to any treatment throughout the study period.

Mycological Results

T. verrucosum was isolated from the microbiological culture prepared from the skin scrapings and hair specimens taken from the skin lesions of all animals included in the study. No microbial growth was observed in the samples collected post-treatment.

DISCUSSION

Dermatophytosis is very prevalent in calves and may

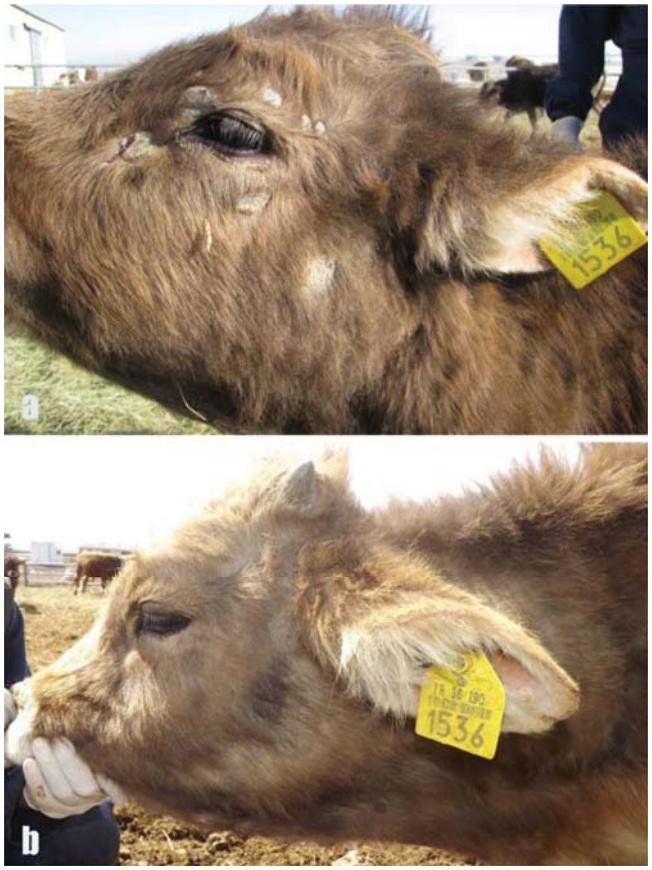

Fig 1. a- Appearance of cattle prior to treatment with tioconazole, **b-** Appearance of cattle after the treatment with tioconazole

Şekil 1. a- Sığırın tiokonazole ile tedavisinden önceki görünümü, **b-** Sığırın tiokonazole ile tedavisinden sonraki görünümü

also be observed in adult bovine animals. This skin disease frequently develops during the weaning period in calves older than 2 months. Although the illness causes superficial damage to the skin, it results in major economic losses due to decreased body weight, poor hide quality, growth retardation and treatment costs [3,4]. In agreement with previous literature reports, the cases investigated in the present study were all observed in young animals and although the general condition of 8 of the animals was good, their body condition scores were poor. The resistance of dermatophytes to environmental conditions helps them survive for prolonged time periods and brings about the possibility of animals becoming infected over long time periods [2,13]. Anamnesis revealed that dermatophytosis outbreaks occurred every year in the holdings the animals were obtained from, which confirmed that these infectious agents survived for long time periods.

Inappropriate housing conditions, and in particular, animals being raised at high stocking density for prolonged periods, as well as high humidity levels of the air circulating in the animal shelter, favour and accelerate the development and spread of dermatophytosis [4-6,9]. In the present study, it was observed that the shelters the animals were raised in were humid and had poor air circulation and that the animals were housed at high stocking density. Due to the cold climate and long winter season of Kars province and its vicinity, animals are confined for prolonged time periods, which results in the disease being encountered frequently in the region.

The fungal species isolated most commonly in bovine dermatophytosis cases is reported as *T. verrucosum* [1,3,5,6,13], yet cases caused by pathogenic agents such as *T. equinum*, *M. gypseum*, *M. nanum, M. canis* and *Epidermophyton* sp. have also been reported [2-4,7,8]. In the present study, *T. verrucosum* was isolated from all of the infected animals.

Various pharmaceutical products and vaccines are used for the treatment and control of dermatophytosis, which is prevalent in Turkey and across the world [3,6,13-15]. In this study, a pomade containing 1% tioconazole, used locally in human medicine for the treatment of fungal infections owing to its effectiveness against certain fungal species was applied to the skin lesions observed in cattle with an aim to determine its effectiveness in the treatment of bovine dermatophytosis. This treatment regimen resulted in recovery by the 5th application in 12 and by the 7th application in 3 of the animals included in the trial group. The local application of tioconazole resulted in the elimination of keratinized tissue and eventually, the healing of skin lesions within a short period of time. Based on these results, it was concluded that 1% tioconazole was rather effective in the treatment of bovine dermatophytosis cases caused by *T. verrucosum*. Accordingly, it is suggested that 1% tioconazole may be used as an alternative to other treatment methods. Furthermore, the results of the present study bear significance in that they are the first to be obtained on the use of 1% tioconazole in the treatment of bovine dermatophytosis.

In conclusion, it has been determined that, owing to its ease of use and strong therapeutic effect, 1% tioconazole is very effective in the treatment of bovine dermatophytosis caused by *T. verrucosum*.

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CASE REPORT

Urorectal Septum Malformation Sequence in A Calf

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Summary

Urorectal Septum Malformation Sequence was described in a 3-day-old, Simmental-Brown Swiss crossbred calf characterized by absence of anal and vaginal openings, ambiguous genitalia, sacral hypoplasia, anuria, colonic atresia and lumbal vertebral scoliosis.

Keywords: *Urorectal septum malformation sequence, Sacral hypoplasia, Anuria, Colonic atresia, Lumbal scoliosis, Calf*

Bir Buzağıda Ürorektal Septum Malformasyon Serisi

Özet

Üç günlük, Simental-İsviçre Esmeri melezi bir buzağıda anal ve vaginal deliklerin bulunmaması, sakral hipoplazi, kuyruk bulunmaması, kolon atrezisi, lumbal omur skoliozisi ve anormal genital organ anomalisiyle belirgin Ürorektal Septum Malformasyon Serisi tanımlandı.

Anahtar sözcükler: *Ürorektal septum malformasyon serisi, Sakrum hipoplazisi, Kuyruksuzluk, Kolon atrezisi, Lumbal skoliozis, Buzağı*

INTRODUCTION

The developmental anomalies may be caused by genetic or environmental factors or combination of both and, in most cases the etiology remains obscured [1]. The occurrence of congenital malformations in food animals has economic importance and might provide material for developing appropriate understanding of the embryologic pathogenesis [2].

URSM (Urorectal Septum Malformation Sequence) in humans is a well-known specific developmental abnormalities originally described in about 25 years ago including the absence of anal, urethral and vaginal openings, ambiguous genitalia and genito-urinary anomalies [1]. Before the introduced the term in human medicine, authors used the terms including 'female pseudohermaphroditism with anorectal malformation, hypoplastic pelvic outlet and persistent cloaca' [2]. The extent of variation in human URSM cases is from complete to partial or milder variant forms characterized by a single openining draining the cloaca might be compatible with life [3].

In veterinary medical literature; the presence and frequency of the URSM in domestic animals was much less documented. To the authors' knowledge, URSM was described in one of the dizygous twin lambs [2], in 6 calves [5-8], in a foal [9] and 40 fetus or aborted pig embryos [10] up to date. Generally, most of the authors in veterinary medicine have used to term of 'persistent cloaca or complex intersex condition' to describe these series of anomalies [5-7], whereas URSM was introduced in veterinary medicine for describing in a lamb with urogenital malformations including abnormal external genitalia imperforate anus, fistulous connection between rectum, bladder and vagina [2].

This study describes the morphologic features of URSM together with colonic atresia, rectum aplasia, anuria, sacral hypoplasia, and lumbar scoliosis in a cross-bred calf.

CASE HISTORY

Three-day-old, Simmental-Brown Swiss crossbred calf was presented to the Animal Hospital of Veterinary Faculty

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of Firat University, with the signs of the absence of perianal opening and tail, and postural abnormality *(Fig.1a)*. There was no record of previous defects and the cow had delivered one normal calf before. External appearance of the calf resembled the male due to presence of phallic structure in normal location for penis *(Fig. 1b)*, however there was neither scrotum nor testes. Urethra was not patent. In radiographical and macroscopical examinations; coccygeal vertebrae were absent, there was scoliosis in 4-5 lumbar vertebrae and the sacrum was moderately hypoplastic.

Upon the owner's request and the situation is not compatible with the life, the calf was sacrificed humanly.

In macroscopic examination; genital and urinary canal was not separated and combined as a cloacal sac measured as12x5x5 cm. The following organs were directly associated with the cloaca: urinary bladder (diameter of 5 cm); urethra (length of 8 cm); and uterus *(Fig. 1c)*. Cloaca, urinary bladder and uterus including the uterine horns and Fallopian tubes were all distended massively by straw-colored fluid accumulation and they considerably filled the pelvic and peritoneal cavity. The total fluid having the characteristics of urine was measured as 2800 ml. There was neither rectum nor anus. The vagina and cervix were also absent.

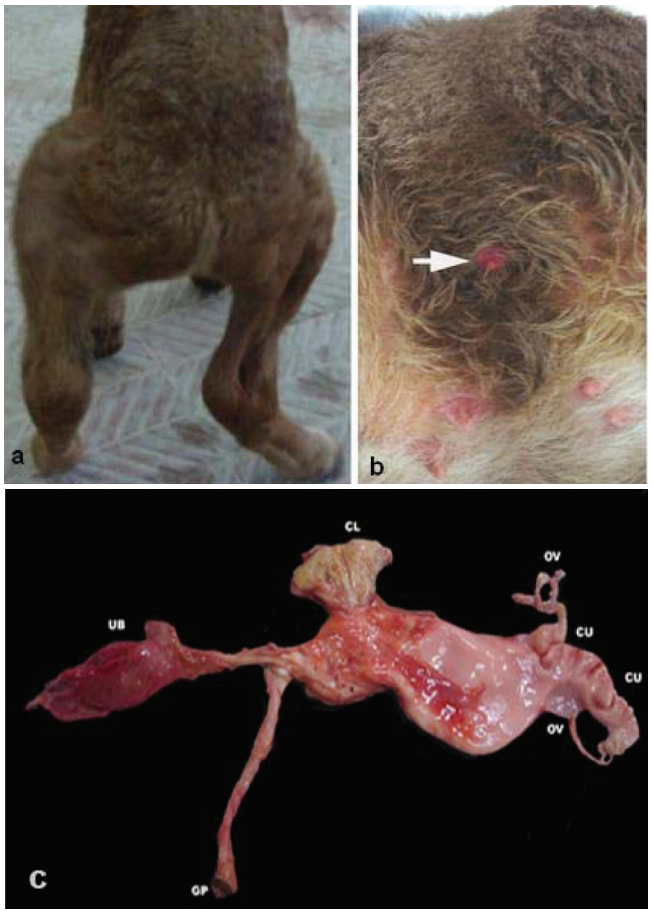

Fig 1. a- Anuria, pelvic hypoplasia and postural abnormality in the calf, **b-** Phallus like structure *(arrow)* and absence of perianal openings, **c-** Dissected organs around the cloaca (CL) including; colon, urinary bladder (UB), cornu uterie (CU), ovaries (O), urether and prepitium (P)

Şekil 1. a- Buzağıda kuyruksuzluk, pelvis hipoplazisi ve duruş bozukluğu, **b-** Penis benzeri yapı *(ok)* ve perianal deliklerin bulunmaması, **c-** Kloaka'yı (CL) içevreleyen dokular; kolon, idrar kesesi (UB), kornu uteri (CU), ovaryumlar (O), üreter ve prepisyum (P)

Colon had no distal portion and ended blindly forming a pouch measuring 2x3x1.5 cm due to meconium accumulation. The urethra was completely atrethic at the point where enter the urinary bladder. Although there was no cervix uteri and vagina; corpus and cornu uteries, oviducts and ovaries were identified. Left cornu uteri was more prominently dilated than the left one *(Fig. 1d)*. The ovaries were in normal size and appearance. No testes, prostate or seminal vesicle was identified.

For histological examination, tissue samples from cloaca, penis, urinary bladder, colon, small intestines, heart, liver, ovary, kidney, and brain were collected and embedded in paraffin wax and then sectioned at 5-6 µm thickness. The prepared sections were stained with haematoxylineosin.

Microscopically; the cloacal wall was lined by a strongly folded, non-keratinized cubiodal epithelium and making villous projections to the lumen *(Fig. 2a)*. It's lamina propria consisted of primitive mesenchymal tissue.

The wall of the urinary bladder consisted of a two to three cells thick transitional epithelium with typical large covering cells having rounded free surface.

The colonic mucosa composed of goblet cells, resorbing epithelial cells and crypts *(Fig. 2b)*. The stroma had highly cellular connective tissue and smooth muscle cells.

The inner surface of the uterus contained thickened regions of the endometrium and the caruncles were present. There was no uterine glands in these areas *(Fig. 2c)*. The mucosa contained single columnar epithelium, lamina propria, and muscularis mucosa. The lamina propria was highly cellular. Myometrium was made up of several layers of smooth muscles, which were demarcated by connective tissue.

The oviducts were lined with simple ciliated cells, cuboidal to columnar in shape *(Fig. 2d)*. The muscularis externa consisted of an inner circular layer of smooth muscles and a less developed outer longitudinal layer. The serosa has a layer of simple squamous epithelium in some connective tissue.

The ovaries covered by single squamous epithelium and tunica albugenea composed of cortex and medulla. Cortex contained numerous primordial, single and multilayered primary follicles *(Fig. 3a)*.

The phallus consisted of corpora cavernous and the corpus spongiosum surrounding the urethra *(Fig. 3b)* and the glans penis covered by prepuce. The corpora cavernous had a thick and tough connective tissue fibers. The sponge-like cavernous tissue consisted of cavernous sinuses and

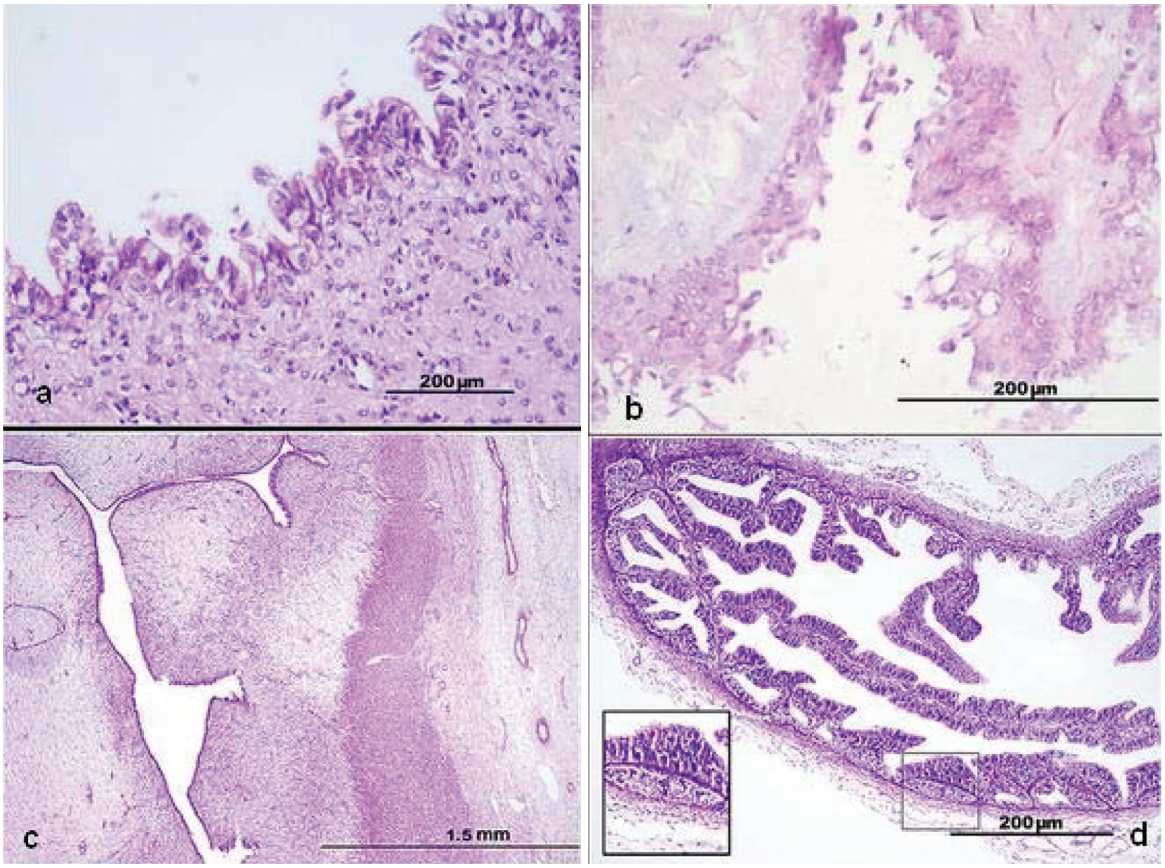

Fig 2. a- Colonic villus composed of goblet and resorptive cells, **b-** Cloacal mucosa containing pseudostatified epithelium and primitive stroma, **c-** The uterine carunculas composed of single columnar epithelial tissue, lamina propria and muscularis mucosa, **d-** The uterine tube lined with simple ciliated cells (inset), cuboidal to columnar in shape, Hematoxylin-eosin (HxE)

Şekil 2. a- Kadeh hücreleri ve rezorbtif hücreler içeren kolon villusu, **b-** Yalancı çokkkatlı epitelyum ve primitif mezenkimden ibaret kloaka mukozası, **c-** Tek katlı kolumnar epitelyum, propria ve musküler tabak içeren üterus karunkulaları, **d-** Silyumlu (inset) kübik-kolumnar epitelyum içeren yumurta yolu, Hematoksilen-eosin (HxE)

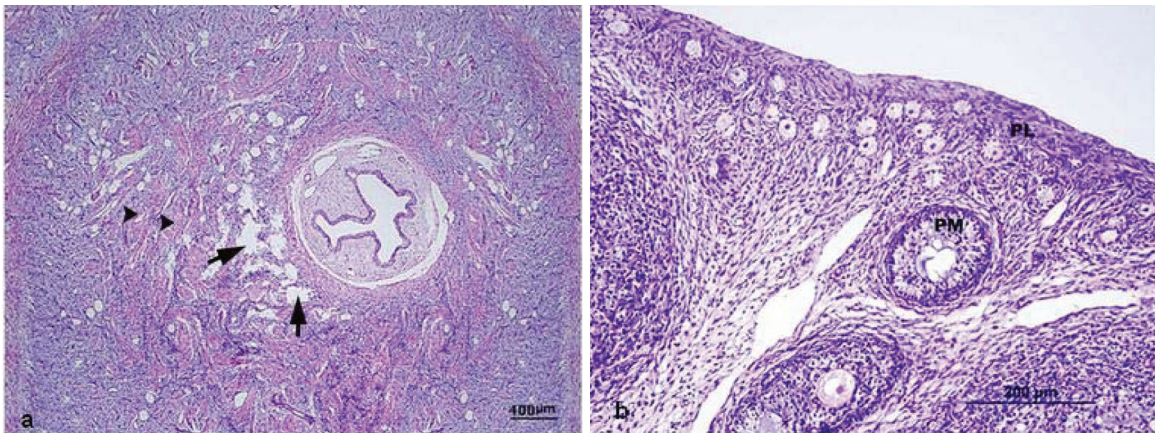

Fig 3. a- The corpora cavernosa *(arrow heads)* and the corpus spongiosum *(arrows)* surrounding the urethra, **b-** The ovarium containing primordial (PL) and primary (PM) follicules, Hematoxylin-eosin (HxE)

Şekil 3. a- Üretrayı çevreleyen korpora kavernoza *(okbaşı)* ve korpus spongiozum *(oklar)*, **b-** Ovaryum'da primordial (PL) ve primer (PM) foliküller, Hematoksilen-eosin (HxE)

septa with smooth muscle fibers. The urethra consisted of a stratified mainly cuboidal urothelium. The lamina propria composed of connective tissue appeared free of glands. The prepuce had stratified keratinized epithelium and showed intraepithelial and subepithelial neutrophilic infiltration.

DISCUSSION

The incidence of URSM is unknown in animals, however, in human neonates, it was reported as one in 250.000 [1]. In this report, we presented URSM sequence in a calf

characterized by absence of anal, urethral and vaginal openings, ambiguous genitalia and genito-urinary anomalies. Additionally, sacral hypoplasie [6,7] anuria and vertebral anomalies [5-7] were described in the present study as in an earlier bovine cases.

Renal changes or defects including horse-shoe kidney [6,8] and renal dysplasia [6], hydronephrosis, hypoplasia or aplasia [5] were commonly reported in bovine and human cases [1], but not such changes were detected in the present report. The absence of this defects in the present case might be explained by the presence of colonic atresia that might protect the obstructive uropathy. Similar to our report, renal defects have not been detected in the lamb [4], in a calf [7], and about 6 of 18 human URSM cases [2].

Originally, it was proposed and widely accepted that the specific sequence is caused by failure of urorectal septum to migrate to and/or fuse with cloacal membrane [1]. However, which factor or factors leading to this failure is unknown. Possible hypothesis including caudal mesodermal deficiency, mutations and teratogens, lateral compression, and vascular steal have been proposed [4].

Similar to the present report, tail abnormalities such as anuria (4 of 6 bovine cases) [5,7] or short and deviated tail (1/6) [6] have been reported co-existently in previous bovine URSM cases. Although anuria seems to be most concurrent lesion in bovine URSM cases, anuria and/or urogenital defects were also co-existently reported in atresia ani in calves [11,12].

Interestingly; amongst the 6 total bovine URSM cases, 3 were Holstein calf [5,7,13], one was Limosin [5], one animal was Simmental [6]. and one was Hereford X Longhorn crossbred calf [8]. The subject of this observation was also Simmental crossbred.

As a result, the case reported here consist of female counterpart of URSM which was reported in two bovine [6,7], and one lamb case and the additional cases are needed to elucidate the mechanism leading to formation of this defects.

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CASE REPORT

Bir Sığırcılık İşletmesinde Coenurosis Salgını

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Makale Kodu (Article Code): KVFD-2012-7599

Özet

Bu çalışmada Türkiye'nin Kuzeydoğu bölgesinde bulunan Kars ilinde 20 başlık bir sığırcılık işletmesinde coenurosis teşhisi konulan 4 dana incelendi. Olgular 2010 yılı Ocak-Şubat ayları arasında görüldü ve klinik muayenede, dönme hareketleri, tek taraflı körlük nedeniyle görme bozukluğu, baş bölgesine yapılan palpasyonda duyarlılık ve opistotonus tespit edildi. Nekropside hayvanlarda beyne yerleşmiş *Coenurus cerebralis* kistlerinin varlığı ortaya konuldu ve beyin dokusu histopatolojik olarak değerlendirildi. Sığırlarda klinik coenurosis ile ilgili olgu sayısı Türkiye'de ve dünyada oldukça sınırlıdır. Günümüze kadar, sığırlarda tek bir çiftlikte kısa bir zaman periyodu içerisinde coenurosisin salgın olarak rapor edildiğine yönelik herhangi bir çalışma bulunmaması nedeniyle bu makalenin klinik veteriner hekimliğe önemli katkı sunacağı kanaatine varılmıştır.

Anahtar sözcükler: *Coenurosis, Çiftlik, Sığır*

An Outbreak of Coenurosis in A Cattle Farm

Summary

This study was evaluated 4 cattle infected with coenurosis in a farm of 20 cattle in Kars province located in eastern region of Turkey. All cases were reported between January and February 2010 and signs noted on clinical examination were, incoordination, circling, impaired vision due to unilateral blindness, pain response on palpation of head and opistotonus. On gross pathology, cysts of *Coenurus cerebralis* were determined in the brains of these cases and brain tissues were histopathologically examined. Clinical cases of cattle with coenurosis are quite rare in Turkey and world as a whole. It was worth publishing such case report as there exists no previous study reporting outbreak of coenurosis in cattle in a single farm within a short period of time as in this report.

Keywords: *Coenurosis, Farm, Cattle*

GİRİŞ

Coenurosis, köpek ve yabani karnivorların ince bağırsağında görülen *Taenia multiceps* (Syn. *Multiceps multiceps*) adlı cestodun larvası olan *Coenurus cerebralis* (*C. cerebralis)* 'in neden olduğu bir hastalıktır. Son konakların dışkısıyla atılan halkaların parçalanmasıyla serbest kalan yumurtalar ara konak olarak koyun ve keçiler, seyrek olarak sığır, at ve insanlarda ağız yoluyla alındığında ince bağırsaklarda serbest kalan onkosfer kan yoluyla beyin ve omuriliğe gitmekte ve bu organlarda *C. cerebralis* adı verilen larvalar gelişmektedir [1-5].

Ara konaklarda akut dönemde çok sayıda yumurta alındığında akut travmatik bir meningoensefalit gelişir ve hayvanlar çok kısa bir süre içinde ölür. Genellikle en sık görülen şekli olan kronik dönemde ise az sayıda yumurta ile oluşan enfeksiyonlarda yumurtaların alınmasını takiben 4-6 ay sonra beyinde 1-2 adet kist gelişebilir. *C. cerebralis* başlangıçta akut dönemde olduğu gibi irinli meningioensefalitise neden olmakla birlikte, daha sonra kistin büyümesine paralel olarak ölümle sonuçlanan merkezi sinir sistemi semptomlarına yol açmaktadır. Kistin lokalize olduğu yere, büyüklüğüne ve dolayısıyla beyne yaptığı basınca bağlı olarak görülen önemli semptomlar görme bozuklukları, dönme hareketleri, opistotonus olarak sıralanabilir. Bu hayvanlar yeterince beslenemedikleri için zayıflayarak ölebilirler [2].

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Yapılan çalışmalarda coenurosisin Türkiye ve dünyada koyun ve keçilerde oldukça yaygın olduğu [2,6-8], ancak sığırlarda nadiren görüldüğü [1,7,9-15] bildirilmiştir. Türkiye'de sığırlarda hastalık prevalansının %0.47 olduğu [4] ve klinik olarak sınırlı sayıda olgunun rapor edildiği görülmektedir [9,15,16]. Ayrıca Kars yöresinde yapılan bir çalışmada ise incelenen 42 köpekten sadece 3'ünde *Taenia multiceps*'in erişkinlerin formlarına rastlanmıştır [17].

Bu olgu sunumunda bir işletmede kısa zamanda ortaya çıkan 4 coenurosis olgusunun klinik, makroskobik ve histopatolojik bulguları ile sunulması amaçlanmıştır.

OLGULARIN TANIMI

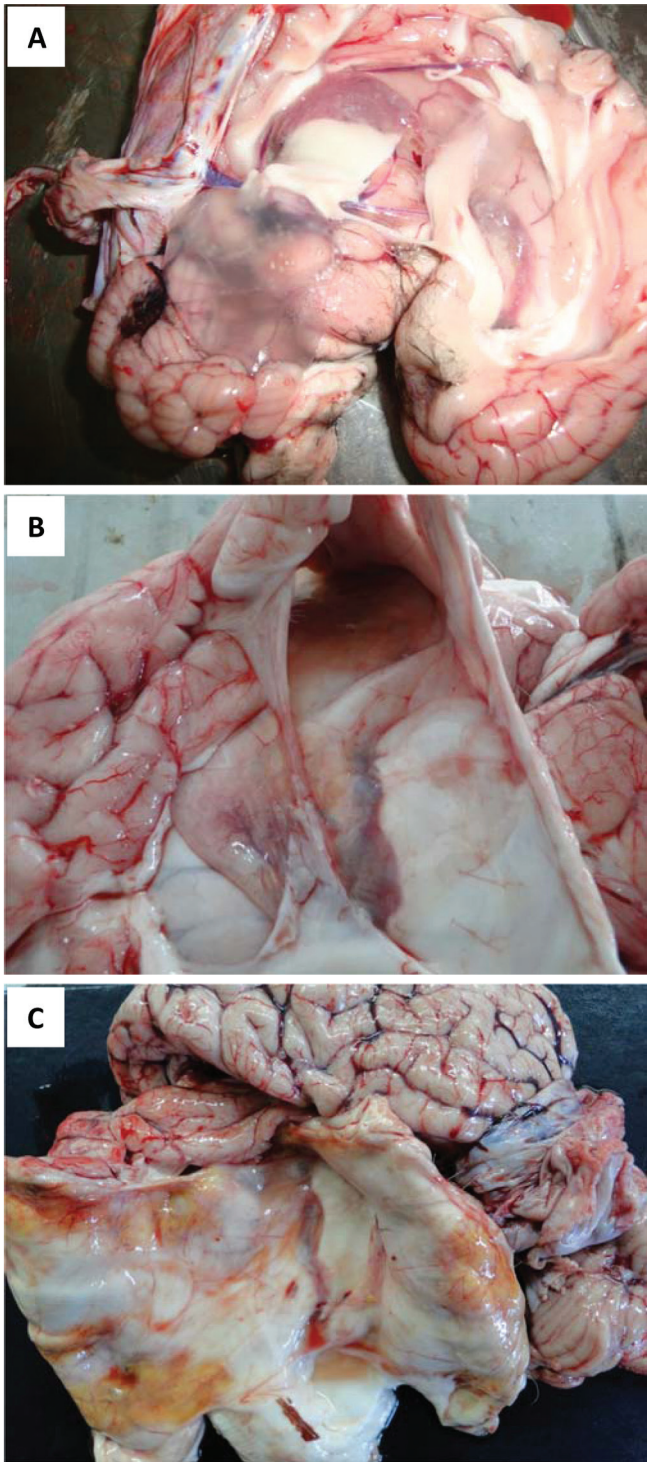
Çalışma materyalini, toplam 20 başlık bir aile işletmesinde klinik olarak coenurosis belirtisi gösteren 4 adet Simental dana oluşturmaktadır. Danaların yaş ve cinsiyetleri *Tablo 1*'de gösterilmiştir.

Yetiştiricinin, 2010 yılı Ocak ayında, işletmesindeki danalarda etrafında dönme ve letarji belirtileri görüldüğünü tarafımıza bildirilmesi üzerine, işletmeye gidilerek hayvanların muayenesi yapıldı. Bu danalarda; koordinasyon bozukluğu, başın sabit objelere dayanması, anormal tutulması, opistotonus, tek taraflı körlük, baş bölgesine palpasyonda ağrı ve diş gıcırdatması gibi coenurosisin kronik formuna benzeyen klinik bulgular tespit edildi. Anamnez bulgularında danaların yaşamlarının ilk 8 aylık dönemlerinde mera veya yaylada köpeklerle birlikte barındırıldığı, daha sonraki kış aylarında klinik bulguların ortaya çıktığı, veteriner hekimler tarafından uygulanan tedavilere cevap alınamadığı öğrenilmiş ve bu durum coenurosis şüphesini güçlendirmiştir. Klinik tablosu ağır olan bir dananın nekropsisi yapılmıştır. Kafatası açıldığında, içerisinde çok sayıda skoleks bulunan berrak sıvı ile dolu tipik *C. cerebralis* kistlerinin beyindeki varlığı makroskobik olarak gözlemlendi *(Şekil 1A)*. Kistlerin etrafındaki beyin dokusunun artan basınçla oldukça incelediği *(Şekil 1B)*, kistin yırtılması ve sıvının boşalmasından sonra beyin hemisferinin çöktüğü görüldü. Bu bölgelerde beyin dokusunun nekrotik, granüler yapıda ve yer yer sarımsı bir görünüm aldığı belirlendi *(Şekil 1C)*. İlk olguda *C. cerebralis* tespiti yapıldıktan sonra diğer dananın da kestirilmesine karar verildi ve yapılan incelemede bu hayvanda da *C. cerebralis*'in varlığı ortaya konuldu. Yaklaşık bir ay içerisinde iki farklı hayvanda yine benzer sinirsel belirtilerin ortaya çıkması üzerine tüm incelemeler tekrarlandı ve *C. cerebralis*'in varlığı saptandı. Kistlerin genellikle sol beyin hemisferinin paryetal ve oksipital beyin loplarına yerleştiği tespit edildi *(Tablo 2)*.

Tüm olgularda beyin ve beyincikten alınan doku örnek-

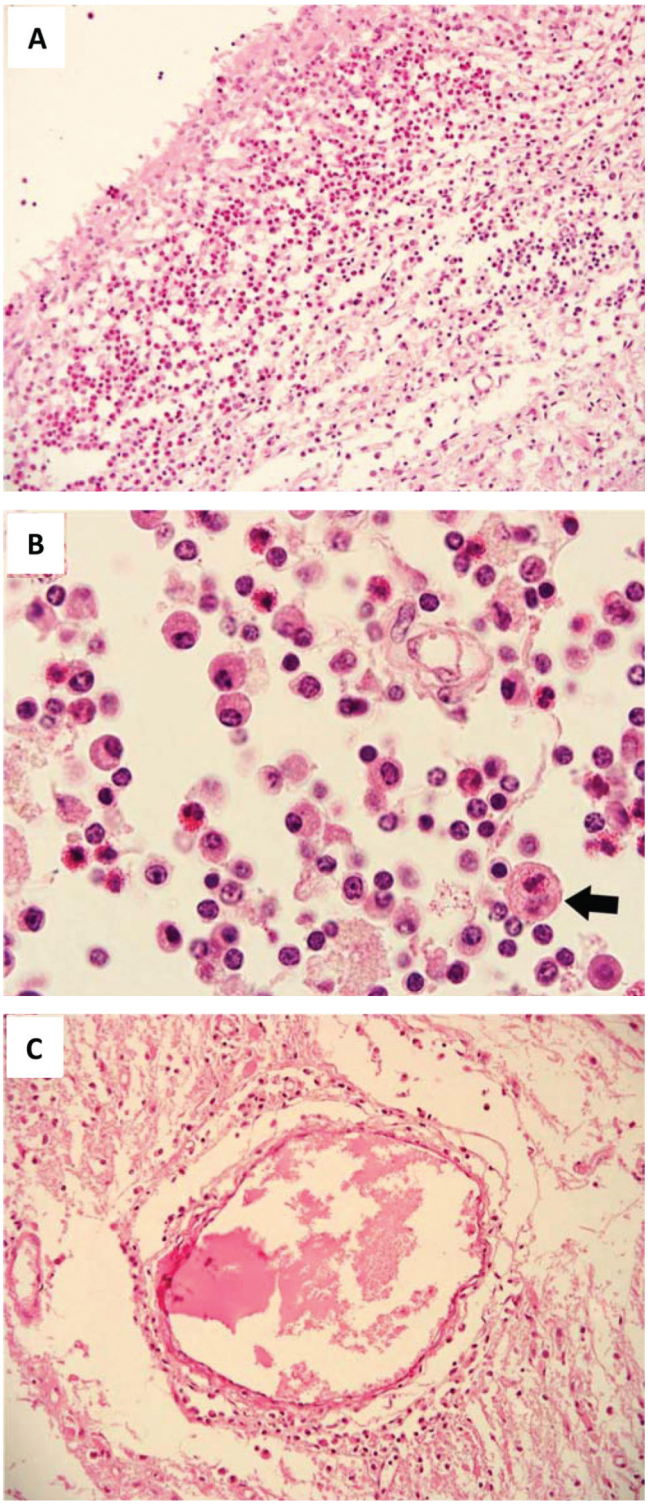
Tablo 1. *Coenurosis belirlenen danaların detayları*
Table 1. *Details on the cattle with coenurosis*

Olgu No	Irk	Yaş	Cinsiyet
1	Simental	11 ay	Erkek
2	Simental	15 ay	Erkek
3	Simental	7 ay	Erkek
4	Simental	16 ay	Dişi



Şekil 1. A- İçerisinde skoleksler bulunan küçük kist, **B-** Yırtılmış serebral kist çevresinde beyin korteksinde belirgin incelme, **C-** Kist şekillenen beyin hemisferinde sarımsı görünümde nekroze alanlar

Fig 1. A- A small cyst containing numerous scolexes, **B-** Thinning of the cerebral cortex of the brain around the cyst ruptured, **C-** Yellowish necrotic areas in cerebral hemisphere in which cyst formed



Şekil 2. A- Nekrotik beyin dokusunda yoğun eozinofil lökosit infiltrasyonu, H&E, 20 x (orijinal büyütme), **B**- Kist çevresinde plazmosit, lenfosit ve eozinofil infiltrasyonu ile sitoplazmasında fagosite eozinofil bulunan makrofaj *(ok)*, H&E, 40 x (orijinal büyütme). **C**- Beyin dokusunda yeni oluşmuş kist. H&E, 10 x (orijinal büyütme)

Fig 2. A- Dense eosinophil leukocyte infiltration in the necrotic brain tissue, H&E, 20 x (original magnification), **B**- Plasmacyte, lymphocyte and eosinophil leukocyte infiltration with a macrophage containing phagocytic eosinophil *(arrow)* around the cyst, H&E 40 x (original magnification), **C**- Newly formed brain cyst. H&E, 10 x (original magnification)

Tablo 2. *Kistlerin yerleşim yerleri*
Table 2. *Location of cysts*

Olgu No	Kist Bulunan Beyin Lobu	Kistin Yerleşimi
1	Sağ hemisfer	Parietal lob
2	Sol ve sağ hemisfer	Paryetal-oksipital lob
3	Sol hemisfer	Paryetal-oksipital lob
4	Sağ hemisfer	Frontal lob

leri %10'luk formalinde tespit edildikten sonra rutin olarak hazırlanan parafin kesitler hematoksilen ve eozin (H&E) ile boyanarak ışık mikroskobunda değerlendirildi. Histopatolojik olarak, kistlerin fibroepitelyal doku ile çevrili olduğu, etrafta beyin dokusunun nekroze olduğu ve bu bölgelerde şiddetli eozinofil lökosit infiltrasyonu ile birlikte çok sayıda lenfosit, plazma hücresi ve makrofajın bulunduğu belirlendi *(Şekil 2A)*. Yine kist çevresinde az sayıda çok çekirdekli dev hücresinin olduğu, gerek makrofajlar gerekse çok çekirdekli dev hücrelerinin sitoplazmasında fagosite edilmiş eozinofillerin bulunduğu görüldü *(Şekil 2B)*. Ayrıca kistlerin çevresinde sıklıkla sinir hücrelerinin dejenerasyonu ile fokal veya diffuz gliozis dikkati çekti. Bunlara ilaveten, beyin parankiminde içlerinde eozinofilik görünümlü homojen madde bulunan yeni şekillenmiş küçük kistler tespit edildi *(Şekil 2C)*.

İşletme sahibi ile yapılan detaylı görüşmeler ve alınan anamnez sonucu, işletmede daha önceki yıllarda da benzer şekilde buzağı kayıpları olduğu ve kesilen bu danalara ait atıkların işletmedeki köpeklere yedirildiği öğrenildi. Tespit edilen olgular sonrasında köpeklere düzenli olarak yapılan antiparaziter ilaç uygulamasının ardından, günümüze kadar bu işletmede herhangi bir coenurosis olgusu da belirlenmedi.

TARTIŞMA ve SONUÇ

Serebral coenurosis arakonak olarak ruminantlar, insan, at ve domuzlarda da görülebilmekte ve bulaşmasında mera ve çoban köpekleri oldukça önem taşımaktadır [3,4,18]. Hastalığın arakonakta meydana getirdiği klinik bulgular çeşitlilik göstermekle birlikte, genellikle kronik seyretmekte ve merkezi sinir sisteminin (MSS) etkilenmesine bağlı olarak görme bozuklukları, dönme hareketleri, ataksi, opistotonus, kafayı sabit objelere dayama, arka ayaklarda felç gibi belirtiler ortaya çıkmaktadır [10]. Sunulan çalışmada aynı işletmedeki dört olguda da benzer MSS bulguları tespit edilmiş olup, benzer semptomlarla seyredebilen diğer hastalıklardan, yapılan nekropsi incelemeleri sonucu makroskobik olarak ayırt edilmiş ve coenurosis teşhisi konulmuştur. Daha önceki araştırmalarda sıkça bildirildiği gibi çalışmamızda da değerlendirilen olgularda kistlerin serebrumda lokalize olduğu belirlendi [4]. Histopatolojik olarak kistlerin fibrovasküler bağ doku ile çevrelenmesi, bu bölgelerde şiddetli eozinofil lökosit infiltrasyonunun bulunması, dış katmanlarda ise mononükleer hücre infiltrasyonu ve

nöron dejenerasyonunun varlığı diğer coenurosis çalışmalarında ortaya konan histopatolojik bulgularla benzerlik göstermiştir [15,16].

Sığırlarda prevalansı oldukça düşük olan coenurosis ile ilgili olarak Doğu Anadolu Bölgesi'nde yer alan Erzurum ilinde 2009-2010 yılları arasında yapılan bir çalışmada bu oran sadece %0.47 olarak belirlenmiştir [4]. Almanya'da yapılan bir araştırmada bu rakamın Türkiye'dekine benzer şekilde %1'in altında olduğu bildirilmiştir [19]. Erzurum'da yine 2012 yılında yapılan başka bir araştırmada aynı mezbahada kesilen ancak farklı işletmelerden gelen coenurosisli üç olgunun tespiti yapılmıştır. Günümüze kadar yapılan çalışmalar coenurosisin bölgemizdeki önemini ortaya koymaktadır ve bu hastalık güncelliğini hala korumaktadır [4,6,16]. Genel olarak değerlendirildiğinde dünyadaki farklı ülkelerde ve Türkiye'de günümüze kadar bildirilen klinik coenurosis ile ilgili olgu sayısının oldukça sınırlı olduğu görülmektedir [12,13]. Yine bilgilerimize göre, koyunculuk işletmelerinde coenurosisin salgın olarak seyredebileceğine yönelik veriler bulunmakla birlikte bu durum sığırlar için bildirilmemiştir [8,20]. Sunulan bu araştırmada elde edilen sonuçlar 20 başlık bir sığır işletmesinde coenurosisli 4 olgunun birden tespit edilmesi (%20), hem coenurosisin bu güne kadarki çalışma sonuçlarının aksine yüksek oranlarda da meydana gelebileceğini hem de doğal kronik klinik coenurosisin salgın olarak da seyredebileceğini göstermektedir. Dolayısıyla bu yönleriyle çalışma dünyada ilk kez elde edilen sonuçları içerdiği söylenebilir.

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CASE REPORT

A Case Report: Recurrent Cystitis in A Mare [1]

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[1] *An earlier version of this case report was presented at the 13th National Congress of Veterinary Surgery Congress (with international participation), 27 June - 01 July 2012, Sarikamis - Kars/TURKEY*

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Summary

A 20 year old Austrian Warmblood mare was presented at the clinic of Vienna Veterinary University for symptoms of polydipsia, polyuria and urinary incontinence and a repeated history of bladder infection. Sampled urine was light yellow, very cloudy, had a low specific weight (1016) and a pH of 9. The dip stick suggested very high hemoglobin concentration. The sediment showed medium numbers of rounded epithelial cells, low numbers of leukocytes. Furthermore, a remarkable quantity of calcium carbonate crystals was present. Urine and plasma chemistry and fractional clearance revealed the following: high blood urea and Ca concentration. The FE of Na and GGT/Creatinine ratio both were increased. These results suggested chronic renal insufficiency and co-existent urinary tract inflammation. Trans-abdominal ultrasound of the kidneys was performed. The left kidney was normal both in size and appearance of medulla, cortex and pyelum. The right kidney appeared morphologically modified such that the border between cortex and medulla could not be identified clearly. At cystoscopy the floor of urinary bladder could not be seen, due to the large quantities of sludge and grainy gravel deposited on it. However, cystic calculi were not identified. The apex vesicae was highly inflamed, with necrotic changes that were coated with gravel and fibrin. Both ureters were highly dilated (thicker than a finger) and appeared to secrete a cloudy fluid. The endoscopic diagnosis was advanced ulcerative sabalous cystitis, and dilated ureters. Because of the poor prognosis of the case the owner decided to have the mare be euthanized. The gross pathology showed a dilated pyelum in both kidneys. The pyelum was filled with gravel. Both ureters were dilated and filled with gravel too. The bladder wall was thickened and just in cranial to its opening, a soft conglomerate of gravel (7x5x0.5cm) was present. The urethra was also filled but not blocked with this gravel. Histopathology showed chronic interstitial nephritis, glomerulonephritis and pyelitis. The muscularis of the bladder was chronically inflamed. The final main diagnosis was chronic sabalous cystitis with subsequent chronic inflammation of the ureters and chronic interstitial nephritis.

Keywords: *Mare, Chronic cystitis, Cystoscopy*

Olgu Sunumu: Bir Kısrakta Tekrarlayan Sistitis

Özet

Geçmişinde tekrarlayan sistitis tedavisi gördüğü bildirilen, 20 yaşlı Avusturya kısrağı polidipsi, polüri ve idrar tutamama şikayeti ile Viyana Üniversitesi Veteriner Üniversitesine sevk edildi. İdrar örneği açık sarı, bulanık, özgül ağırlığı 1.016 ve pH'sı ise 9 olarak tespit edildi. İdrar analizinde yüksek konsantrasyonda hemoglobin varlığı ölçüldü. İdrar kalsiyum içeriği orta derecede olup, sedimentte yuvarlak epitel hücresi belirlendi ve düşük sayıda lökosit hücresi sayıldı. Ayrıca sediment analizinde belirgin derecede kalsiyum karbonat kristalleri mevcuttu. Kan üre, kalsiyum ve sodyum seviyelerindeki artış ile GGT/Kreatinin oranı kronik böbrek yetmezliği ve tekrarlayan sistitis ile uyumluydu. Böbreklerin transabdominal ultrasonografisi yapıldı. Sol böbrek normal büyüklükte olup medulla, korteks ve renal pelvisler de normal görünümdeydiler. Sağ böbrekte ise kortex ve medulla sınırı açıkça belirgin değildi. Kısrağın sistoskopik incelemesi yapıldı. Fazla miktarda sediment oluşumundan dolayı kese alt duvarı görülemedi ve sistik kalkül oluşumuna rastlanmadı. Kesenin apeksi oldukça yangılı olup burada tortu ve fibrin ile kaplı değişimler gözlendi. Her iki üreter genişlemişti (bir parmağın eninden daha fazla). Bulgulara dayanarak bu vakaya ulseratif sistitis teşhisi koyuldu. Prognozun iyi olmamasından ötürü hasta sahibinin onayı ile ötenazi kararı alındı ve kısrak ötenazi edildi. Makroskopik muayenede, her iki böbreğin renal pelvisinde genişleme olup içinde idrar tortusu mevcuttu. Kese duvarı kalınlaşmış ve ön tarafında 7x5x0.5 cm ebadında yumuşak kum kitlesi tespit edildi. Üretra dolgundu fakat tıkanıklık mevcut değildi. Böbrek histopatolojisinde, kronik interstitiyel nefrit, glomerolonefritis ve piyelit belirlendi. Kese duvarında kronik yangı mevcuttu. Teşhis kronik üreter yangısı ile birlikte seyreden kumlu sistitis ve kronik interstisyel nefritis olarak koyuldu.

Anahtar sözcükler: *Kısrak, Kronik sistitis, Sistoskopi*

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INTRODUCTION

Cystitis commonly occurs secondary to urinary outflow disturbances caused by urolithiasis, bladder paralysis or tumour, anatomical defect, or iatrogenic trauma [1-5]. In male horses, cystitis is usually the result of calcium deposits in the bladder, commonly known as bladder stones. In mares vaginal infection may ascend and lead to cystitis. In some instances, if any injury has occurred, such as when the bladder or urethra is damaged in the course of a foaling, cystitis may occur. Repeated urinary catheterization is also a risk factor [5-7]. Horses grazing on pastures with sudan grass, sorghum or sorghum-sudan hybrid grass may develop cystitis too [5,6,8]. Some of the more obvious signs are excessive urination, hematuria, or dribbling of urine without full voiding of the bladder, urine scalding of the perineum in mares or the front of the hind limbs of male horses. Cystitis tends to affect mares more so than stallions [3,5].

Diagnosis of cystitis is by physical examination, transrectal palpation, cystoscopy, ultrasonography, urinalysis, and culture of urine. Transrectal examination is helpful in case of cystolithiasis or bladder neoplasm. An internal examination is usually required, and this can be performed diagnostically by endoscopy, which uses a slender tube with an attached camera. X-ray or ultrasound imaging can also be useful for viewing the internal structure of the bladder, as the bladder stones can often be seen specialized equipment [5,9,10]. Cystoscopy is beneficial for visualizing mucosal irritation or masses in cystitis[11].

CASE HISTORY

A 20 year old, 568 kg weighing Austrian Warmblood mare was presented for symptoms of polydipsia, polyuria, incontinence and a repeated history of bladder infection. In the clinical examination, dermatitis, likely by urine contract, was revealed in the thigh area, as well as the presence of urine crystals on the hind limbs and the vulvar area. It was decided to perform a urinalysis completed with estimation of kidney function. Collected urine was light yellow, very cloudy and with a low specific weight (1016, RI: 1020-1050) and pH= 9.00. A dip stick test revealed a very high content of hemoglobin/myoglobin. Analysis of the sediment revealed medium number of rounded epithelial cells, a low leukocytes count and a high quantities of calcium carbonate. In *Table 1* the results of urine and plasma chemistry and the calculation of kidney function indices are given.

The high values of blood urea, blood Ca, FE of Na, high GGT/Creatinine ratio and low specific weight of the urine all lead to a diagnosis of a incipient, still compensated chronic renal insufficiency with tubular alterations, hydronephrosis, as well as recurrent cystitis.

By ultrasound of the urinary apparatus a closer diagnosis could be established. The left kidney seemed normal both in size and appearance of the medulla and cortex and the pyeleum did not appear to be enlarged. The right kidney, however, appeared abnormal. There was low definition between cortex and medulla. The pyelum appeared strongly dilated and was filled with a hypoechogenic liquid. The dilated pyelum continued into a dilated ureter.

Table 1. *Electrolyte composition and enzymatic activity of urine and blood taken at the simultaneously. The fractional excretion (FE) of various electrolytes is also given*

Tablo 1. *Aynı zamanda alınan kan ve idrar örneklerinin enzimatik aktivite ve elektrolitkompozisyonu. Çeşitli elektrolitlerin fraksiyonal salınımlarıda verilmiştir*

Parameter	Value of Analysis	Reference Range
Sodium (Pl)	140 mmol/L	126-157
Potassium (Pl)	4.4 mmol/L	3.5-4.5
Chloride (Pl)	101 mmol/L	98-107
Phosphor (Pl)	0.96 mmol/L	3.5-4.5
Urea (Pl)	56.5 mg/dl	20-40
Creatinin (Pl)	1.5 mg/dl	<2
Calcium (Pl)	3.47 mmol/L	2.0-3.2
FE Sodium	1.16	0-0.7
FE Potassium	170	15-200
FE Chloride	3.20	0.7-2.1
FE Phosphor	0.51	0-0.2
Urea index	13.9	renal
GGT/ Creatinin ratio	0.62	0-0.25
Creatinine clearance	1.28	1.24-2.59
Urine Specific Gravity (USG)	1016	1020-1050
Urine pH	9.00	7.5-8.5

Pl: *Plasma ,* ***FE:*** *Fractional excretion*

At cystoscopy, the bladder floor could not been visualized, due to large quantities of sludge and grainy gravel, one or more bladder calculi could not be identified. The apex vesicae was highly inflamed, with necrotic mucosa that was coated with gravel and fibrin. Furthermore, partially ulcerated and hemorrhagic lesions were seen, while the rest of the bladder wall was covered with large streaky erosions. Both ureters were highly dilated (thicker than a finger) and were secreting urine in a pulsating manner (> 6 x/min). This urine contained gravel particles. The diagnosis of an advanced ulcerative cystitis, with high urinary gravel content, as well as dilated ureters was established *(Fig. 1, 2)*.

The end-result of these investigations was the diagnosis sabulous cystitis, with an ascending infection and incipient chronic renal failure. Based on the bad prognosis, the owner took the decision to have the mare euthanized.

Bilateral dilated pyelae with gravel were seen at necropsy. Both ureters were dilated and filled with gravel. Histologically chronic interstitial nephritis and medium membranous glomerulonephritis was present. Due to chronic inflammation the bladder wall was highly thickened. Directly in front of

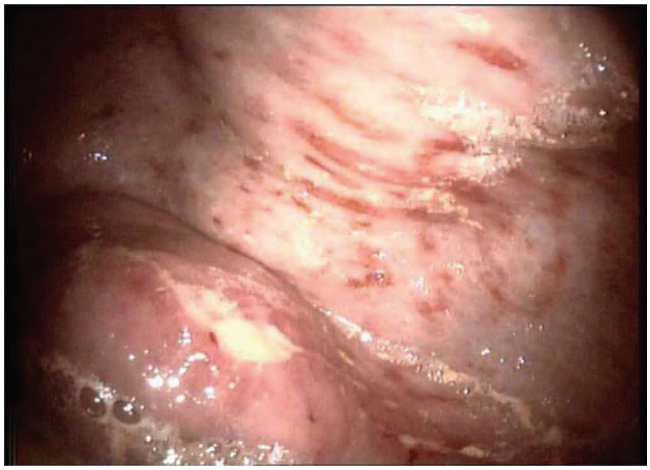

Fig 1. Linear erosions in the bladder wall

Şekil 1. Kese duvarında linear erozyon oluşumları

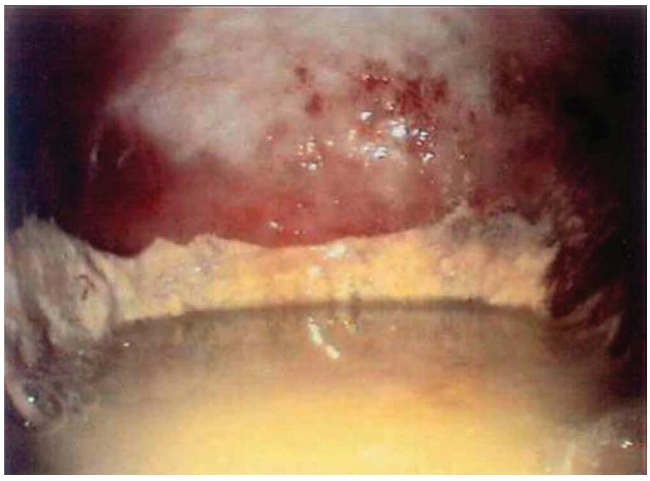

Fig 2. Ulcers and sever inflammation of the roof of bladder. At the bottom of the bladder sabulous urine was present

Şekil 2. Kese üst duvarında şiddetli yangı oluşumu ve ülserli alanlar. Kese alt duvarında kumlu idrar oluşumu

the bladder opening a soft conglomerate of urinary gravel (7x5x0.5 cm) was present. The urethra was partially filled with gravel. In the left uterine horn 20 cm large white mass was present, that later was characterized histologically as adenoma of the uterine glands. A discreet filamentous perihepatitis and a moderate level of lung stasis were seen. The final diagnosis was chronic cysto-urethro-pyelitis chronic interstitial nephritis and glomerulonephritis was made. Furthermore the mare suffered from uterine gland adenoma *(Fig. 3-4)*.

DISCUSSION

The observed skin abnormality, as well as the presence of urine crystals on the hind limbs and the vulvar area [12-15] was likely a urine-induced contact dermatitis.

In horses, urolith formation and cystitis often accompany each other. The most appropriate antibacterial agents for

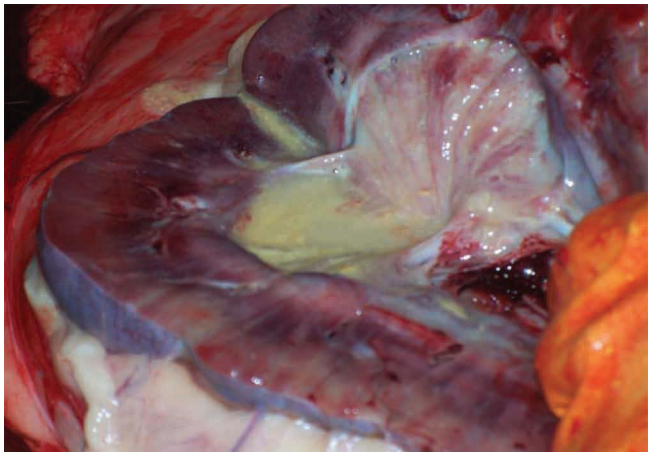

Fig 3. Sabulous urine in the pyelum and necrosis area in the cortex

Şekil 3. Pelvis renaliste kumlu idrar oluşumu ve böbrek korteksinde nekrotik alanlar

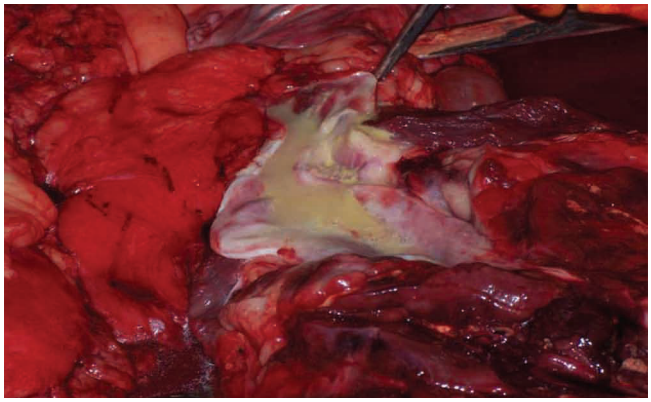

Fig 4. Sabulous urine and dilated ureters

Şekil 4. Kumlu idrar ve dilate üreterler

treating this cystitis form can only be determined after bacteriological cultivation and subsequent sensitivity testing of aseptically collected urine samples. After invasive procedures, the irrigation of the bladder with nonirritating (antiseptic) solutions is a possibility to treat or prevent secondary cystitis [16]. The bacteria most commonly isolated from horses with ascending cystitis include *E coli*, *Proteus*, *Klebsiella*, *Enterobacter*, *Corynebacterium*, *Streptococcus*, *Staphylococcus*, and *Pseudomonas* [17]. The interpretation of a positive culture result is not straight forward since both in healthy horses and those with cystitis, it is not uncommon to isolate multiple organisms from the urine [17,18]. Bactria in absence of cystitis symptoms is called bacteriuria and mostly disappear without treatment. In addition to the microorganisms commonly associated with ascending cystitis, growth of *Actinobacillus equuli*, *Streptococcus equi*, *Rhodococcus equi*, and *Salmonella* spp has been observed in septic foals and may presumably contribute to the incidence of septic nephritis of hematogenous origin [18]. Due to the transcriptional control that alters the temporal expression of fimbrial protein, uro-pathogenic *E coli* strains from cases of cystitis display different patterns of fimbrial expression that are those strains isolated in pyelonephritis [19].

Horses suffering cystitis should be treated with antimicrobials that are excreted in high concentration in urine, such as penicillin, gentamicin, amikacin, enrofloxacin, or trimethoprim-sulfa, depending on the sensitivity pattern of the cultured microbes [20].

To prevent iatrogenic urinary tract infection it is also important to clean and disinfect the penis or vulva as well as the endoscope before entering the urethra and the bladder [21]. Transurethral electrohydraulic lithotripsy has been used successfully to treat obstructive urolithiasis in horses. It may be contraindicated in long-standing cases of cystic calculi associated with chronic cystitis and cases where an inflammatory fibrinomucoid lattice, adherent to the bladder lining, is present [22]. In the cases of sabulous cystitis, apart from flushing the gravel out of the bladder and treating the cystitis with antimicrobials, other alternatives are not existent.

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CASE REPORT

Melez Bir Köpek Yavrusunda Ektrodaktili Olgusu [1]

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[1] *XIII. Ulusal Veteriner Cerrahi Kongresi (Uluslararası Katılımlı)'nde (27 Haziran - 1 Temmuz 2012, Sarıkamış/Kars, Türkiye) poster olarak sunulmuştur*

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Özet

Bu raporda estetik kusur ve yürüme zorluğu şikayeti bulunan melez, 2 aylık, erkek bir yavru köpekte karşılaşılan ektrodaktilinin klinik ve radyolojik muayene bulgularının sunulması amaçlanmıştır.

Anahtar sözcükler: *Ektrodaktili, Köpek*

A Case of Ectrodactyly in A Mixed Breed Puppy

Summary

In this report aimed to determine the clinical and radiological examination findings of ektrodactyly; of hybrid, 2 months old, male puppy which is suffering from an aesthetic defect and difficulty in walking.

Keywords: *Ectrodactyly, Dog*

GİRİŞ

Ektrodaktili kedi ve köpeklerde nadir olarak karşılaşılan kongenital bir anomali olup [1] yalnız karpal kemiklerin deformitesi ile ortaya çıkabileceği gibi radius-ulna ve metakarpal kemikler arasında çoğu zaman yumuşak dokuyu da içeren bir bölünme veya yarık ile karakterizedir [1-3]. Ektrodaktili aynı zamanda distal ekstremitenin bir ya da daha fazla komponentinin kongenital anomalisi olarak da tanımlanabilir [3].

İnsanlarda kongenital deformiteler arasında çok nadir rastlanan el ve ayak deformiteleri yaklaşık 1/90.000 sıklıkta görülmektedir [4]. Olguların orta hattan başlayarak bir veya birden fazla parmakları içeren bazen metakarpal ve metatarsallarla birlikte deformiteleri söz konusudur [4,5].

Bu olgu sunumunda kedi ve köpeklerde ender olarak karşılaşılan ve ekstremite anomalilerinden biri olan ektrodaktili olgusunun sunulması amaçlanmıştır.

OLGUNUN TANIMI

Olgumuzu Kafkas Üniversitesi Veteriner Fakültesi Cerrahi Kliniğine estetik kusur ve yürüme zorluğu şikayeti ile getirilen, melez, 2 aylık, erkek bir yavru köpek oluşturdu. Gerekli klinik ve radyolojik muayenelerden sonra ön sağ ekstremitede ektrodaktili tespit edildi.

Yapılan klinik muayenede olgunun ön sağ ekstremitesini karpal eklemden itibaren yarım fleksiyon pozisyonda tutarak yere basmaya çalıştığı, ancak başarılı olamadığı görüldü *(Şekil 1-A, 1-B)*.

İlgili ekstremitenin inspeksiyonunda patilerden karpal ekleme kadar olan bölge derisinin yarık olduğu ve karpal kemiklerden itibaren ekstremitenin iç bükey eğik olarak durduğu anlaşıldı. İğne pikürüne (pin-prick) karşı ekstremiteye ait derinin her yerinde normal tepki alındı. Elle yapılan düzeltme hareketi ile ekstremite normal pozisyona getirilmeye çalışıldıysa da dirençle karşılaşıldı. A/P ve M/L pozisyonda alınan radyogramda metakarpal ve falanksların üçünün medial segmentte, ikisinin ise lateralde olduğu ve ekstremitenin distalde yarık olarak şekillendiği saptandı *(Şekil 2)*. Ayrıca radius ve ulna da birbirinden ayrık idi.

Olgu operatif tedaviye karar verilerek hospitalize edildi ise de bu süreçte parvoviral enteritis gelişerek 2 gün sonra boksunda ölü bulunduğu için operasyon gerçekleştirilemedi.

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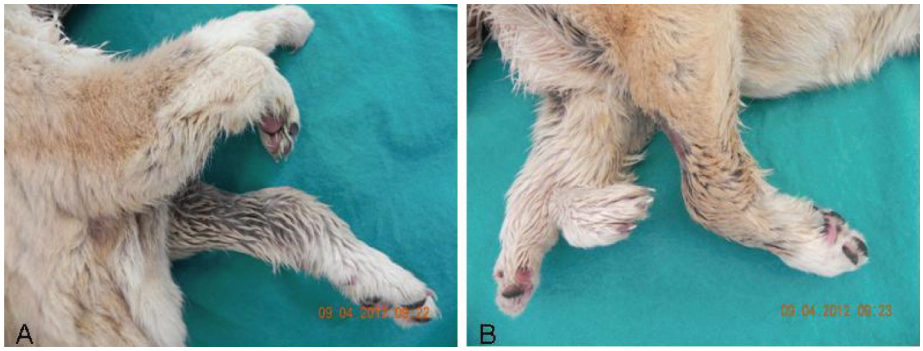
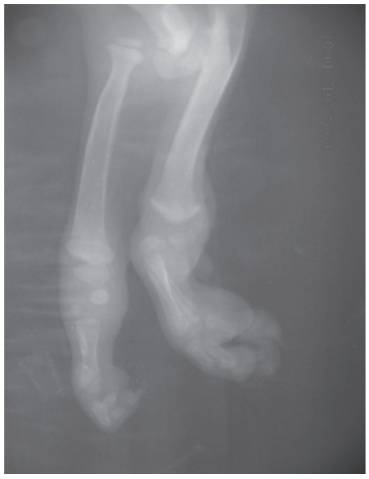


Şekil 1. A, B. Sağ ön ekstremitede ektrodaktili

Fig 1. A, B. Ectrodactyly deformity in the right forelimb



Şekil 2. Radius ve ulnanın radyografideki ayrık görünümü

Fig 2. Radiograph showing the division of the radius and ulna

TARTIŞMA ve SONUÇ

Ektrodaktili olarak da bilinen el veya ayak yarığı kongenital bir malformasyon olup el veya ayağın median hat yokluğu veya median hatta derin bir yarıkla karakterizedir [5]. Olgumuzun klinik ve radyolojik değerlendirilmesinde metakarpal ve falanksların üçünün medial segmentte, ikisinin ise lateralde olduğu ve ekstremitenin distalde yarık olarak şekillendiği saptandı. Bu yönüyle olgunun kaynaklarca [1-3] yapılan tanımlamaya uyduğu görülmektedir.

Ektrodaktili tek başına bir malformasyon olabileceği gibi bir sendromun parçası olarak da şekillenebilir. Her iki formda da genellikle kromosomal yer değiştirme veya silinme gibi yeniden düzenlenmeler söz konusudur. Fare modeli üzerinde yapılan deneysel çalışmalarda ektrodaktili için en belirleyici faktörün genetik olduğu ortaya konulmuştur [5]. Olgumuzda da bu malformasyonun genetik bir nedene bağlı olarak gelişmiş olabileceği düşünülmektedir. Zira gebeliğin 23 ve 35. haftaları arasında mezenşimal kemik hücrelerinin gelişimindeki yetersizlikler veya intrinsik malformasyonların ektrodaktili anomalileri ile sonuçlanabileceği bildirilmiştir [4,5]. Genetik mutasyon veya hastalık, diyet, aşı, radyasyon gibi çevresel faktörlerin nedenler arasında sayılabileceği bildirilmiştir. Ayrıca laboratuar çalışmalarında defekt oluşumunda kadmiyumun da rolünün olabileceği ortaya konulmuştur [5].

Ektrodaktilinin insanlarda özellikle damak yarığı gibi diğer kongenital anomalilerle bir arada görüldüğü bildirilirken, köpeklerde böyle bir rapora rastlanmamıştır [1,3]. Olgumuzda da malformasyonun sadece distal ekstremitede ektrodaktili olarak görülmesi mevcut literatürleri destekler niteliktedir.

Metakarpal kemikler arasında bir yarığın varlığı ile karakterize olan ve çoğu zaman 1 ve 2. metakarpal kemikler arasında görülebileceği belirtilen ektrodakitilide aynı zamanda bir veya daha fazla metakarpal ve falankslarda gruplanmalar olabileceği bildirilmiştir. Ulnanın genellikle normalden daha kısa olmasının bu tip olgularda dirsek ekleminde luksasyon veya subluksasyona predispoze kıldığı bildirilmiştir. Radyogramda radius ve ulnanın ayrık olduğu görülmektedir [1]. Sunulan olguda malformasyonun unilateral olarak sağ ön ekstremitede görüldüğü ve deri ile birlikte seyreden ayrıklığın patilerden karpal ekleme kadar uzandığı, ayrıca metakarpal ve falanksların literatürde de bildirildiği gibi kendi aralarında gruplaştığı saptandı. Radius ve ulnanın ise klinik anlamda belli olmasa da radyogramda birbirinden ayrık olduğu tespit edildi. Bütün bu bulguların literatür bilgileriyle örtüştüğü söylenebilir.

Ektrodaktilinin cerrahi tedavisi sınırlı olmakla birilikte sağaltım deformitenin özellikleri ve şiddetine göre düzenlenebilir. Sağaltımda elde edilecek başarı olgunun şiddetine ve tercih edilen yönteme göre değişebilir [1,2,6]. Olgumuz parvoviral enfeksiyon sonucu öldüğünden planlanan operasyon tekniği uygulanamamıştır.

Sonuç olarak olgunun operatif müdahale ile düzeltilerek sağlığına kavuşturulabileceği öngörülse de ölüm nedeniyle operasyon gerçekleştirilemese de ender görülen böyle bir vakanın literatüre katkı sunabileceği düşünülmektedir.

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CASE REPORT

Bir Buzağıda Dermatosparaksis Olgusu [1]

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Özet

Bu olguda bir buzağıda karşılaşılan dermatosparaksis olgusunun klinik ve histopatolojik olarak tanımlanması ve operatif sağaltım sonuçlarının bildirilmesi amaçlandı. Olgumuzu boyunun sol orta hattında, 3x4x4 cm büyüklüğünde bir şişkinlik ile getirilen 20 günlük simental ırkı dişi bir buzağı oluşturdu. Anamnez bilgilerinden kitlenin, doğumu izleyen birinci haftanın sonunda fark edildiği ve giderek büyüdüğü öğrenildi. Aynı zamanda buzağının tüm vücut deri yüzeyinin irinle kaplı olarak dünyaya geldiği bilgisine ulaşıldı. Total olarak ekstirpe edilen kitlenin histopatalojik olarak değerlendirilmesi sonucu dermatosparaksis tanısı konuldu. Operasyondan 1 ay sonra biri boynun sağ tarafında aynı büyüklükte diğeri ise sağ regio mandibulanın arka tarafında nispeten daha küçük birer kitlenin geliştiği tespit edildi. Bu kitleler de operasyonla uzaklaştırıldı. Bu iki kitlenin histopatalojik değerlendirilmesinde de aynı sonuca varıldı. Sonuç olarak ender olarak görülen dermatosparaksis olgusunun takdimi ile klinik pratiğe olduğu kadar literatüre de katkı sağlanacağı söylenebilir.

Anahtar sözcükler: *Dermatosparaksis, Buzağı*

A Case of Dermatosparaxis in A Calf

Summary

The purpose of this case is to present the results of clinical and histopathological diagnosis and surgical treatment in a case of dermatosparaxis encountered in a calf. Our case consisted of a 20-day-old female Simmental calf brought in due to a swelling the size of a 3x4x4 cm on the left medial line of the neck. It was ascertained form anamnesis information that the mass was noticed at the end of the first week after birth and grew continually. We also learned that the calf had been born with purulence covering the surface of the calf's skin. After the mass was completely extirpated, it was evaluated histopathologically and determined to be dermatosparaxis. One month after the operation, it was determined that one the same size had developed on the right side of the neck and a relatively smaller mass behind the right mandibular region. These masses were surgically removed. Reached the same conclusion in the histopathologically evaluation of these two masses. In conclusion, the presentation of a rare case of dermatosparaxis could be a contribution to the literature and to clinical practice.

Keywords: *Dermatosparaxis, Calf*

GİRİŞ

Kalıtsal kollajen displazisi olan dermatosparaksis, derinin dayanıklılığının azalması, kolay yırtılması, eklemlerde aşırı oynaklık ve etkilenen bölgedeki derinin iyileşmesindeki gecikme ile karakterize resesif kalıtsal bir bağ doku bozukluğu olarak tanımlanmaktadır [1-4]. Sığır, koyun, köpek, kedi ve atta rastlanan bu bozukluğun insanlardaki Ehlers-Danlos sendromuna benzediği bildirilmiştir [1,2,5].

Resesif özellikte olan dermatosparaksis Belçika'da White-Blue sığırlarında ve Hereford, Charolis ve Simmental ırklarında tanımlanmıştır [1]. Hasta sığırların dermisinde amino terminal prokollagen peptidaz enziminin yetersizliğine bağlı olarak prokollagen birikimi söz konusudur. Fazla miktarda biriken bu prokollagen, dermise kontraksiyon gücü kazandıran kollagen fibrillerin paketlenmesini engelleyerek kollagen fibriller çözülmüş halde kalır ve derinin dayanıklılığı kaybolur [1,6,7].

Dermatosparaksisin tanısında klinik bulguların yanında kollajenik oluşumların morfolojik olarak değerlendirilmesinin de önemli olduğu bildirilmiştir [1]. Işık mikroskobunda,

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etkilenen alanda kollajen fibrillerin çap ve sayısının azalması, fibriler yapıda çözülme ve aynı zamanda dermisteki kollajen fibrillerinin diziliminde gözlenen bozukluklar hastalık için tanımlayıcıdır [1].

Bu gözlemde bir buzağıda karşılaşılan dermatosparaksis olgusunun klinik ve histopatolojik olarak tanımlanması ve operatif sağaltım sonuçlarının sunulması amaçlandı.

OLGUNUN TANIMI

Olgumuzu boyunun sol orta hattında, 3x4x4 cm büyüklüğünde bir şişkinlik varlığı nedeniyle getirilen simental ırkı, 20 günlük dişi bir buzağı oluşturdu. Anamnez bilgilerinden buzağının vücut yüzeyinin irinle kaplı olarak dünyaya gelmiş olduğu öğrenildi. Ayrıca gerek annenin gerekse daha önceki doğan kardeşlerde böyle bir durumla karşılaşılmadığı bilgisi alındı. Doğumdan bir hafta sonra boynunun sol tarafında giderek büyüyen bir kitlenin fark edildiği anlaşıldı.

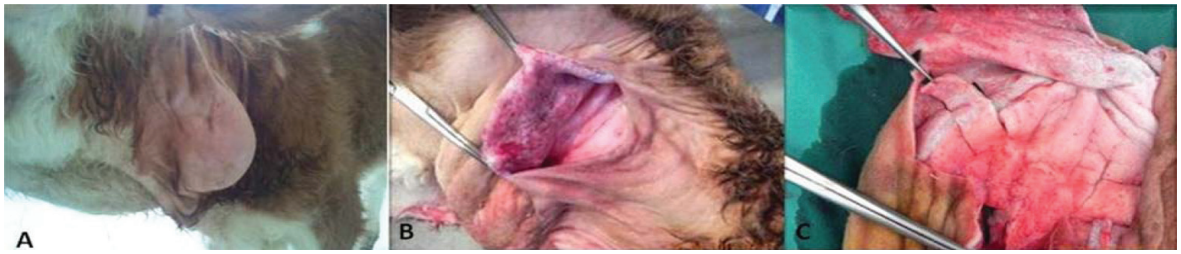
Klinik muayenede bölge derisinin esnekliğini kaybetmiş olduğu ve çekme sırasında kolayca yırtıldığı görüldü. Ayrıca deri altında poşlu bir yapının oluştuğu ve içinde açık renkli seröz bir sıvının bulunduğu tespit edildi *(Şekil 1. A, B,C)*.

Sağaltım için operasyona karar verildi ve rutin operasyon hazırlıklarından sonra lokal infiltrasyon anestezisi eşliğinde problemli görülen deri bölgesi diseke edilerek uzaklaştırıldı. Açıklık rutin yöntemlerle kapatıldı ve postoperatif olarak 3 gün süre ile kas içi olarak 10.000 IU benzil penisilin prokain ve 10 mg dihidrostreptomisin/kg (Reptopen-S, 50 ml flakon, DİF, TÜRKİYE) kullanıldı.

Operasyondan bir ay sonra boyunun sağ tarafında aynı büyüklükte ve sol regio mandibulanın kaudalinde nispeten daha küçük ve içerisinde eksudat bulunan yeni bir kitlenin geliştiği tespit edildi. Bu kitleler de operasyonla uzaklaştırıldı ve postoperatif antibiyoterapiye devam edildi. Uzaklaştırılan kitlelerin tamamı histopatalojik olarak değerlendirildi.

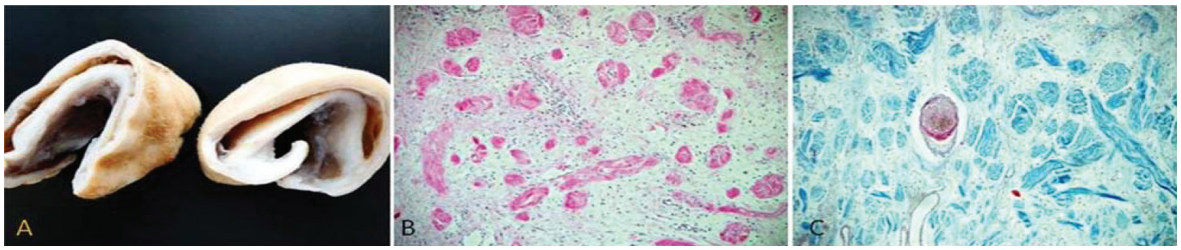
Alınan doku örneklerinin makroskobik olarak incelenmesinde epidermis ve dermisin subkutisten ayrıldığı ve ayrılma noktasında jelatinöz yapıda eksudanın varlığı görüldü *(Şekil 2. A)*. Dokudan alınan örnekler %10 formalin solusyonunda tesbit edildi ve rutin olarak hazırlanan parafin bloklardan alınan kesitler hematoksilen-eozin (H&E) ile boyanarak ışık mikroskopta değerlendirildi. Ayrıca bazı kesitler Mallory üçlü boyama tekniği ile boyandı. Kesitlerin histopatolojik değerlendirilmesinde epidermal katmanların normal görünmesine rağmen, dermiste ter ve yağ bezleri ile kıl folliküllerinin pembe homojen görünümde kollajen veya düz kas hücre ile uyumlu demetler tarafından çevrelendiği görüldü *(Şekil 2. B)*. Mallory üçlü boyama tekniği ile boyanan kesitlerde dermal tabakanın, oval veya silindirik görünümlü mavimsi boyanmış kollajen demetlerden oluştuğu tespit edildi *(Şekil 2. C)*. Subkutis ile dermis arasında gelişen genç granülasyon dokusuna ilaveten, fibrinöz efuzyon, yaygın hemorajiler ve nötrofil lökosit eksudasyonu saptandı. Dermiste özellikle perivasküler yüzeylerde artış gösteren eozinofil lökosit infiltrasyonu dikkat çekici bulundu. Ayrıca epidermisin oldukça inceldiği, stratum spinozum tabakasının 2-3 hücre ve granulozum un sadece bir hücre kalınlığında olduğu görüldü. Elde edilen klinik ve histopatolojik bulgulara dayanılarak, olgu dermatosparaksis olarak tanımlandı.

Postoperatif altıncı ayda yapılan kontrolde hayvanın yaşamını sorunsuz bir şekilde sürdürdüğü tespit edildi *(Şekil 3)*.



Şekil 1. A, B, C- Olguya ait klinik görünüm

Fig 1. A, B, C- Clinical appearance of the calf



Şekil 2. A- Ekstirpe edilen dokuda epidermis ve dermisin subkutisten ayrılması **B-** Dermiste yoğun kollajen bağdoku artışı. H&E x 10 *(Orijinal Büyütme)* **C-** Dermiste diffuz kollajen bağ doku artışı. Mallory üçlü boyama tekniği x 10 *(Orijinal Büyütme)*

Fig 2. A- Seperation from subcutis of the epidermis and dermis in excised tissue **B-** Increased collagenous tissue in the dermis. H&E x 10 *(Original Magnification* **C-** Diffuse collagenous connective tissue in the dermis. Triple x 10 *(Original Magnification)*

Şekil 3. Olgunun postoperatif 6. aydaki görünümü
Fig 3. Postoperative appearance at 6th month of the calf

TARTIŞMA ve SONUÇ

Dermatosparaksisli hayvanlardaki en belirgin klinik bulgu derinin kolay yırtılması ve esnekliğini kaybetmesidir [1]. Kuzularda etkilenen derinin yırtılması veya dökülmesiyle bu alanlarının yaraya dönüştüğü ve birkaç gün içinde yaraların enfekte olması sonucu sepsis geliştiği bildirilmiştir [2]. Tek bir buzağıda saptanan dermatosparaksis olgumuzda ise etkilenen bölge derisinin ilkinde derinin açılmasına rağmen herhangi bir enfeksiyon gelişmediği, ancak deri altında içinde açık renkli seröz bir sıvının doldurduğu poşlu bir yapının bulunduğu görüldü. Deri altında ise yer yer hemorajik alanlar saptandı. Daha sonra gelişen oluşumlarda ise deride açılma söz konusu değildi. Dermatosparaksisli kuzularda da etkilenen alanların bacak ve boyun bölgelerinde yer aldığı, bölgedeki derinin alttaki dokulardan kolayca ayrıldığı ve deri altında hemorajilere rastlandığı bildirilmiştir [2]. Bu yönüyle sunulan olgunun kuzularda bildirilen dermatosparaksisin kliniğine benzerlik gösterdiği söylenebilir.

Sığırlarda dermatosparaksisin resesif özellikte olduğu ve Belçika'da White-Blue sığırları ile Hereford, Charolis ve Simmental ırklarında tanımlandığı bildirilmiştir [1]. Bizim olgumuzun da simintal ırkına mensup olması ve anne veya kardeşlerinde böyle bir durumun görülmemesiyle literatürü destekler niteliktedir.

Dermatosparaksisli koyunların derisinin histopatolojik bakısında; corium'un normale göre daha kalın ve ödemli görüldüğü, kollagen ipliklerin gevşek bir şekilde bir araya geldikleri, kollajen bantlarda bir örnekliliğin kaybolduğu, fragmentasyon, kısalma ve şişmenin dikkati çektiği bildirilmiştir [2]. Olgumuzda ise epidermal katmanların normal görünmesine rağmen, dermiste ter ve yağ bezleri ile kıl folliküllerinin pembe homojen görünümde kollajen veya düz kas hücre ile uyumlu oval doku arasında yerleşik olduğu görüldü.

Sonuç olarak, derinin hem cerrahi manipülasyonlar sırasında hem de ekstirpe edildikten sonra çekildiğinde elastikiyetinin kaybolması ve kolayca yırtılmasının gözlenmesi; ayrıca Mallory üçlü boyama tekniği ile boyanan kesitlerde tüm dermiste oval veya silindirik yapıda mavimsi boyanmış kollajen bağdoku demetlerinin bulunmasına dayanılarak olgu dermatosparaksis olarak tanımlanmıştır.

Bir buzağıda saptanan dermatospraksis olgusunun sunulması ile klinik veteriner hekimliğe ve literatüre katkı sağlayacağı kanaatine varılmıştır.

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CASE REPORT

İki Buzağıda Karşılaşılan Ektopik Böbrek Olgusu [1]

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[1] *XIII. Ulusal Veteriner Cerrahi Kongresi (Uluslararası Katılımlı)'nde (27 Haziran - 1 Temmuz 2012, Sarıkamış/Kars, Türkiye) poster olarak sunulmuştur*

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Makale Kodu (Article Code): KVFD-2012-7725

Özet

Bu olgu sunumu ile iki buzağıda karşılaşılan ektopik böbrek olgularının klinik, histopatolojik bulguları ve operatif sağaltım sonuçlarının sunulması amaçlandı. Kafkas Üniversitesi Veteriner Fakültesi Cerrahi kliniğine getirilen buzağılardan ilkini kuyruk kısalığı ve arcus ischiadicus düzeyinde bir kitle bulunan Montafon ırkı, bir günlük dişi bir buzağı oluşturdu. Diğer olguyu ise perineal bölgede bir şişkinlik bulunan simental ırkı, dişi bir aylık bir buzağı oluşturdu. Her iki olguda da kitleler total olarak ekstirpe edildi ve histopatolojik olarak ektopik böbrek tanısı konuldu. İlk olgunun bir hafta sonra öldüğü diğer olgunun ise postoperatif 6. ayında sorunsuz bir şekilde yaşamına devam ettiği tespit edildi. Sonuç olarak yapılan literatür taramalarında ektopik böbrek olgularının perineal bölgede lokalize olduğuna dair kayıt bulunmamıştır. Bu yönüyle bakıldığında orijinal olarak görülen olguların literatüre katkı sağlayacağı söylenebilir.

Anahtar sözcükler: *Ektopik böbrek, Buzağı*

Case of Ectopic Kidney in Two Calves

Summary

The purpose of this report is to present the results of clinical and histopathological results and surgical treatment of ectopic kidney encountered in two calves. The first was a one-day-old female Montafon calf brought to the Kafkas University School of Veterinary Medicine Surgical Clinic because of a mass at the arcus ischiadicus and the fact that its tail was malformed. The other case was a one-month-old female Simmental calf. It had swelling in the same area. After the masses were completely extirpated in both cases, they were evaluated histopathologically and ectopic kidney was identified. The first calf died one week later. The other calf was determined to be alive and well six months after the surgery. In conclusion, no record was found of ectopic kidney cases in the perineal region during the literature review. When viewed in this light, these original cases could be seen as a contribution to the literature.

Keywords: *Ectopic kidney, calf*

GİRİŞ

Üriner sistem anomalileri genellikle kolumna vertabralis, genital ve alt gastroistestinal kanal veya spinal kord ve meningis anomalileri ile birlikte gelişebilir [1,2]. Domuz, köpek ve kedilerde sık rastlanan böbrek ektopisinde bir veya her iki böbreğin çoğunlukla pelvis ve inguinal bölge başta olmak üzere vücudun farklı bölgelerine lokalize olabileceği bildirilmiştir. Böbrekler normal büyüklükte olabileceği gibi anormal derecede küçük de olabilirler [1,3].

Böbrek ektopilerinin çoğunlukla ünilateral ve sol böbrekte daha yaygın olduğu bildirilmiştir. Pelvik bölge ektopilerinde böbrekler displaziktir [2].

Ektopik böbrek olgularında üreterler normalden daha kısa ve anormal görünümlü olup damarlaşma da fazladır. Böbreğin yapısı ve fonksiyonunun normal olduğu olgularda herhangi bir semptom ortaya çıkmayabilir [4]. Üreterlerin genital kanala açıldığı durumlarda üriner inkontinans mevcuttur [1]. Üreterlerin malforme olduğu olgularda ise hidronefroz veya pyolonefrozise predispozisyon gelişir [1,4]. Rektal veya vaginal muayene ile böbreğin pelvisdeki yeri tespit edilebilse de kesin tanı için urografiden faydalanılır [4].

Bu olgu sunumu ile iki buzağıda karşılaşılan ektopik böb-

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rek olgusunun klinik ve histopatolojik bulguları ve operatif sağaltım sonuçlarının bildirilmesi amaçlandı.

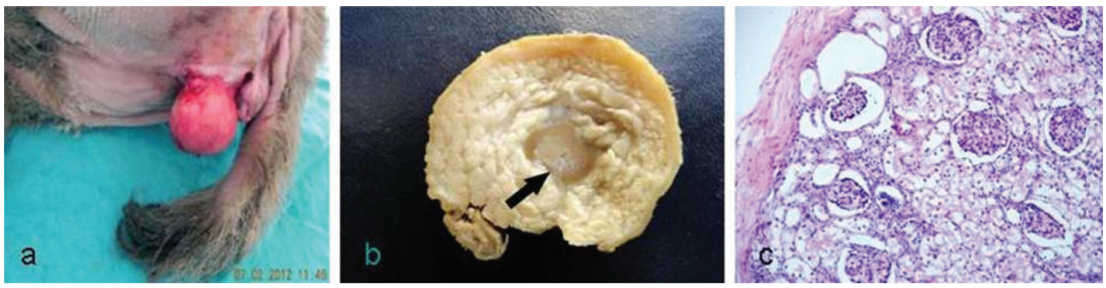
OLGULARIN TANIMI

Olgularımızın ilkini kuyruk kısalığı ve arcus ischiadicus düzeyinde bir kitle (3x4x4) varlığı nedeniyle getirilen montofon ırkı, 1 günlük dişi bir buzağı; diğerini ise perineal bölgede kitle bulunan simental ırkı, 1 aylık dişi bir buzağı oluşturdu.

Klinik muayenede her iki olguda da arcus ishiadicus düzeyinde zarsel bir yapıyla kaplı kitle saptandı *(Şekil 1. a)*. Anamnezde buzağıların idrarını normal bir şekilde yapabildikleri, ancak birinci olgunun henüz defekasyon yapmadığı öğrenildi. Ayırıcı tanı açısından intravenöz pyelografi (İVP) yapıldı. İVP'de renal fossa içerisinde fonksiyonel iki böbreğin bulunduğu görülürken söz konusu kitle içerisinde kontrast madde geçişi saptanamadı. Operasyona karar verilen buzağılarda Marcaine® Spinal Heavy %0.5 (Astra Zenaca) kullanılarak spinal anestezi eşliğinde kitleler total olarak ekstirpe edildi ve histopatalojik olarak değerlendirildi.

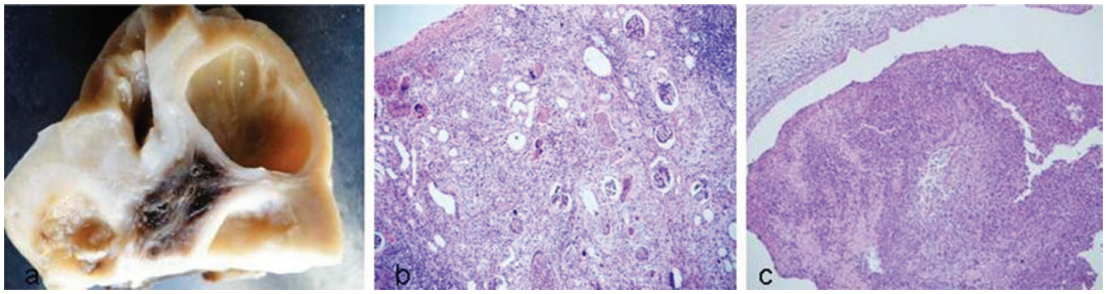
Kitlelerin makroskobik değerlendirilmesinde birinci olguda yaklaşık 3x4x4 cm çapında oval yapı ve gevşek kıvamdaki doku kesildiğinde dıştan kapsülle çevrili açık görünümlü ve ortasında yaklaşık 1x1cm çapında koyu görünümlü doku saptandı *(Şekil 1. b)*. Doku örneklerinden hazırlanan kesitlerin histopatolojik muayenesinde, kitlenin ortasında lokalize dokunun böbrek dokusu ve etraftan mikzomatöz görünümlü lipoid doku ile çevrelendiği görüldü. Kitlenin dış yüzeyinde nekroz, bakteri kümeleri, şiddetli nötrofil infiltrasyonu, fibrin eksudasyonu ve yer yer kanama odaklarının bulunduğu görüldü. Ektopik böbrek dokusunun, normal dokuda olduğu gibi kapsülle çevrili ve kortikal ve medullar kısımlar içerdiği; kortekste çok sayıda glomerulus bulunduğu ve medullar bölgede yaygın tubullerin olduğu saptandı *(Şekil 1. c)*. Böbrek tubul epitellerinde nekrozun yanı sıra altın sarısı görünümünde hemosiderozise rastlandı.

İkinci olguda ise yaklaşık 5x5x6 cm ebadında ve sert kıvamlı kitleye kesit yapıldığında kesit yüzünün irin ihtiva eden oldukça sert ve kıkırdaksı görünümde kavernöz yapılardan oluştuğu görüldü *(Şekil 2. a)*. Yapılan histopatolojik muayenede, kitlenin ektopik böbrek dokusundan oluştuğu ve enfekte olduğu saptandı. Kortikal bölgede glomeruluslarla birlikte yaygın fokal lenfoid infiltrasyonlar, çok sayıda ve değişen büyüklüklerde kist ve apse oluşumları görüldü *(Şekil 2. b)*. Özellikle intertubuler alanlarda artan fibrozise bağlı olarak tubul sayısı ve büyüklüğünde azalma, yoğun lenfoid hücre infiltrasyonları, yer yer tubuler lumenlerde bakteri kolonileri, kalsifikasyon ve asidofilik görünümde ve protein kalıntısı



Şekil 1. a- I nolu olguda kitlenin klinik görünümü, **b-** Ekstripe edilen kitlenin kesit yüzeyinde korteks ve medullası ile birlikte ektopik böbrek dokusu *(ok)*, **c-** Ektopik böbrek dokusun histopatolojik görünümü. HE. 20x *(Orijinal büyütme)*

Fig 1. a- The clinical appearance of the mass of No. I, **b-** Ectopic kidney tissue showing cortex and medulla in the cut surface of the extirped mass *(arrow)*, **c-** Histopathological appearance of the ectopic kidney tissue. HE. 20x *(Original magnification)*



Şekil 2. a- Geniş bağdoku ile çevrili kavernöz yapılar içeren ektopik böbrek dokusunun kesit yüzeyi, **b-** Ektopik böbrek dokusunda şiddetli lenfoid hücre infiltrasyonu, apse oluşumları ve kistik alanlar. HE. 10 x *(Orijinal büyütme)*, **c-** Pelvis renalis lumeninde apse, mukozada epitelyal kalınlaşma. HE. 10 x *(Orijinal büyütme)*

Fig 2. a- Cut surface of the ectopic kidney showing caverns surrounded by a wide connective tissue, **b-** Severe lymphoid cell infiltrations, apscess formation and cysts in the ectopic kidney tissue. HE. 10 x *(Original magnification)*, **c-** Abscess in lumen of the renal pelvic, epithelial thickening of the mucosa. HE. 10 x *(Original magnification)*

olduğu düşünülen homojen yapılar gözlendi. Pelvis renalis lumeninde apse kitlesi, mukoza çeperinde kronik yangı hücreleri ve epitelyal kalınlaşma dikkati çekti *(Şekil 2.c)*.

Olguların takibinde, ilk vakanın bir hafta sonra öldüğü ikinci olgunun ise postoperatif 6. ayında sorunsuz bir şekilde yaşamına devam ettiği öğrenildi.

TARTIŞMA ve SONUÇ

Diğer evcil hayvanlarda olduğu gibi buzağılarda da farklı ürogenital sistem anomalilerine rastlanabileceği belirtilirken [5-8] böbreğin renal fossa dışında bulunması olarak tanımlanan ektopik böbrek anomalilerine dair fazla bilgiye rastlanmamaktadır. Böbrek ektopilerinde bir veya her iki böbreğin çoğunlukla pelvis veya inguinal bölge başta olmak üzere karın ve göğüs boşluğunun herhangi bir bölümünde serbest ya da diğer organlara yapışık olarak bulunabileceği [1,3,5,6] ve üreter obstrüksiyonu ya da atrezisi olmadıkça çoğunlukla herhangi bir klinik belirti vermediği bildirilmiştir [4]. Tanı radyografi, ultrasonografi ve İVP ile konabilir [4]. Tarafımızca değerlendirilen her iki olguda da ektopik böbrek arcus ischiadicus düzeyinde ve pelvik kanal dışında saptanmıştır. IVP'de fossa renaliste normal konumunda bulunan her iki böbrekte kontrast madde saptanırken pelvik kanal dışında yer alan kitlede kontrast madde geçişinin olmamasıyla bu ektopik böbreğin fonksiyonel olmadığı kanaatine varıldı. Postoperatif 7. günde ölen birinci olgunun klinik muayenesinde kuyruk kısalığı da mevcuttu. Konuya ilişkin kaynaklarda üriner sistem anomalilerinin çoğunlukla inguinal kanal içerisinde olabileceği ve farklı sistem anomalilerine eşlik edebileceği bildirilmiştir [2].

Yapılan literatür değerlendirmelerinde buzağılarda ektopik böbrek anomalilerinin ırk ve cinsiyete göre dağılımını konu edinen istatistiki bir veriye rastlanmamıştır. Sunulan olguların birinin montofon diğerinin simental ırkına mensup olması ve her ikisinin de dişi olması yönüyle sonraki çalışmalara katkı sunacağı düşünülmektedir.

Sonuç olarak buzağılarda ektopik böbrek olgularının perineal bölgede lokalize olduğuna dair kayda rastlanmamıştır. Bu yönüyle bakıldığında orijinal olarak görülen bu olguların sunulmasıyla literatüre katkı sağlanması umulmaktadır.

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CASE REPORT

Paraplejili Bir Köpekte Spinal Kord Lezyonunun Belirlenmesinde Klinik, Radyolojik ve Patolojik Bulguların Değerlendirilmesi [1]

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Miktat KAYA ** Hayati AYGÜN *** Mahmut SÖZMEN ****

[1] *XIII. Ulusal Veteriner Cerrahi Kongresi (Uluslararası Katılımlı)'nde (27 Haziran - 1 Temmuz 2012, Sarıkamış/Kars, Türkiye) poster olarak sunulmuştur*

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Makale Kodu (Article Code): KVFD-2012-7834

Özet

Bu raporda 2 yaşlı erkek melez bir köpekte ateşli silah yaralanmasına bağlı gelişen ilginç bir spinal kord lezyonunun tanımlanması amaçlandı. Klinik olarak parapleji tanısı konulan olgunun direkt radyografisinde 9-10. interkostal aralıkta ve korpus vertabralara 2-3 cm uzaklıkta bir adet kurşun saptandı. Direkt ve kontrast radyografi (myelografi) ile spinal kordda herhangi bir anormal bulguya rastlanmazken manyetik rezonans görüntüleme (MRG) ile T_9-T_{10} düzeyinde lezyonlu bir alan tespit edildi. 10 gün sonra ölen köpeğin nekropsisinde lezyonlu spinal kord incelendiğinde MRG bulguları ile uyumlu 0.5 cm'lik hasarlı bir bölge belirlendi. Histopatolojik değerlendirmede ise spinal kordda myelomalasi tespit edildi. Sonuç olarak, karşılaşılabilecek benzer olgularda gerek tanı gerekse hastalığın prognozunun belirlenmesinde faydalı olabileceği inancı ile bu olgu sunulmuştur.

Anahtar sözcükler: *Parapleji, Ateşli silah yaralanması, Köpek, Röntgen, MRG, Histopatoloji*

Evaluation of Clinical, Radiological and Pathological Findings in the Identification of a Spinal Cord Lesion in a Paraplegic Dog

Summary

The aim of this report describes the identification of an interesting spinal cord lesion developing in a 2 years old, male, hybrid dog due to being injured by gunshot. In the direct radiography of the patient diagnosed with clinic paraplegia was detected a bullet in the 9^{th}-10^{th} intercostal space at a distance of 2-3 cm away from the corpus vertebrae. While no abnormal findings were determined from direct and contrast radiography (myelography), through MRI, an area with a lesion at the T9-T10 level was identified on the spinal cord. At necropsy of the dog dying on the 10^{th} day of case, a damaged area of 0.5 cm compatible with MRI findings was determined while examining the injured spinal cord. Besides, myelomalacia on spinal cord was recognized during histopathology evolution. As a result, this case report is presented so that it would be beneficial in the identification of both diagnosis and prognosis of the disease in similar cases our colleagues may come across.

Keywords: *Paraplegia, Gunshot wounds, Dog, Rontgen, MRI, Histopathology*

GİRİŞ

Küçük hayvan pratiğinde sıkça karşılaşılan ve acil olarak değerlendirilmesi gereken medulla spinalis lezyonları birçok intrinsik ve ekstrinsik nedene bağlı olarak ortaya çıkmaktadır [1-4]. Ateşli silah yaralanmaları ise ekstrinsik nedenler içerisinde özel bir yere sahiptir [5-7].

Spinal lezyonların şekline ve derecesine göre klinik belirtiler değişmekle birlikte çoğu olgularda parapleji tipiktir. Spinal lezyonlarda prognoz etiyolojinin bilinmesi, lezyonun yeri ve şiddetinin belirlenmesine bağlı olduğundan diagnostik prosedürler önemlidir. Bu amaçla önem sıra-

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sına göre bir dizi laboratuar analizleri, direkt radyografi, myelografi, bilgisayarlı tomografi (BT) ve MRG tekniklerinden faydalanılabilir [1,2].

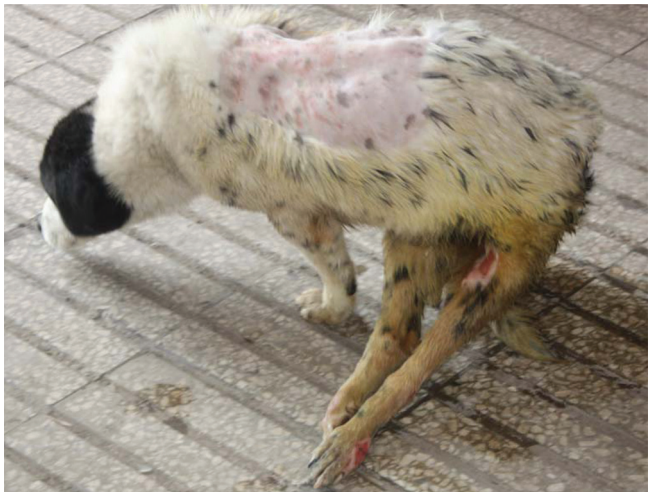
Bu makalede, benzer olgularda tanıya ışık tutabileceği düşüncesiyle bir köpekte saptanan spinal kord lezyonu öyküsü sunulmuştur. Zira klinik olarak parapleji tanısı konulan olguda ne anamnez bilgilerinden ne de radyolojik muayene bulgularından lezyonun yeri ve şiddeti hakkında kesin bir bilgiye ulaşılamamıştır. Kesin tanı ise nekroskopi sonrası yapılan makroskopik ve histopatolojik incelemelere dayanılarak konulabilmiştir.

OLGUNUN TANIMI

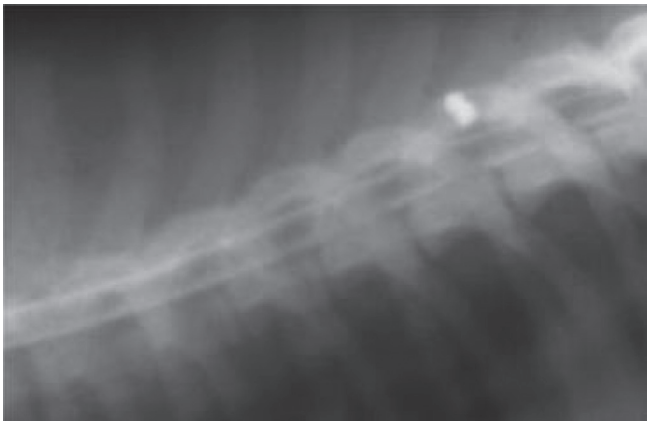
Olgumuzu 2 yaşlı, erkek, melez bir köpek oluşturdu. Anamnez bilgilerinden köpeğin mevcut proplemlerle birlikte bahçede bulunduğu ve nedene yönelik herhangi bir bilgi sahibi olunmadığı, ancak paraplejinin birden bire şekillendiği ve kliniğimize olaydan bir hafta sonra getirildiği anlaşıldı. Klinik muayenede parapleji ve üriner inkontinans görüldü. Lumbosakral bölgeden servikal bölgeye kadar yapılan deri yüzeyinin inspeksiyonunda herhangi bir lezyon ya da deformasyon saptanmadı.

Öncelikle her iki ekstremitenin distalinden başlayarak genunun proksimaline kadar iğne pikürü ve elektrikli üvendireyle yüzeysel ağrı duyusunun olup olmadığına bakıldı. Yüzeysel ağrı duysunun saptanamaması üzerine yapılan ekstensiyon itme, fleksiyon geri çekme, perineal ve panniculus refleks muayeneleri ile de derin ağrı duyu kaybının şekillendiği belirlendi *(Şekil 1)*.

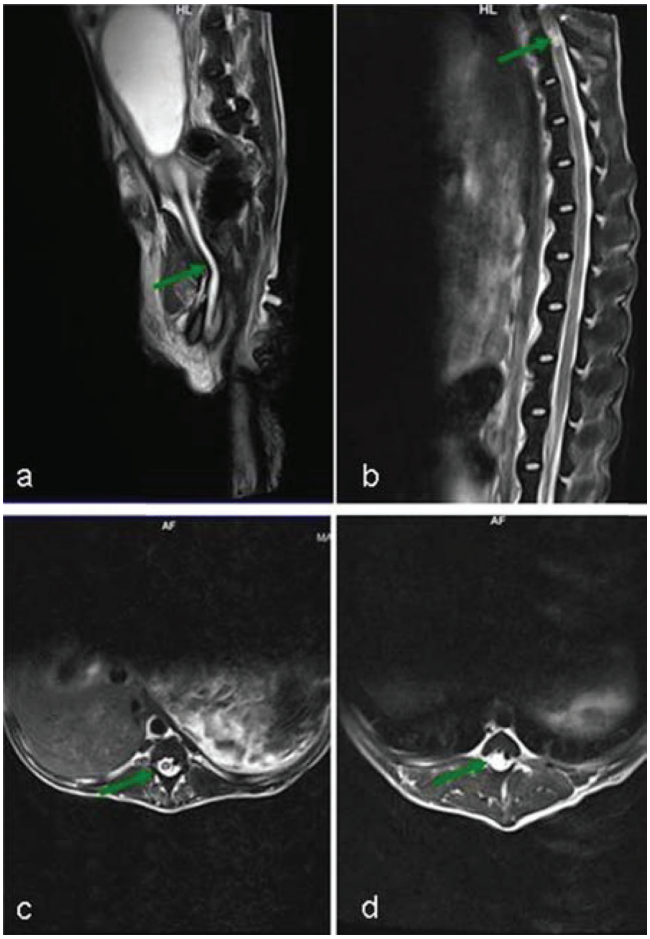
V/D ve L/L pozisyonlarda alınan direkt radyografide dokulara özgü herhangi bir anormal bulgu saptanamazken 9-10. interkostal aralıkta ve korpus vertabralara 2-3 cm uzaklıkta bir adet kurşun saptandı. Atlanto-occipital (sisternal) teknik kullanılarak elde edilen myelogramda kontrast maddenin spinal kord boyunca sakral düzeye kadar muntazam bir şekilde ilerlediği ve herhangi bir lezyona işaret etmediği anlaşıldı *(Şekil 2)*. MRG'de T_2 aksiyal ve sagittal kesitlerde üriner inkontinans varlığını da gösteren nörojenik mesane *(Şekil 3.a)* ve T_9-T_{10} düzeyinde spinal



Şekil 1. Olgunun klinik görünümü
Fig 1. Clinical appearance of the case

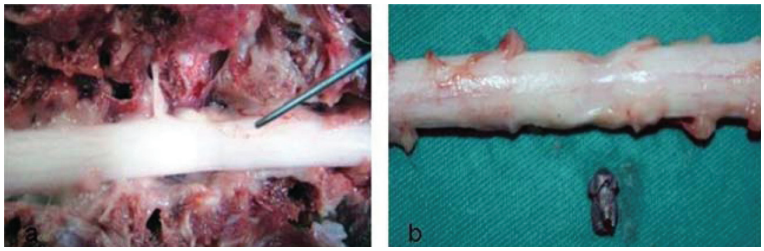


Şekil 2. Olgunun myelografik görünümü
Fig 2. Myelographic appearance of the case



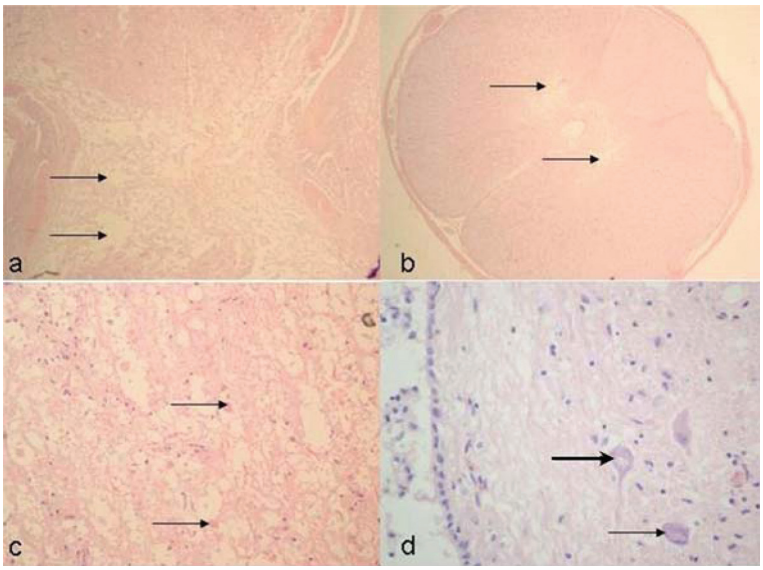
Şekil 3. Olguya ait MRG görüntüsü **a-** Üriner inkontinans (nörojenik mesane) **b-** T_2 aksiyal kesitte hiperdens lezyon *(ok)* **c-** Spinal kordun T_2 sagittal kesitte normal görünümü *(ok)*, **d-** T_2 sagittal kesitte spinal kord üzerinde hiperdens lezyon *(ok)*

Fig 3. MRI of the case **a-** Urinary incontinence (neurogenic bladder) **b-** Hyperdense lesion of the spinal cord on the T_2 axial cross-sections *(arrow)* **c-** Normal view of the spinal cord on the T_2 sagittal cross-sections *(arrow)* **d-** Hyperdense lesion of the spinal cord on the T2 sagittal cross-sections *(arrow)*



Şekil 4. a-b. Spinal kord üzerinde lezyon

Fig 4. a-b. Lesion on the spinal cord



Şekil 5. Histopatolojik bulgular, **a-** Likefaksiyon nekrozu sonucu gelişen boşluklar *(oklar)*. Longitudinal kesit. H&E x20 **b-** Medulla spinalisde nekroz ve nöron kaybı sonucu gri maddede şekillenen bilateral simetrik kavi-tasyonlar *(oklar)*. Transversal kesit. H&E x20 **c-** Yaygın sferoid formasyonları *(oklar)*. H&E x20 **d-** Marjinal yerleşimli çekirdek ve periferal granüller ile karakterize santral kromatolizis *(ok)*. Çekirdekte şişme ve hücrenin kenarına yerleşme ile karakterize olan nöron dejenerasyonu *(kalın ok)*. H&E x40

Fig 5. Histopathological findings, **a-** Growing gaps due to liquefaction necrosis *(arrows)*, Longitudinal section. H&E x20, **b-** The appearance bilaterally symmetric of cavitations, as a result of necrosis and neuronal loss in the gray matter of the spinal cord *(arrows)*. Transversal section. H & E x20, **c-** Common sferoidale formations *(arrows)*. H & E x20, **d-** Central kromatolizis characterized by the nukleus (located marginal) and the peripheral granules *(arrow)*. Neuron degeneration characterized by swelling of the cell and settlement on the edge of the nukleus *(thick arrow)*. H & E x40

kordu tamamen dolduran hiperdens özellikli lezyonla uyumlu bir alan *(Şekil 3.b-d)* saptandı.

Kesin tanı konuluncaya kadar medikal olarak herhangi bir sağaltım önerilmezken sadece deri altı yolla 4 mg/kg karprofen (Rimadyl®, Pfizer) verildi. Ertesi gün için randevu verilmesine rağmen getirilmeyen, ancak olaydan on gün geçtikten sonra sahibi tarafından kulübesinde ölü bulunan ve isteğimiz üzerine tekrar kliniğimize getirilen köpeğin makroskopik incelenmesinde şüpheli bölge düzeyinde deriye ait gözle görülür herhangi bir lezyona rastlanmadı. Deri altında serbest olarak lokalize olan kurşun incelendiğinde uç kısmının bombeli olduğu ve herhangi bir şekil değişikliğine uğramadığı görüldü. Laminektomi ile açığa çıkarılan korda ait zarsel yapıda (Duramater, Arachnoid ve Piamater-DAP) ve çevre dokularda herhangi bir yangı belirtisi saptanamadı, ancak zarsel yapının hemen altında, belli belirsiz bir renk farkı ile anlaşılabilen ve kord materyalinin yaklaşık 0.5 cm mesafede bütünlüğünün bozulmuş olduğunu gösteren bir alan tespit edildi *(Şekil 4)*. MRG ile de saptanan bu alanın direkt röntgende belirlenen ve nekroskopi sırasında derinin hemen altında bulunan kurşunla aynı doğrultuda olduğu anlaşıldı.

Bütünlüğü bozulan kordun mikroskobik incelenmesinde etkilenen bölgenin yaygın bir şekilde nekroze olduğu görüldü. Bu bölgede meydana gelen erime (likefaksiyon, kollikuasyon nekrozu) nedeni ile beyaz ve gri madde arasındaki sınırın ortadan kalktığı ve geniş boşlukların oluştuğu saptandı *(Şekil 5.a)*. Lezyonlu bölgenin kranial ve kaudal kısımlarındaki medulla spinalisin hem gri maddesi *(Şekil 5.b)* hem de beyaz maddesinde yoğun dejeneratif değişiklikler ve nekroz saptandı. Bu bölgedeki aksonların ovoid ya da sirküler eozinofilik yapıda (sferoid formasyonları) olduğu *(Şekil 5.c)* ve myelin kılıfı ile birlikte şiştiği görüldü. Çoğu nöronun ortadan kaybolduğu, kalanların ise sitoplazmalarının büzüştüğü, yuvarlaklaştığı ve eozinofilik karakterde oldukları ya da çekirdeklerinin ve Nissl granüllerinin periferik konumda oldukları tespit edildi (santral kromatolizis) *(Şekil 5.d)*. Diğer nöron çekirdeklerinin parçalandığı (karyoreksis) ya da tamamen kaybolduğu (lizis) belirlendi. DAP'ın bütünlüğünde ise bir bozulma görülmedi. Kordun kopan uçlarının her iki tarafında da yaygın nekroz alanları ve travma sonrası gelişen myelomalasi (omurilik nekrozu) tespit edildi.

Nekropside diğer sistem veya organlarda herhangi bir anormal bulguya rastlanmadı. Muhtemel ölüm nedeninin spinal kordda gelişen myelomalasinin kraniale doğru ilerlemesi sonucu ortaya çıkan solunum ve dolaşım yetersizliğinden kaynaklanmış olabileceği kanısına varıldı.

Tüm bulgular birlikte değerlendirildiğinde, kurşunun intervertebral aralıktan kavum medullarise girerek kord zarlarını esnetip geçtiği ve kord bütünlüğünü tamamen bozarak karşı intervertebral aralıktan çıkmış olduğu varsayımına ulaşıldı.

TARTIŞMA ve SONUÇ

Hayvanlarda ateşli silah yaralanmaları sadizm sonucu kasten olabileceği gibi rastlantısal ya da av sırasında yan-

lışlıkla da meydana gelebilir. Anamnezde etkenin ne olduğu konusunda bilgi sahibi olunmadığı ve hayvanın kısa süre önce bahçede sağlıklı bir şekilde dolaşırken birden bire arka bacaklarını sürükler halde görüldüğü ifade edilmiştir. Klinik muayene ile spinal kordda meydana gelen lezyonların yeri belirlenebilse de lokalizasyonunun tam olarak saptanması her zaman mümkün olmayabilir. Bu amaçla direkt ve kontrast radyografi (myelografi), bilgisayarlı tomografi ve MRG gibi ileri muayene yöntemlerine başvurulabilir [1-4]. Olgumuzun klinik değerlendirilmesinde lumbosakral bölgeden servikal bölgeye kadar deri düzeyinde herhangi bir lezyon saptanamamıştır. Asıl etkenle birlikte mevcut klinik tablonun oluşumuna neden olan lezyonun yeri ve derecesinin saptanabilmesi amacıyla muayene ve tanı yöntemleri kaynaklarda [1,2] da belirtildiği gibi en basitten komplekse göre sırasıyla uygulanmış, ancak parapleji tanısı kesin olarak konulmasına ve muhtemel etkenin belirlenmesine rağmen, lezyonun kesin yeri ve derecesinin saptanmasında tereddüte düşülmüştür.

Spinal kord farklı doku ya da organları innerve eden periferik sinirlerin köken aldığı segmentler itibariyle dört kısma ayrılmakta ve bu segmentlerin her birinde meydana gelen lezyonlarda farklı klinik semptomlar ortaya çıkmaktadır [1,2,4]. Bununla birlikte ortaya çıkan semptomların derecesi lezyonun şiddeti ve derecesi ile doğru orantılıdır [2]. Dolayısıyla spinal travmalı hastalarda çoğu kez lezyonun hangi segmentte olduğunun anlaşılması organ ve dokularda belirlenen klinik semptomların doğru bir şekilde yorumlanmasıyla anlaşılabilir. Nitekim tartışmaya konu olan olgunun klinik muayenesinde ön plana çıkan bulgular parapleji ve üriner inkontinenstir. Her iki bulgunun birlikte görülmesi spinal kordun ya sakral ya da T_3-L_3 düzeyindeki lezyonlarında sözkonusu olmakla birlikte T_3-L_3 düzeyindeki lezyonlarda her iki bulgunun birlikte görülmesi için spinal kord bütünlüğünün tamamen bozulması gerekir [2]. Bu bilgiler ışığında yapılan tüm klinik ve radyografik muayane prosedürlerine öncelikle sakral düzeyden başlanarak kraniale doğru ilerlenmiş, ancak direkt radyografi ve myelografi bulguları klinik tabloyu açıklamaya yetmemiştir. Zira sisternal teknik kullanılarak elde edilen myelogramda kontrast maddenin spinal kord boyunca sakral düzeye kadar muntazam bir şekilde yayıldığı ve herhangi bir lezyona işaret etmediği anlaşılmıştır. Lezyonun hangi segmentte şekillendiği ise MRG'de T_9-T_{10} düzeyinde spinal kordu tamamen dolduran hiperdens özellikli bir alanın görülmesi ile belirlenmiştir.

Kedi ve köpeklerde spinal kord lezyonlarının tanısında myelografiden sıkça faydalanılmaktadır [1,2,4,8]. Sunulan olguda ise myelografi bulguları tanıda etkili olamazken nekroskopide spinal kordu saran zarsel yapıların bütünlüğünün tamamen korumasının bunda etkili olduğu söylenebilir. Ayrıca nekroskopide zarsel yapıların sağlam olmasına rağmen MRG'de T_9-T_{10} düzeyinde spinal kordun bütünlüğünü tamamen kaybettiği anlaşılmıştır. Mikroskobik değerlendirmede dura-araknoid ve piamaterin yapısını tamamen koruduğu, buna rağmen, bütünlüğü bozulan spinal kordun her iki tarafında yaygın likefaksiyon ve kollikuasyon nekrozunun görülmesi MRG bulgularını teyit ederken bu tip olgularda prognozun neden olumsuz olarak değerlendirilmesi gerektiğine de ışık tutmaktadır. Dolayısıyla eldeki veriler dikkate alınarak spinal kord lezyonunun yeri ve şiddetinin bir an önce saptanmasıyla prognozun belirlenmesi ve/veya hasta hakkında ki son kararın verilmesi hekim, hasta ve hasta sahibi açısından önemlidir.

Dokularda meydana gelen lezyonun büyüklüğünün merminin türüne, ateş mesafesine ve geliş açısına bağlı olarak değişebileceği bildirilse de [5-7,9,10] ortaya çıkan komplikasyonların ciddiyetinin lezyonun lokalizasyonu ile yakından ilgili olduğunu açıklaması bakımından sunulan olgu oldukça ilginç olarak değerlendirilmiştir. Nekroskopide DAP yapının bütünlüğünü tamamen koruduğu görülürken kord materyalinin bütünlüğünün bozulmuş olduğu zorlukla fark edilebilmiştir. Kurşunun muhtemelen foramen vertebra düzeyinden girdiği ve DAP yapıyı esnetip geçerken kord materyalini tamamen koparark karşı taraftaki vertebral aralıktan çıkmış olabileceği varsayımına ulaşılmıştır.

Sonuçta kurşunun dışarı çıkması durumunda asıl etkenin ne olduğu anlaşılamayacağından belki de kesin tanıya ulaşılamayabilirdi. Klinik tecrübe açısından ilginç olarak değerlendirilerek benzer olgularda gerek farkındalık oluşturularak tanının konmasında gerekse hasta hakkında son kararın belirlenmesinde faydalı olabileceği inancı ile bu vaka sunulmuştur. Ayrıca, adli olguların aydınlığa kavuşturulmasında da izlenen yolun meslekdaşlarımıza rehber olabileceği söylenebilir.

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CASE REPORT

İsviçre Esmeri Bir Buzağıda Atipik Vulva Atrezisi Olgusu [1]

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[1] *XIII. Ulusal Veteriner Cerrahi Kongresi (Uluslararası Katılımlı)'nde (27 Haziran - 1 Temmuz 2012, Sarıkamış/Kars, Türkiye) poster olarak sunulmuştur*

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Özet

Bu makalede, bir buzağıda atipik bir form gösteren vulvar atrezi olgusunun sunulması amaçlanmıştır. Olgunun yapılan muayenesinde vulvanın şekillenmiş olduğu ancak vulva dudaklarının tamamına yakınının kapalı olduğu gözlendi. Sonrasında vajinoplasti operasyonuna karar verildi. Sonuç olarak, sıkça rastlanmayan vulva atrezisinde operatif yöntem uygulamasının yararlı olabileceği kanaatine varıldı.

Anahtar sözcükler: *Vulva atrezisi, Doğmasal anomali, Buzağı, İsviçre Esmeri*

The Case of Atypical Vulvar Atresia in A Brown Swiss Calf

Summary

Aim of this presentation, shows a form of atypical a calf race is to present a case of vulvar atresia. The examination of the case, but the vulva lips of the vulva is formed by almost all were found to be closed. After the examination, vaginoplasty operation was decided. As a result, the reporting of this phenomenon rarely seen in the literature is thought to be appropriate for vaginoplasty operation.

Keywords: *Vulvar atresia, Congenital anomaly, Calf, Brown Swiss*

GİRİŞ

Doğmasal anomalilerin patogenezi tam olarak bilinmemekle birlikte, bu anomalilerin genetik veya genetik olmayan faktörlerden (çevresel faktörler) kaynaklandığı bildirilmektedir. Bunun yanında teratojenik etkiye sahip virusların, intrauterin dönemdeki beslenme hatalarının ve intoksikasyonların asıl anomali nedenleri arasında sayıldığı düşünülmektedir [1]. Anomalilerin birçok hayvan türünde tek bir organı veya vücudun bir kısmını etkileyebildiği görülmektedir [2]. Buzağılarda ise sıklıkla atresia ani, atresia recti, rektovaginal fistül, hidrosefalus, göz kapağı ve üriner sistem anomalilerine rastlandığı belirtilmektedir [3,4]. Buzağılarda, vulva atrezisinin de eşlik ettiği multiple kongenital anomalileri konu alan yayınlar olmakla birlikte vulva atrezisine dair ayrıntılı bilgiler içeren çok fazla olgu kaydı yoktur [5].

Bu olguda bir buzağıda saptanan atipik vulva atrezisinde kapalı olan vulva dudaklarının vajinoplasti ile düzeltilmesi yoluna gidilmiştir.

OLGUNUN TANIMI

Olgumuzu, atipik bir form gösteren vulva atrezisine sahip 90 günlük İsviçre Esmeri bir buzağı oluşturdu. Yapılan muayene sonrasında vulvanın şekillendiği ancak vulva dudaklarının tamamına yakınının kapalı olduğu *(Şekil 1. A)* ve anüse yakın bölgede yaklaşık olarak 0.5 cm genişlikte açıklık bulunduğu, hayvanın bu açıklıktan idrarını yapabildiği gözlendi *(Şekil 1. B)* ve vajinoplasti operasyona karar verildi. Operasyon öncesi bölgenin asepsi antisepsi sağlandıktan sonra 15 ml %2'lik lidokain (Adokain®, Sanovel) vulvar bölgeye uygulandı. Kapalı olan vulva dudakları 4 cm'lik bir ensizyonla açılıp, yara dudakları 1 USP polyglactin (Medsorb PGLA®, Medeks) ile dikildi *(Şekil 1. C, D, E)*. Dikiş sonrası yapılan spekulum muayenesi ile buzağının genital organlarında (vagina, vestibulum vagina ve serviksin girişinde) herhangi bir anormal durum saptanmadı. İyileşme süreci 7 gün takip edildi ve 10. gün yapılan muayeneler sırasında vulva dudaklarında herhangi bir probleme rastlanmadı. Ayrıca iyileşmenin de komplikasyonsuz olduğu gözlendi *(Şekil 1. F)*.

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Şekil 1. Doğmasal vulva atrezisi (A, B), vajinoplasti (C, D) ve postoperatif görünüm (E, F)

Fig 1. Congenital vulvar atresia (A, B), vaginoplasty (C, D) and postoperative view (E, F)

TARTIŞMA ve SONUÇ

Doğmasal anomalilerin sebepleri tam olarak bilinmemektedir ancak çevresel faktörlere bağlı annenin toksik maddeler tüketmesi ve bu maddelerin teratojenik etkiye sahip olması veya gebelik döneminde föto-maternal viral enfeksiyonlar sonucu anomalilerin şekillendiği bildirilmiştir. Anomaliler tek bir organı veya bir sistemi etkileyebildiği bildirilmektedir [6]. Kılıç ve ark.[7], üç buzağıda çoklu ürogenital organ anomalisi (atrezia ani, atrezia vulva, mega vagina ve rekto-vaginal fistül) bildirmişlerdir. Sunulan olguda ise yalnızca vulva dudaklarında bir anomalinin olduğu görülmekteydi.

Yalnızca bir organı veya bazı sistemleri (sindirim sistemi gibi) etkileyen ve fazla komplike olmayan anomalilerin operatif yöntemlerle tedavi edilebildiği bildirilmektedir [8]. Yapılan bir çalışmada [9], atrezia vulva, atrezia ani ve rekti olgusu cerrahi müdahale ile tedavi edilmiş ve olumlu sonuçlar alınmıştır. Sunulan olguda da vulvada doğmasal olarak şekillenen atrezi, operatif müdahale ile giderildi *(Şekil 1 C, D)* ve olgunun problemsiz olarak iyileştiği gözlendi *(Şekil 1 F)*.

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CASE REPORT

Holstein Irkı Bir İnekte Karşılaşılan Erken Dönem Fetal Maserasyon Olgusu [1]

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[1] *XIII. Ulusal Veteriner Cerrahi Kongresi (Uluslararası Katılımlı)'nde (27 Haziran - 1 Temmuz 2012, Sarıkamış/Kars, Türkiye) poster olarak sunulmuştur*

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Makale Kodu (Article Code): KVFD-2012-8295

Özet

Bu olgu sunumunda, bir inekte başlangıç aşamasında karşılaşılan fetal maserasyon'un ve operatif sonuçlarının değerlendirilmesi amaçlandı. Yapılan rektal muayenede yavruya ait canlılık belirtileri saptanmayıp, uterusta krepitasyon ile beraber duvarında kalınlaşma ve vaginal muayenede ise serviksin kısmen açık olduğu belirlendi. Sonrasında sezaryen operasyonuna karar verildi. Sonuç olarak olgu, rapor edilen literatürlerin aksine sürecini tam olarak tamamlamamış fetal maserasyon olarak tanımlandı.

Anahtar sözcükler: *Fetal maserasyon, İnek, Holstein*

A Case of Early Fetal Maceration Encountered in A Holstein Cow

Summary

This case report describes a case of a cow shaped fetal maceration and operative results are presented in the early period. Undetected signs of vitality of the fetus by rectal examination, the uterus and vaginal examination, crepitus in the cervix with the uterus wall thickening were closed. Then, the cesarean operation was decided by operator. As a result, cases are reported in the literature have not completed the process of contrast, defined as a fetal maceration.

Keywords: *Fetal maceration, Cow, Holstein*

GİRİŞ

Fetusun maserasyonu bütün çiftlik hayvanlarında görülse de ineklerde görülme sıklığı daha fazladır [1] ve gebeliğin hemen hemen her döneminde görülür. Gebeliğin üçüncü ayından itibaren şekillenen fetal maserasyon olgularında çoğunlukla yavru bakteriyel parçalanmaya uğrar ve serviks bir miktar açık konumdadır [2]. Çoğu fetal maserasyon olgusunda vaginadan mukopurulent bir akıntı gelir. Ayrıca operasyon sırasında uterustan yavruya ait kemik parçaları çıkarılabilmektedir [3]. Bu tür olgularda, başarılı bir sağaltım şekli olmamakla birlikte prostaglandinler ve östrojen uygulamaları denenebilir [1]. Bunun yanında operatif müdahale ile yavruya ait kısımların uterustan temizlemesine başvurulabilinir. Bu olgularda prognoz kötüdür ve gebe kalma oranını olumsuz etkilemektedir [2].

Bu olgu sunumunda, bir inekte başlangıç aşamasında karşılaşılan fetal maserasyon'un ve operatif sonuçlarının değerlendirilmesi amaçlandı.

OLGUNUN TANIMI

Olgumuzu, doğum yapacağı (sancılarından dolayı) şüphesiyle Kafkas Üniversitesi Veteriner Fakültesi Doğum ve Jinekoloji Kliniğine getirilen 5 yaşlı Holstein ırkı bir inek oluşturdu. Yapılan klinik muayeneler sonrasında serviks önü torsiyo uteri'nin de şekillenmiş olduğu belirlendi. Rektal muayenede yavruya ait canlılık belirtileri saptanmazken, uterusta krepitasyon ve duvarında kalınlaşma saptandı. Spekulum ile yapılan vaginal muayene sonrasında ise serviksin kısmen açık olduğu görüldü ve sonrasında sezaryen operasyonuna karar verildi. Operasyona başlamadan bölgenin asepsi ve antisepsisi sağlandıktan sonra lokal infiltrasyon anestezisi (%2 lidokain, Adokain®, Sanovel) uygulandı. Bölge yaklaşık olarak 30-40 cm'lik dikey bir ensizyon ile açılıp uterusa ulaşıldı. Uterusta yavruya ait çoğu yumuşak dokuların neredeyse tamamen eridiği ve yapışkan bir hal aldığı gözlendi. Bunun yanında yavruya ait akciğer, böbrekler ve kısmi olarak da yüz kaslarında erime olmadığı gözlendi *(Şekil 1)*.

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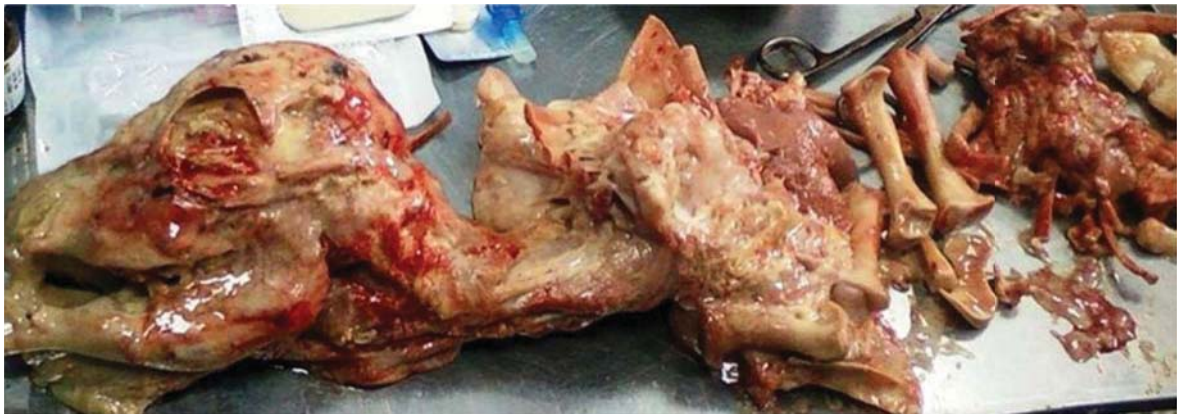

Şekil 1. Erken dönem fetal maserasyon
Fig 1. Early fetal maseration

Uterus boşaltıldıktan sonra serum fizyolojik ile yıkandı ve antibiyotikli oblet (Metrisiklin®, Phenix) konulup, Schmiden ve Lembert dikişleri uygulanarak kapatıldı. Karın kasları ve deri altı bağ dokuya basit sürekli, deriye ise Horizontal Matres dikişi uygulanarak bölge kapatıldı. Postoperatif olarak oksitosin (Vetaş Oksitosin®, Vetaş) ve antibiyotik uygulandı (Primamycin LA®, Pfizer). Yapılan postoperatif muayenelerde herhangi bir komplikasyona rastlanmadı.

TARTIŞMA ve SONUÇ

Fetal maserasyon olgusuna, çiftlik hayvanları başta olmak üzere tüm hayvan türlerinde gebeliğin herhangi bir döneminde rastlanabildiği bildirilmektedir. Genellikle bu tür olgularda medikal tedavi endike olup sonuç alınamadığı durumlarda operasyon önerilmektedir [4]. Sunulan olguda da operasyona karar verilip yavruya ait kısımların çıkarılması hedeflendi. İncelediğimiz literatürlerde operasyon sırasında sıklıkla uterusta sadece yavruya ait kemik parçalarına rastlandığı bildirilmektedir [4,5]. Olgumuzda ise, literatür bilgilerine ek olarak yavrunun tamamen masere olmadığı, maserasyonun başlangıç aşamasında olduğu, kemiklerle beraber tırnaklar ve bazı iç organların da (akciğer ve böbrekler) operasyon sırasında uterusta bulunduğu gözlendi.

Sonuç olarak olgu, rapor edilen literatürlerde belirtilenlerin aksine sürecini tamamlamamış fetal maserasyon olarak tanımlanmıştır. Daha sonra karşılaşılabilecek fetal maserasyon olgularında isimlendirilme yapılırken olgumuzun dikkate alınacağı kanısındayız.

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CASE REPORT

Urinary Calculus in A Guinea Pig

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Summary

Guinea pigs (*Cavia porcelles*) are susceptible to formation of urinary tract calculi. Uroliths can be located anywhere in the urinary tract and are typically composed of calcium salts. A pet, female guinea pig, aged 5 years and weighing 0.5 kg was referred to Internal Medicine Department Clinics for stranguria and recurrent haematuria over 15 days. The guinea pig was emaciated, dehydrated and hypothermic, also demonstrated pain on caudal abdominal palpation and a small thickened bladder wall was discovered. Gastric dilatation and urinary calculus was detected with thoracoabdominal radiography. 2 days later death was learned and then it was brought for necropsy to Department of Pathology. Necropsy revealed calculus in the lumen of urinary bladder with the dimensions of 0.7x0.5 cm though no distinct changes were grossly evident in the urinary tract. The bladder was empty and the mucosa was moderately thickened suggestive of fibrosis and edema resulting from a possible chronic condition. Physical and chemical analysis of the calculus was done by laboratory. Calcium and oxalates are the main risk factors for stone formation, but the calculus of this guinea pig included calcium carbonate and oxalate in combination with other minerals; including struvite. To the authors' knowledge, it was the first guinea pig including urinary signs and calculus, presented in Turkey.

Keywords: *Hematuria, Stranguria, Guinea pig*

Bir Kobayda İdrar Kesesi Taşı

Özet

Kobaylar (*Cavia porcelles*) idrar yolu taşlarını oluşturmaya yatkındırlar. İdrar taşları idrar yolu kanalının herhangi bir yerinde lokalize olabilirler ve genellikle kalsiyum tuzlarından oluşurlar. Pet hayvanı olarak bakılan dişi, 5 yaşlı, 0.5 kg ağırlığındaki kobay 15 günden fazladır süregelen strangüri ve tekrarlayan hematüri nedeniyle İç Hastalıkları Anabilim Dalı Polikliniği'ne getirildi. Klinik muayenede kobayın zayıflamış, dehidre ve hipotermik olduğu belirlenirken, ayrıca kaudal abdominal palpasyonda ağrı tespit edildi. İdrar kesesi duvarının küçülmüş ve kalınlaşmış olduğu saptandı. Torakoabdominal radyografi ile gastrik dilatasyon ve idrar kesesi taşı saptandı. 2 gün sonra hayvanın ölmüş olduğu öğrenildi ve ardından nekropsi için Patoloji Anabilim Dalı'na getirildi. Nekropside idrar kesesinin lümeninde 0.7x0.5 cm boyutlarında idrar kesesi taşı tespit edilirken, idrar yolu kanalında gözle görülür belirgin bir değişim saptanamadı. İdrar kesesinin boş olduğu ve idrar kesesi mukozasının muhtemel bir kronik durum sonucu şekillenen fibrozis ve ödeme bağlı olarak kalınlaşmış olduğu belirlendi. İdrar taşının fiziksel ve kimyasal analizi laboratuvar tarafından yapıldı. Kalsiyum ve okzalatlar taş oluşumunda temel risk faktörleri olarak kabul edilmektedir. Buna rağmen, bu vakayı oluşturan hastanın idrar kesesi taşı kalsiyum karbonat, okzalat ve strüviti de içeren bir mineral kombinasyonundan oluşmaktadır. Olgunun Türkiye'de üriner belirtiler gösteren ve idrar kesesi içerisinde taş tespit edilen ilk kobay olduğu saptanmıştır.

Anahtar sözcükler: *Hematüri, Strangüri, Kobay*

INTRODUCTION

Urinary tract disease is relatively common in pet rodents. Diagnostic principles and management regimens for these species are essentially the same as those used for dogs and cats with urinary disease [1]. Guinea pigs *(Cavia porcelles)* and rabbits are susceptible to formation of urinary tract calculi. Urinary calculi may occur in guinea pigs, but it's a rather uncommon incidental finding. Clinical signs are observed if there is an obstruction or inflammation occurs. These clinical signs can include uremia, anuria or oliguria, pyuria, depression and anorexia. Hematuria may be observed even

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without obstruction [2]. Uroliths can be located anywhere in the urinary tract and are typically composed of calcium salts: calcium phosphate, calcium oxalate. The etiopathogenesis of uroliths in these species is poorly understood [3,4] but it is related to the urinary concentration of ions and crystals and crystal aggregation [3].

CASE HISTORY

A pet female guinea pig aged 5 years and weighing 0.5 kg was referred to Internal Medicine Department Clinics for inappetence, stranguria and recurrent haematuria over 15 days *(Fig. 1)*. The guinea pig was emaciated, dehydrated and hypothermic (T: 33°C, reference range: 37.2-39.4°C). The patient also demonstrated pain on caudal abdominal palpation and a small thickened bladder wall was discovered. Because of these findings, thoracoabdominal radiography was taken in laterolateral (LL) view. Gastric dilatation and urinary calculus was detected with radiography *(Fig. 2)*. Diagnosis was based on radiographic findings. Ultrasound could be inconclusive in this patient because there was a large amount gas in the gastrointestinal tract and the bladder was empty in palpation. Urine sample couldn't also be taken because the bladder was empty. General condition status was not also good enough to collect blood. Because of hypothermia, guinea pig was supported with fluid therapy and vitamins in intensive care unit of our department. Lactated Ringer solution were administered subcutaneously (SC) in a dosage of 10 ml, Vitamin B_{12} (0.1 ml SC) and Vitamin C (0.1 ml SC) were also administered intramuscularly (IM). After two hours, the body temperature was elevated (T: 36.0°C). After these therapies, it was learned that the guinea pig started to move and turned in a normal condition at the first day, but 2 days later, death was learned and it was brought for necropsy. Necropsy was done at the Department of Pathology. Necropsy revealed calculus in the lumen of urinary bladder with the dimensions of 0.7x0.5 cm though no distinct changes were grossly evident in the urinary tract *(Fig. 3)*. The bladder was empty and the mucosa was moderately thickened suggestive of fibrosis and edema resulting from a possible chronic condition. The kidneys were slightly enlarged with subtle border irregularity and mottled surface beneath the capsule. The stomach was

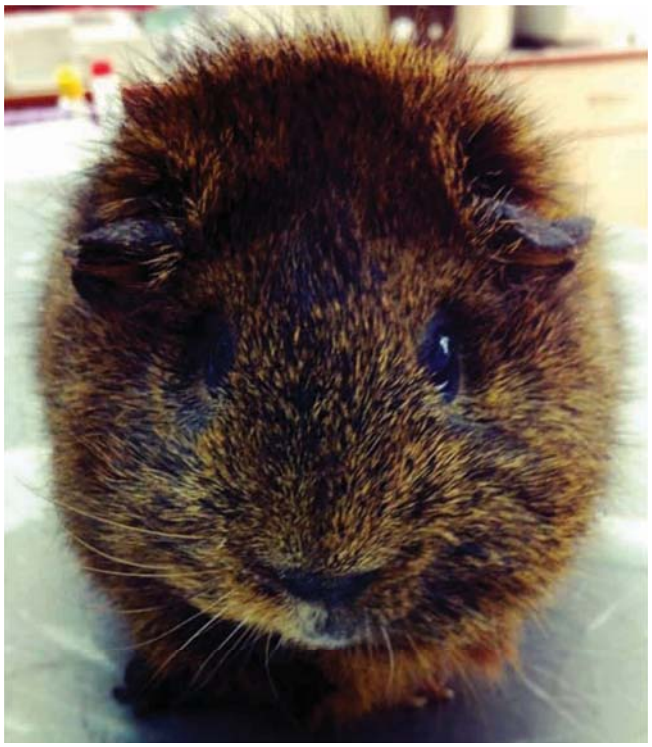

Fig 1. Guinea pig
Şekil 1. Kobay

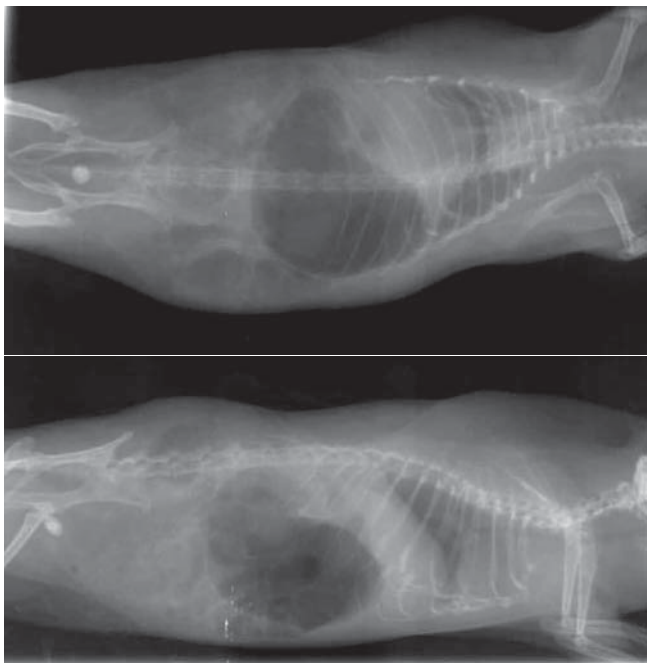

Fig 2. LL and VD radiographies of the patient where the calculus was clearly seen inside the urinary bladder
Şekil 2. Hastanın idrar kesesi içerisinde kalkülün belirgin olarak görüldüğü LL ve VD radyografileri

Fig 3. Urinary calculus of the guinea pig
Şekil 3. Kobayın üriner kalkülü

greatly enlarged though empty and filled with yellowish mucous fluid and the wall was observed to become quite thin suggestive chronic atrophic gastritis. The liver, lungs and the spleen were markedly congested *(Fig. 4)*. Tissue samples were collected from all visceral organs and particularly from the urinary tract and submitted for histopathology for further evaluation of the lesions. Physical and chemical analysis of the calculus was done at veterinary laboratory. Calculus material was submitted to the laboratory of Vetlab, Veterinary Analysis Laboratory, Avcilar, Istanbul for chemical analysis which was revealed ammonium, magnesium, phosphate, calcium, carbonate and oxalate reactions gave positive result. The urinary calculus was beige colored and its surface was rough.

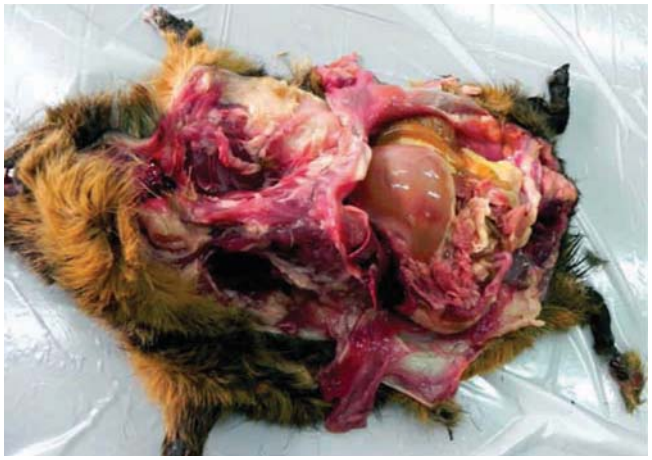

Fig 4. An in situ gross pathological examination of guinea pig revealed severely congested vital organs (the liver, the heart and the lungs), distended stomach and intestines

Şekil 4. Kobayın doğal durumunda bütün patolojik muayenesinde hayati organların (karaciğer, kalp ve akciğerler) konjeste olması, mide ve bağırsaklarda şişme

DISCUSSION

Urolithiasis is a common health problem in many species including guinea pigs. Historically, middle-aged to older females are believed to be overrepresented among affected guinea pigs [4]. Our patient was female and 5 years old.

In a retrospective study, they were systematically examined the organs of deceased or euthanized guinea pigs [5]. With this way, they found urinary calculi in 1 male and 5 female of 170 (74 male, 96 female) guinea pigs. It could correspond to an incidence of about 1.3-5.2% [5,6]. However, the reasons why females are predisposed to cystitis remain poorly understood. Most investigators believe that it is related to the difference in anatomical structure of the urinary tract between female and male. In female guinea pigs, the urethra is close to the anus, so that intestinal bacteria, especially gram-negative organisms, are more likely to enter the urethra and the urinary bladder where they may cause disease if conditions are appropriate [5].

Diet is also likely to play a role in calculi formation [1]. Our patient was being fed with a diet high in calcium and supplemented with greens rich in calcium. Intestinal hyperabsorption of dietary calcium may lead to excessive excretion of calcium in the urine. Calcium and oxalates are the main risk factors for stone information in the guinea pig [1]. These calculi are radiodense and easy to identify radiographically. The calculus of the guinea pig was seen clearly with radiography.

The stones consisted of two or more minerals in examinations. Against this in dogs more than 60%, in humans only 28% of the stones are monomineralic. The components of stones agree with those, described in literature, similar to rabbits, minerals containing calcium are predominant [6]. Our case's calculus consisted different types of minerals: ammonium, magnesium, phosphate, calcium, carbonate and oxalate.

In addition, this region is often ignored in physical examination and clinical symptoms are often missed. Female guinea pigs with urinary clinical symptoms should be exactly examined. Most of the bacteria are normally washed away before they invade the urethral mucosa with urine passage. The injury of mucosa and stagnation of urine are the important predisposing factors [5]. Urolithiasis can be very subtle and should be on the differential diagnosis list for any guinea pig that presents "sick" [7].

Although it is known that calcium and oxalates are the main risk factors for stone information in guinea pigs, the calculus of the guinea pig included calcium carbonate and oxalate in combination with struvite. Finally, urinary tract disorders in guinea pigs may show the same symptoms like many of our pet species. Diagnostic tools and general therapies used in small animal practice are appropriate for the pet rodent.

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CASE REPORT

A Case of Complicated Sole Ulcer and Its Treatment in A Calf

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Summary

In this report, a case of complicated sole ulcer and its treatment in male, aged of 1.5 months, Holstein calf has been subjected. According to radiologic and clinical findings, it was complicated with purulent arthritis in coffin joint, osteophyte formations 1st, 2nd, 3rd phalanx, and tendinitis of profound and superficial tendons. After two months from the beginning of the treatment, calf was able to walk without any lameness and there was no pain symptom on palpation. The aim of this case report was to contribute to the literature data for complicated sole ulcer that can be also seen in cows.

Keywords: *Calf, Sole ulcer, Treatment*

Bir Buzağıda Komplike Taban Ulkusu Olgusu ve Sağaltımı

Özet

Bu gözlemde 1.5 aylık erkek Holstein ırkı bir buzağıda karşılaşılan komplike taban ülseri olgusu ve sağaltımı konu edildi. Radyolojik ve klinik bulgularda olgunun ayak ekleminin purulent yangısı, 1. 2. ve 3. falankslarda osteofitik üremeler, profund ve süperfisiyal tendoların yangısı ile komplike olduğu görüldü. Tedavi başlangıcından 2 ay sonra buzağı topallamadan ayağını kullanabildi ve lezyonlu bölgede ağrı yoktu. Bu olgu sunumunun amacı sığırlarda sıklıkla gözlenen taban ülserlerine ilişkin bilgi birikimine katkı sağlamaktır.

Anahtar sözcükler: *Buzağı, Solea ülseri, Sağaltım*

INTRODUCTION

Sole ulcers are generally observed in highly efficient and heavy-bodied adult dairy cows and it is most common foot-hoof diseases [1-4]. Lack of stall hygiene, nutrition, laminitis, gestation and calving, incorrect hoof trimming, hoof deformities, biomechanical factors, walking on small stony floors, sharp objects, and loss of quality in hoof production are the main causes of sole ulcers [1,4-6]. Sole ulcers are usually located in the region of the sole/bulb junction, nearer the axial margin compared to abaxial one, and seen as lesions with around 1-1.5cm diameter on the lateral claw of hind limbs. They are classified as superficial or complicated (deep) sole ulcers. Because of the insufficiency of immune response and lack of hoof trimming procedure and delayed diagnosis, superficial sole ulcers are developed as complicated sole ulcers. Coffin joint, profound tendon and its sheet, navicular bone and bursa, and coffin bone, a few or all of them are effected during complicated sole ulcers [4,6]. Sole ulcers are the mostly encountered in hoof lesions of lame cows. These cows generally lag behind the herd while they are rushing for feeding and milking parlor; also they usually prefer to lie down. Cows loose bodyweight and their milk yield decreases [2,4,7]. Severe lameness, swelling in the digit (if tendons effected swelling can developed at the level of distal metacarpals or metatarsals), asymmetry between digits are evident in complicated sole ulcers [4]. These incidents cause serious economic losses [2,7]; Therapeutic hoof trimming increases the effectiveness of the treatment. During hoof trimming, pressure on the lesion must be eliminated and necrotic tissues must be removed. Body weight must be transferred to the unaffected claw by means of plastic slippers, wooden or plastic blocks [4,6,8]. Intravenous regional antibiosis can also be effective [6]. Partial resection of profound tendon and navicular bone, arthrodesis of coffin joint, amputation of effected digits; are considered as operative techniques applied for the treatment of complicated sole ulcers [4,6,8].

Sole ulcers are usually reported in high milk yielded cows [4,6,8,9], and it causes lameness. As lameness an important

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problem for adult dairy cows, little attention is paid to lameness in the calves. Infectious arthiritis, congenital joint and/or tendon deformities, genetic diseases of the limbs (mule foot, polydactylism, dactylomegaly, marfans and osteogenesis imperfects), dystocia and calving-related injuries, injection site paresis, and feeding defects can be a causes for lameness in calves. In our knowledge there is no report about lameness caused by sole ulcers in the calves. This complicated sole ulcer case is deemed as useful, since it was encountered on a calf.

CASE HISTORY

A male, aged of 1.5 months, Holstein calf was brought to our clinic due to lameness. According to the anamnesis, the calf's left front leg has been started to lame a month ago and foot gradually swollen. Clinically, left front leg was suffering from lameness at moderate to severe level. In front view, medial phalanx was swollen towards fetlock joint and medial hoof turned too medially. Ulcer was measured 5mm in diameter lesion and purulent discharge was seen on the sole-bulb junction with severe pain in palpation. Forceps, inserted through lesion, was head towards approximately 3-4 cm to coffin joint; there was cavity and forceps touched to the bone tissue *(Fig. 1c)*. Based on these findings, it was considered that coffin joint degradation has been developed. Furthermore, there were signs of pain during palpation of both profound and flexor tendons at level of distal metacarpus. Increased volume of soft tissue of medial digit and osteoarthritis of coffin joint were apparent in radiographs. Significant osteophyte formations were identified on the medial side of first phalanx, both sides of second and third phalanx in radiographs *(Fig. 1b)*. According to radiological and clinical findings, the case was assessed as complicated sole ulcer. It was complicated with purulent arthritis in coffin joint, osteitis in 1st, 2nd, 3rd phalanx with osteophyte formations, and tendinitis of profound and superficial tendons.

The calf was sedated with 0.1mg/kg im Xylazine HCl (Alfazyne®, Egevet). During clinical examination of the foot, suppuration discharge was observed from the lesion. Lesion cavity was irrigated *(Fig. 1d)* via catheter with 10% povidon – iodine solution (Betakon®, Aroma). Irrigation was continued until clean solution came back from the lesion cavity. Then, coffin joint and bone ends forming the joint were curetted and cavity was irrigated again. Crystal penicilline 1.000.000 IU (Penicilin G®, İ.E. Ulugay) was injected in the lesion cavity. Foot was dressed with antiseptic wet compress with 0.1% ethacridine lactate solution (Rivanolum EPG®, Galenik). Amoxicillin - clavulanic acid (Synulox®, Pfizer) was applied and recommended as parenterally (1 ml/20 kg/12 h). The owner was advised to apply wet compress with antiseptic solution for three times a day. For a three-week period, anticeptic wet dressing were replaced one a week and irrigation was applied. At the end of three weeks, purulent discharge was disappeared, and size of

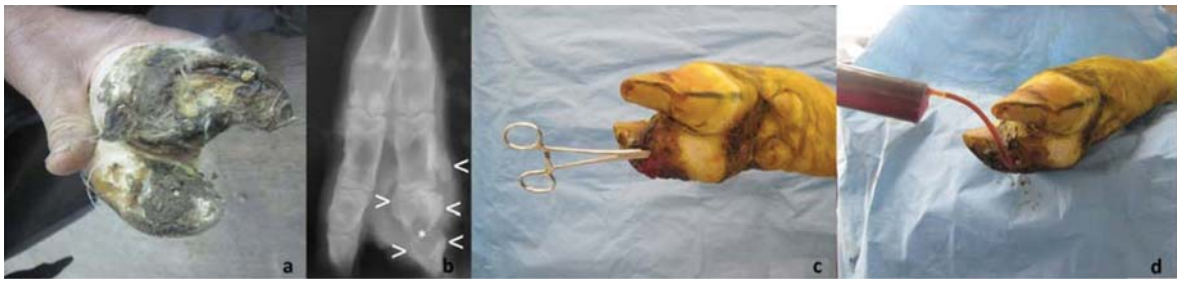

Fig 1. a- First glance of the sole, **b-** X-Ray image of the effected foot (>: osteophyte formations, * osteolysis in the coffin joint), **c-** Forceps, inserted through the lesion, **d-** Irrigation of the lesion cavity

Şekil 1. a- Tabanın ilk görünümü, **b-** Etkilenmiş ayağın radyografik görünümü (>: osteofitik üremeler, * Art. interphalangea distalis'te osteoliz), **c-** Lezyondan içeriye ilerletilen hemostatik pens, **d-** Lezyon boşluğunun irrigasyonu

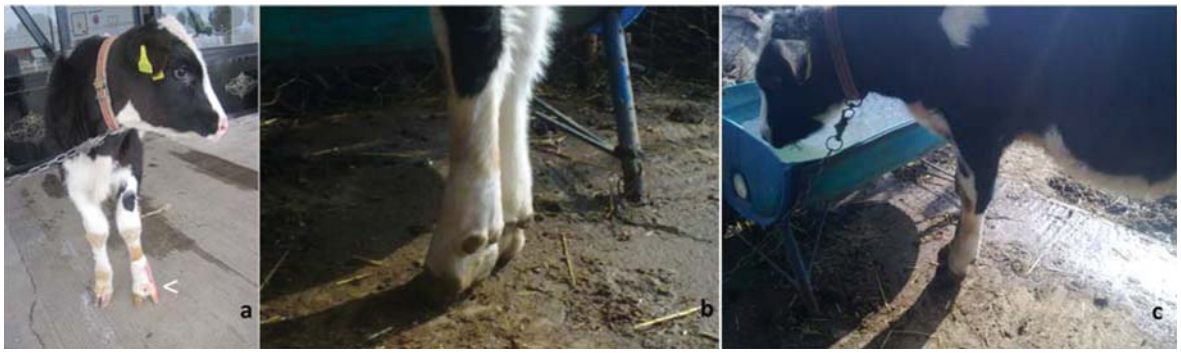

Fig 2. a- Sixth week of the treatment (<: effected foot), **b** and **c-** After two months views of the case, taken by owner

Şekil 2. a- Tedavinin altıncı haftası (<: etkilenmiş ayak), **b** ve **c-** Olgunun iki ay sonraki görüntüleri, hasta sahibi tarafından çekilerek gönderildi

the lesion decreased; however there was still mild pain. At the end of the sixth week *(Fig. 2a)*, there was slight pain on lesion and no lameness; also swelling was decreased. Foot was dressed again for protection of the lesion region. The calf was brought from another city to our clinics and the owner had difficulties about it. Therefore, it was suggested to owner that, it is required to open the dressing and applying juniper or pine tar to the hoof, by himself. After two months from the beginning of the treatment, calf was able to use its leg without any lameness; and there was no any sign of pain on the lesion area as it is learned from the owner by telephone dialogue. At this period, photos (*Fig. 2b* and *2c*) were taken by the owner and submitted to us.

DISCUSSION

Sole ulcers are generally observed in adult dairy cows due to environmental, nutritional and infectious reasons [1-4]. In this presentation, we considered that a trauma caused by a sharp object can be possible reason of complicated sole ulcer in a 1.5-months-age calf. Beside, sole ulcers are more frequently encountered in the medial hoof than lateral in front limbs [6]; this is similar with this case.

All clinical signs of the reported case were similar with in adult cows. Sole ulcers may be complicated with navicular bursitis, tendinitis, arthritis and/or osteitis [4,6]. In this case, tendinitis determined with palpation and osteoarthritis of the coffin joint was determined on the radiographs. After inserting forceps through to the lesion, a cavity was identified and it had been developed till to the bone tissue; thus, it gave the impression of coffin joint degradation with neighboring tissues.

In this presentation, the case was treated as described for adult dairy cows [4,6,8,9]. However we were not able to apply plastic slipper on unaffected hoof, because slippers did not fit the hoof. It is reported [4] that uncomplicated sole ulcers could be treated in 45 days (6 weeks); and complicated sole ulcers required longer period of time compared to superficial ulcers encountered in dairy cows. In this presented case report, at the end of the sixth week, there was symptom of slight pain on the lesion but no lameness observed. After two months, it was reported by the owner during the telephone inquiry that the calf was completely healed.

As a result, although complicated sole ulcers encountered in adult cows, in our knowledge there is no report lameness cases related to the sole ulcers in the calves. In this report, the treatment of the case was performed in same as cows, which takes part in classical books and in literature. Sharing this case is deemed as useful for treatment of complicated sole ulcer that can also be seen in calves as a cause of lameness.

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CASE REPORT

Bilateral Malignant Seminoma in Two Dogs

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Summary

In this report, the cases of bilateral diffuse malignant seminoma encountered on testes of 12 and 14 years old, male, cross-breed two dogs were evaluated clinically and pathologically. Both cases were referred to surgery clinic with the complaint of swelling in scrotum. Through microscopic examination, bilateral diffuse malignant seminoma was diagnosed in both cases.

Keywords: *Dog, Scrotal enlargement, Malignant seminoma*

İki Köpekte Bilateral Malign Seminom

Özet

Bu gözlemde, 12 ve 14 yaşlı, erkek, melez iki köpekte karşılaşılan bilateral malign seminom olgusu klinik ve histopatolojik olarak değerlendirildi. Her iki olgu da skrotumda büyüme şikayeti ile cerrahi kliniğine getirildi. Mikroskobik incelemede her iki olguya da bilateral diffuz malign seminom tanısı konuldu.

Anahtar sözcükler: *Köpek, Skrotal büyüme, Malign seminoma*

INTRODUCTION

Scrotal enlargement occurs in dogs when testis tumour, hernia scrotalis, funiculus spermaticus torsion, spermatocele, hydrocele, and orchitis cases are evident [1-3]. Testis tumours usually occur in dogs at advanced age (mean 10) [4-6], and they are classified into three groups, namely gonadostromal tumours (interstitial-Leydig cell and sertoli cell tumours), germ cell tumours (seminoma, teratoma, and embryonal carcinoma) and other tumours (mesothelioma and vascular tumours) [4,5]. While seminomas account for 33% of testis tumours in dogs, they develop unilaterally and are mostly benign, and to a lesser degree in malignant [4,5,8,9]. In its malignant form, its common symptoms are enlargement of testis, bleeding and necrosis [5].

The aim of this report is to describe clinical course, and to present istopathological evaluation results of bilateral malignant diffuse seminoma cases observed in the two dogs.

CASE HISTORY

The first case was a cross-breed, male, 12-year-old dog whose weight was 26 kg. Regarding its history, it is an indoor dog and it was not taken out while there was enlargement experienced in scrotum within the last year, which progressed to the degree that it would limit its movement ability. In terms of clinical symptoms, the scrotum causes difficulty walking such that the dog was unable to step on hind legs which led unwillingness for walking. Through palpation, it was determined that the scrotum was quite large *(Fig. 1A)* with soft consistency. There was no pain evident during palpation of the funiculus spermaticus and testis. Scattered ulcerative areas were seen on the scrotum.

The second case was a 14-year-old, croos-breed dog whose weight was 24 kg. In the anamnesis, the dog was taken out, scrotal enlargement developed within the last 6-8 months and finally it was referred to our clinic. Clinically, a marked volume increase was observed in the right half of the scrotum compared to the opposite half. The testis with hyperthropy and at hard consistency and funiculus spermaticus were felt during palpation and there was no symptom of pain. Dog was able to walk as usual. For

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both cases, brucella results were negative based on the agglutination test. During the ultrasonographic and the radiological examinations, no pathological finding was observed in the abdomen and thorax regions of the animals. As a result of the clinical, radiological and laboratory examinations, it was decided to remove scrotal tissue and testis together in the first case; and only the testis in the second case. For the operation, the dogs were sedated with 1 mg/kg intramuscular Xylazine HCl injection (Alfazine® Egevet, Turkey). Then, 4 mg/kg intravenous propofol (Propofol 1%® Fresenius, Sweeden) was administered for induction. With intubation, anesthesia was maintained at 1.5-2% concentration isofloran (Isoflurane® Adeka). The funiculus spermaticus were accessed with a blunt dissection. Occurred hemorrhagies *(Fig. 1B)* were controlled by means of hemostatic forceps and ligatures (2/0 polyglactin 910, Vicryl®, Ethicon, Edinburg). Testes were removed by placing hemostatic forceps approximately 1 cm below the trans-fixation ligatures and by cutting between ligatures and hemostatic forcep, and then the scrotum was removed with incision through the incision line. The incision line on the regional skin was closed with simple sutures (2/0 silk suture, Silk® Kruuse); and the operation was completed *(Fig. 1C)*. In the second case, the orchiectomy operation was performed. After the operation, the tissue samples obtained from the testes and scrotum were fixed in 10% neutral buffered formalin solution; embedded in parafin wax, sectioned at 5 µm, and stained by routine methods with haematoxylin and eosin (H&E) and examined under the light microscope [6].

Macroscopically, in the first case, while the left testis was at the size of 5.9x3.9x3.4 cm and its weight was 60 g, the size of the right testis was 3.6x3.4x2.4 cm and its weight was 39.2 g. A homogenous appearance was evident on the sections of both testes but grayish-white multilobular areas were located in all of left testis while the diameter of the right testis was 1.3 cm *(Fig. 2A)*. The weight of the scrotum removed with testis was 1.900 g and its size was 22x 23x8 cm. Its exterior was covered with skin and its texture was presenting soft consistency. Homogenous structure of the srotum section was quite edematous with grayish white color *(Fig. 2B)*. In the second case, the size of the right testis was 12x7.5x3.8 cm and it was weighted 300 g while the size of the left testis was 5.4x4.9x3.4 cm and it was weighted 65 g. The exterior of both testes were presenting nodular appearance; and at their cross sections, the multilobular grayish white areas were in various sizes with well defined borders *(Fig. 2C)*. In terms of microscopic examination, the tumour tissues in the testes of both cases were consisted of anaplastic cells diffusely spread in various sizes. These cells were in polyhedral shape with sharp edges, vasicular nuclei, which was usually about 1-2 nucleoli, and slightly basophilic cytoplasm *(Fig. 2D)*. The number of mitotic figures in tumour area was between 2 and 3. In many areas, single or multi nucleated tumour type giant cells can be observed. In view of pathological findings, both cases were diagnosed as malignant diffuse seminoma. In the first case, together with edema in the dermis layer of scrotum, the hyperemia in vessels and the inflammatory cell infiltrations consisting of plasma cells and lymphocytes were remarkable.

DISCUSSION

In animals, hernia scrotalis, funiculus spermaticus torsion, spermatocele, hydrocele, orchitis and testis/testes tumours are the most common causes of scrotal enlargement [2]. In the first case, both testis had normal anatomical structure, funiculus spermaticus torsion, spermatocele and hydrocele were not present and no finding regarding infection was found in microbiological and pathological examinations, which suggested that scrotal enlargement was not associated with infection. The facts revealed as a result of the histopathological examination that the tumour was formed in both testes, and that the dermis was quite edematous, suggested that the enlargement in scrotum was a complication of the existing tumour.

For the malignant seminoma cases with metastasis, radiotherapy and chemotherapy are recommended; and for the cases without metastasis, castration is referred [7]. In the first case, testes were removed together with the scrotum while only testes were removed in the second case. During the first operation of the first case, abnormal vascularization and bleeding were observed until funiculi spermaticus were reached after the incision. Bleeding was controlled through hemostatic pens, cauter and ligatures.

Fig 1. A- Appearance of the scrotum before the operation, **B-** Apperance of the testes removal from the inside of scrotum (*arrowhead:* left testis, *arrow:* right testis, ***:** skrotum), **C-** Postoperative incision line (case no 1)

Şekil 1. A- Operasyon öncesi skrotumun görünümü, **B-** Skroum içerisinden çıkarılan testislerin görünümü (*ok başı:* sol testis, *ok:* sağ testis, ***:** skrotum), **C-** Postoperatif ensizyon hattı

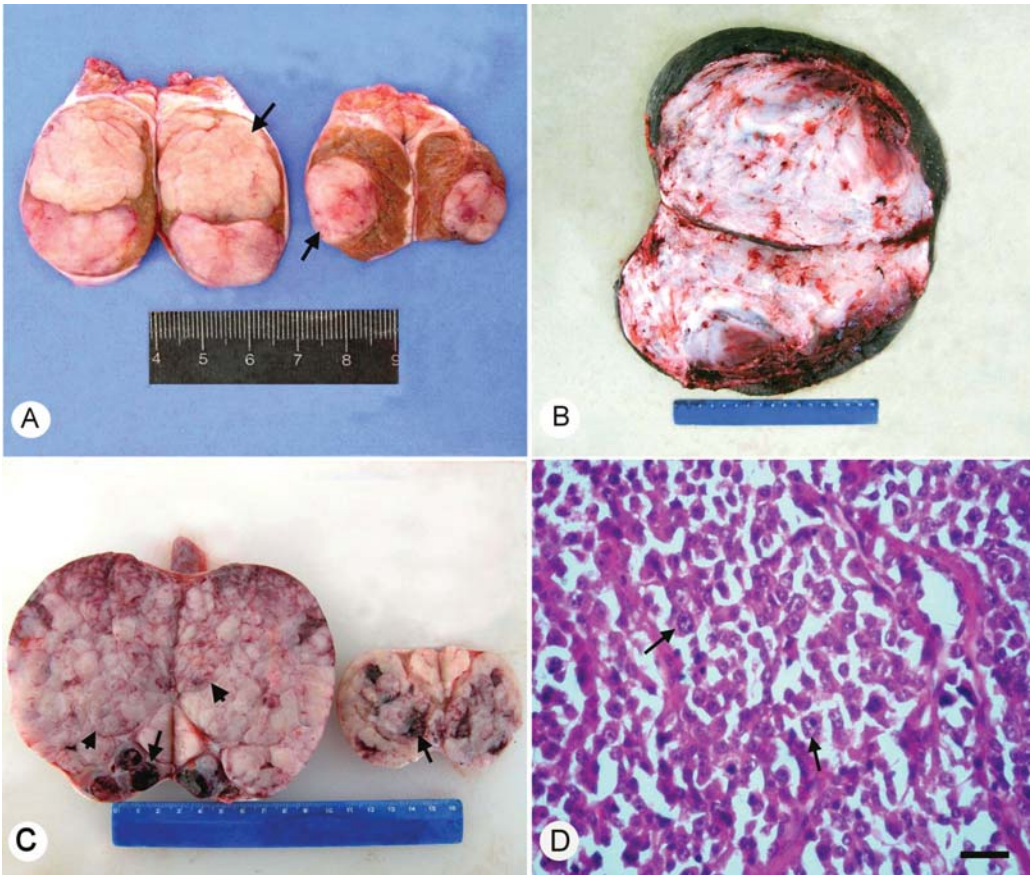

Fig 2. A- Multilobular grayish white areas at various sizes in the cross section of the both testes, (case no; 1), **B-** Macroscopic appearances of the cross section of the scrotum (case no; 1), **C-** Multilobular grayish white areas at various sizes *(arrows)* with bleeding areas *(arrows)* in the cross section of the both testes (case no; 2), **D-** The tumour was consist of had polyhedral shape, sharp edges and vasicular nuclei with slightly basophilic cytoplasm (case no; 1, right testis, arrows), HE. Bar: 50 µm

Şekil 2. A- Her iki testsin kesit yüzünde değişen büyüklüklerde boz beyaz renkte alanlar (olgu no: 1), **B-** Skrotumun kesit yüzünün makroskobik görünümü (olgu no: 1), **C-** Testislerin kesit yüzlerinde değişen büyüklüklerde boz beyaz renkte alanlar *(ok)* ile birlikte kanamalar *(oklar)*, (olgu no: 2), **D-** Tümör dokusunda diffuz dağılımlı, değişen büyüklüklerde, polihedral şekilli, keskin kenarlı, veziküler çekirdekli, hafif bazofilik sitoplazmalı anaplastik hücreler (olgu no: 1, sağ testis, o*klar*), HE. Bar: 50 µm

During the operation of the second case, no abnormal bleeding was observed. It was concluded that in cases similar to first one, in which marked scrotal enlargement is present and testis are decided to be removed, some precautions must be taken before the operation againist excessive bleeding which may occur during operation.

Seminomas arise from the germ cells that constitute the spermatogenic epithelium within the seminiferous tubules, and are subdivided on the basis of their histological appearance into intratubular and diffuse types [5,12]. The tumors in both cases, in the present study, was diagnosed as a tubular diffuse type malignant seminoma based on histopathological patterns [5,12,13]. Seminomas are classified as benign or malignant according to pleomorphic changes, mitotic activity and metastatic characteristics [8-10,13]. In both cases, although pleomorphic changes are evident, testis tumours in both cases were evaluated as malignant seminoma based on these characteristics and that mitotic activity was found rather low [7-9,11]. Although malignant seminomas are reported to metastasis in many cases [5,14,15], no such finding was seen before operation and during the examination on the 6th month.

Based on evaluation of the clinical and histopathological findings, it can be concluded that bilateral seminoma cases that do not metastasize in spite of long development process were found significant to be reported; and uncomplicated postoperative healing was emphasized.

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CASE REPORT

Granulosa Theca Cell Tumor in An Arabian Mare: Are Immunohistochemically Loss of GDF-9 and BMP-6 Proteins Associated with High GATA-4, Inhibin-α, AMH Expressions?

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Summary

Granulosa-theca cell tumor (GTCT) in an Arabian mare was diagnosed by clinical, pathomorphological and immunohistochemical (IHC) examinations. Immunohistochemically, it was tried to clarify the possible roles of Transforming Growth Factor–β superfamily members [Growth Differentiation Factor-9 (GDF-9), Bone Morphogenetic Protein-6 (BMP-6), anti-Müllerian Hormone (AMH) and inhibin-α], GATA family members (GATA-4 and GATA-6) and Insulin-like Growth Factor family (IGF-1 and IGF-2) on GTCT and results was compared with two negative control ovarian tissues. Moderate positivities with AMH, inhibin-α, IGF-2, GATA-4, and mild positivities with IGF-1, GATA-6 were obtained whereas no positivity could be shown with GDF-9 and BMP-6 in neoplastic ovarian tissue. Additionally, mild positivities were obtained with all markers in control stainings. Further molecular studies for transcription factors regulating activation of genes in response to mitogenic and stress signals in equine GTCT tumorigenesis are needed to elucidate whether the high GATA-4, AMH, and inhibin-α immunopositivities play a significant role on GDF-9 and BMP-6 deficiency.

Keywords: *Mare, Granulosa-Theca Cell Tumor, GATA-4, GATA-6, AMH, Inhibin, GDF-9, BMP-6, Immunohistochemistry*

Arap Kısrakta Granuloza-Teka Hücre Tümörü: İmmunohistokimyasal Olarak GDF-9 ve BMP-6 Protein Kayıpları Yüksek GATA-4, İnhibin-α ve AMH Ekspresyonları İle İlişkili mi?

Özet

Bir Arap kısrağında granuloza-teka hücre tümörü (GTHT); klinik, patomorfolojik ve immunohistokimyasal (İHK) incelemelerle teşhis edildi. İmmunohistokimyasal olarak GTHT'de; Transforming Growth factor-β ailesi [GDF-9 Growth Differentiation Factor-9 (GDF-9), Bone Morphogenetic Protein-6 (BMP-6), anti-Müllerian Hormone (AMH) ve inhibin-α], GATA ailesi (GATA-4 ve GATA-6) ve Insulin-like Growth Factor ailesi (IGF-1 and IGF-2) üyelerinin olası rolleri ortaya konmaya çalışıldı ve bulgular iki negatif kontrol ovaryum dokusuyla karşılaştırıldı. Neoplastik ovaryum dokusunda AMH, inhibin-α, IGF-2, GATA-4 orta derecede, IGF-1, GATA-6 ise hafif derecede pozitif sonuç verirken GDF-9 ve BMP-6 pozitiflik göstermedi. Ayrıca kontrol grubu dokuların boyamalarında tüm belirteçler ile orta derecede pozitif sonuçlar elde edildi. Kısraklarda GTHT tümörogenezisinde yüksek GATA-4, AMH ve İnhibin-α düzeylerinin, GDF-9 ve BMP-6 eksikliğindeki önemli rolünü belirlemede mitojenik ve stres sinyallerine yanıttaki genlerin aktivasyonundan sorumlu transkripsiyon faktörlerinin de yeni moleküler çalışmalar ile incelenmesi gerekliliğini ortaya koymuştur.

Anahtar sözcükler: *Kısrak, Granuloza-Teka Hücre Tümörü, GATA-4, GATA-6, AMH, İnhibin, GDF-9, BMP-6, İmmunohistokimya*

INTRODUCTION

Granulosa-theca cell tumor (GTCT) is one of the most common ovarian neoplasm found in the mare [1-4] and represents 2.5% [5,6] to 5.6% [3,7] of equine neoplasms. GTCT is a steroidogenic tumor in domestic animals [1,3,4,8,9] which

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originates from the sex cord or specialized ovarian stroma [6]. Diagnosis is based upon history, findings on clinical examinations, macroscopical and microscopical evaluations of excised tissue [4,7].

Transforming growth factor-β (TGF-β) superfamily members are extracellular signaling molecules which regulates biological processes as cellular growth, differentiation, motility, and apoptosis. TGF-βs are subdivided into TGF-βs/Activins/Nodals, and Bone Morphogenetic Proteins (BMPs)/Growth Differentiation Factors (GDFs)/Anti-Müllerian Hormone (AMH) or Müllerian Inhibitory Substance (MIS) [10]. TGF-β superfamily members have important efficacy in intra-ovarian molecular interactions. These proteins play active roles in primordial follicle development, granulosa and theca cell proliferation, and follicular atresia, development of gonadotropin receptors in somatic cells, oocyte maturation, ovulation, luteinization, and corpus luteum formation [11,12].

Inhibins are proteins produced by gonads which act in an endocrine manner to inhibit follicle stimulating hormone (FSH) synthesis and secretion from pituitary [13]. Subtype inhibin-α is expressed from most of GTCTs and is used as a marker as AMH [14].

GATA family (GATA-1 to-6) is a member of zinc finger transcription factors, and GATA-1,-2,-4,-5, and -6 have been implicated to reproductive development or function in mammals [15,16]. Expression of some essential gonadal genes is regulated by transcription factors GATA-4 and GATA-6 [14].

Insulin-like growth factors (IGFs) are peptides which are related to proinsulin. IGFs regulate mitosis, differentiation, and growth as well as survival of cells when FSH concentrations are declining or low. IGFs regulate follicular growth and also promote the actions of gonadotropins on ovary. IGF-I plays important role in folliculogenesis. IGF-II stimulates thecal cell steroidogenesis, thereby determines the ability of androgen synthesis of thecal cells [17].

Although the molecular pathogenesis of GTCT in mares have not been fully understood, defects in ovarian and intrafollicular paracrine/autocrine signaling pathways have been considered to be important. However, the influence of AMH and inhibin-α on equine GTCT have been studied recently [18] as in human medicine, the effects of other growth and differentiation factors on tumoral pathobiology are not clear. Therefore, the aim of this study was to search the availability of immunolabeling some TGF-β superfamily, IGF, and GATA family members (GDF-9, BMP-6, AMH, inhibin-α, GATA-4, GATA-6, IGF-1, IGF-2) and to discuss the possible role of these proteins in pathogenesis of GTCT in mares as a first report.

CASE HISTORY

An 8-year-old, non-parity Arabian mare was referred to Equine Hospital with complaints of anestrus, lameness, and aggressive behaviour. On rectal examination, the right ovary was identified as a rough mass; approximately 15 cm in diameter, the left ovary was detected to be firm and inactive. Transrectal ultrasonographic examination revealed irregularly shaped multicystic areas with a honeycomb appearance. The left ovary confirmed to be small and inactive. A presumptive diagnosis of GTCT was made. Ovariectomy via a right ventral, diagonal paramedian approach was performed under general anaesthesia. The gross and histopathological examinations of the tumor were performed after removal of affected ovary.

Two healthy ovaries were used as negative controls in immunohistochemical evaluations. Control ovaries were obtained from normally cycling but humanely euthanized mares due to incurable emergency conditions in accordance with the decision of authorized veterinarian.

Materials were evaluated macroscopically and tissue samples taken from ovary were fixed in 10% neutral buffered formalin. Tissue samples were evaluated with routine tissue process and embedded in paraffin. Tissue sections were stained with Hematoxylin-Eosin (H&E) and IHC.

For immunohistochemistry, Avidin-Biotin Complex Peroxidase (ABC-P) was applicated to tissue sections. The deparaffinized and dehydrated sections were kept for 3 min in microwave 700 W with 0.1 M citrate buffer as antigen retrieval solution, and hold in 3% hydrogen peroxide-methanol solution for 20 min. After blocking with non-specific blocking sera (DAKO, X1010) at 4°C overnight, the sections were incubated with polyclonal primary sera [GDF-9, BMP-6, AMH, inhibin-α, IGF-I, IGF-II, GATA-4, GATA-6, *(Table 1)*] at the same conditions and time. After washing with PBS, the sections were incubated for 45 min with biotinylated goat anti-rabbit antibodies at room temperature. After washing with PBS again, the immune complexes were detected by secondary antibodies marked with horseradish peroxidase (HRP) (DAKO LSAB+System HRP- kit, cat. no: K0679). DAB chromogen for revealing the reaction (Cat no. RE7105 and RE7106, Novocastra Lab.), and Mayer's haematoxylin for counter staining was used. Negative control sections were treated as described above by being exempted from primary antibodies. All results were evaluated under light microscope (Leica, DM 4000B) and imagined by camera attachment (Leica, DFC-420). Positivities which obtained from all primary sera were counted on 10 different microscopic fields at x100 magnification. The immunopositivities were scored as: 0 (no reaction), 1+ (mild), 2+ (moderate) and 3+ (strong).

The diagnosis of GTCT was confirmed by macroscopical and histopathological examinations of the excised tissue. Macroscopically, the ovary was weighed of 827 g. and measured 18x11x8.5 cm in diameter. Its consistency was generally firmness but some areas was fluctuated. Its surface was roughly, well vascularizated and tan yellow coloured. On cut surface, there were come across multiple

Table 1. *Antibody panel of primary sera*
Tablo 1. *Temel serumların antikor tablosu*

Primary sera	Optimal Dilution	Catolog Codes
Polyclonal goat anti mouse GDF-9	1:100	Santa Cruz Biotechnology, C-20, sc-7407
Polyclonal goat anti mouse BMP-6	1:100	Santa Cruz Biotechnology, S-20, sc-27408
Polyclonal goat anti mouse AMH	1:100	Santa Cruz Biotechnology, C-20, sc-6886
Polyclonal goat human inhibin-α	1:100	Santa Cruz Biotechnology, T-17, sc-22048
Polyclonal goat anti mouse IGF-I	1:100	Santa Cruz Biotechnology, G-17, sc-1422
Polyclonal goat anti mouse IGF-II	1:100	Santa Cruz Biotechnology, N-20, sc-1415
Polyclonal goat anti mouse GATA-4	1:100	Santa Cruz Biotechnology, G-4, sc-1237
Polyclonal goat anti mouse GATA-6	1:100	Santa Cruz Biotechnology, G-6, sc-7244

fluctuated cyst, seized from 0.3 mm to 2 cm in diameter and filled with yellowish fluid. Histopathologically, sections were generally composed of neoplastic granulosa cells with hyperchromatic nuclei and polygonal shape. These neoplastic cells were surrounded by compact fibrous stroma, contained also theca cells.

Immunohistochemically, AMH and inhibin-α were more dense in cytoplasms of neoplastic granulosa cells *(Fig. 1-a,-b)*. Furthermore, IGF-2 and GATA-4 with moderate positiveness (> 50%/10 microscope areas) were also observed in both granulosa and theca cells *(Fig. 1-d,-e)*. However, it was not attended to any positive reaction with GDF-9 and BMP-6 in neoplastic cells, mild positivities (20-50%/10 microscope areas) with IGF-I and GATA-6 markers were detected in neoplastic granulosa and theca cells, respectively *(Fig. 1-c,-f)*.

In negative control sections, mild positivities (20-50% /10 microscope areas) were obtained from whole antibodies which mentioned above in follicular epithelial cells, thecal cells and stromal components in few areas.

DISCUSSION

The oocyte plays an important role in growth and differentiation of the follicle and in directing its own fate [19]. Gene expression profiles of GTCTs in women found to be similar to that of granulosa cells of preantral and small/medium antral follicles [14]. This situation may be similar in another monovular species, in mares too.

Among BMPs, BMP-6 is demonstrated to be strongly expressed in granulosa cells of tertiary follicles and oocytes in human. BMP-6 is found to stimulate gene expression of inhibin/activin βA and βB subunits but not inhibin-α subunit in cultured human granulosa cells. BMP-6 is also found to stimulate mRNA expression of FSH receptor and AMH. Follicles with high BMP-6 expression may be more likely to survive FSH decrease in serum and to reach dominant follicle stage [20]. GDF-9 is an oocyte-derived factor which plays important roles in primordial follicle recruitment, granulosa and theca cell proliferation/atresia, steroidogenesis, oocyte maturation, ovulation, luteinization, and corpus luteum formation [19]. GDF-9 promotes follicular growth and synergistically inhibits FSH-induced granulosa cell differentiation in primates, ruminants and rodents. It is also demonstrated that follicular development was improved when GDF-9 function was upgraded, whereas ovarian dysfunctional follicular abnormalities leading to infertility were detected when GDF-9 expression was reduced [21].

In the case, expressions of BMP-6 and GDF-9 in GTCT cell lines were not observed in contrast to positivities in controls. It was thought that the ovary quietly progressed in the way of neoplastic changes, and tumor cells were not under effects of growth and differentiation stages of follicles. As BMP-6 is present in healthy tertiary follicles and GTCTs are mostly consisted of small and multicystic follicles, it may be expected to show low immunopositivity. Deficiency in GDF-9 may play a role in tumoral development in this case because of emerging abnormalities on granulosa and theca cells in its insufficiency.

In recent studies, immunolabeling for AMH was not detected in small, primordial follicles with a single layer of flattened granulosa cells, but was detected in follicles consisted of granulosa cells with more than one layer in normal equine ovaries. AMH immunolabeling increased in antral follicles with multiple layers of granulosa cells. The intensity of AMH immunolabeling was decreased in antral follicles with diameter >30 mm compared to small antral follicles. Follicles undergoing atresia had a slight AMH immunolabel, while atretic follicles without granulosa layer did not. Corpora lutea also did not show AMH immunolabeling [18,22].

In this case, moderate positivities with AMH were detected in neoplastic granulosa cells. The result obtained from GTCT with small and multiple cystic structures were compatible with the abundant expressions of AMH in healthy preantral and small antral follicles. This similarity is thought to be related with size and number of follicles. Ball et al.[18] reported that AMH immunostaining may be a useful tool for detection of GTCT in mares because of high AMH expression characteristics of such tumors. Elevation

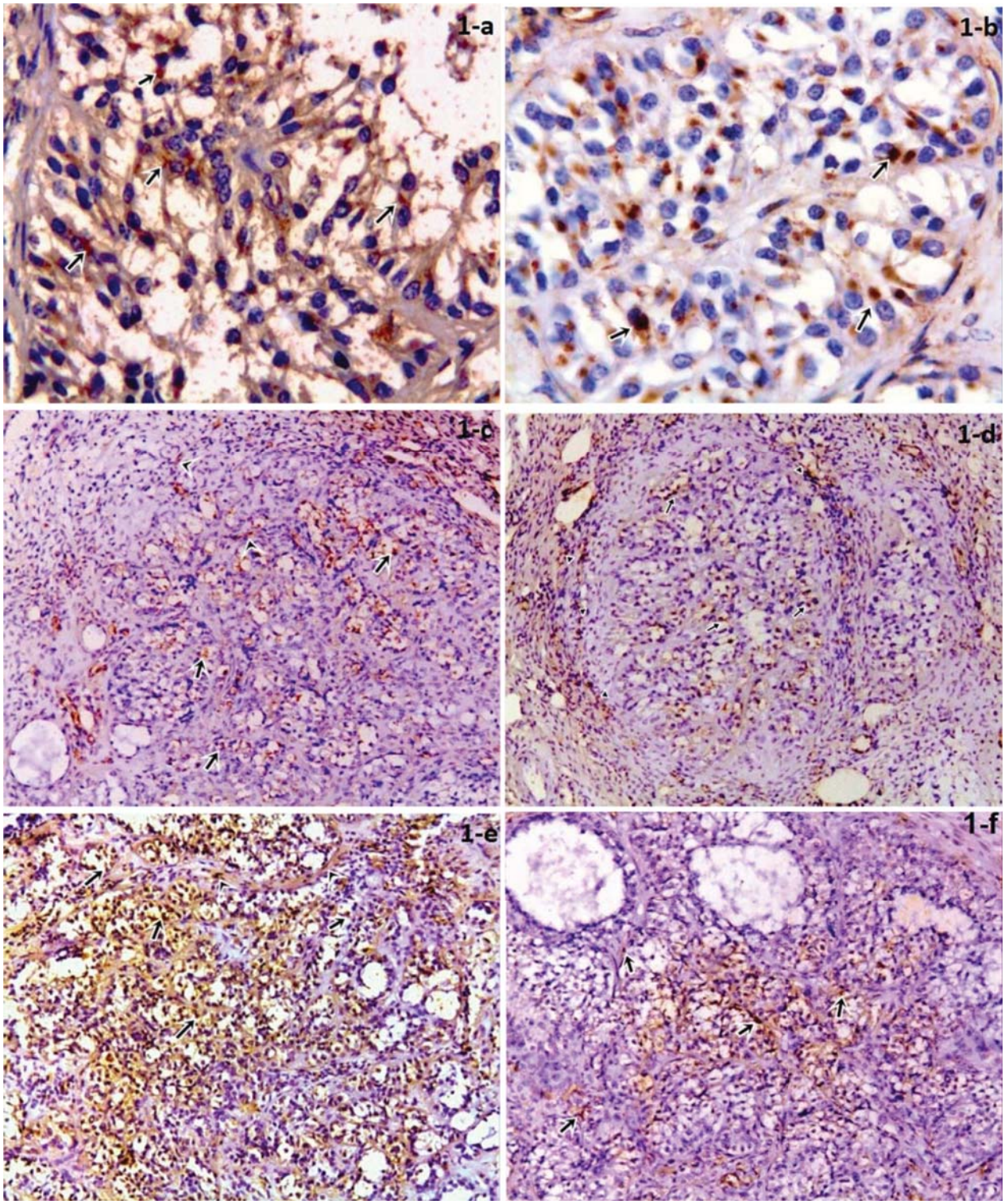

Fig 1. Immunohistochemical stainings with AMH, Inhibin-α, IGF-1, IGF-2, GATA-4 and GATA-6, respectively, **a-** Moderate positiveness with AMH in cytoplasm of neoplastic granulosa cells (arrows), ABC-P method, x400, **b-** Moderate positiveness with Inhibin-α in cytoplasms of neoplastic granulosa cells *(arrows)*, ABC-P method, x400, **c-** Mild positiveness with IGF-1 in cytoplasms of neoplastic granulosa cells *(arrows)* and spindle shaped cells *(arrowheads)*, ABC-P method, x40, **d-** Moderate positiveness with IGF-2 in cytoplasms of neoplastic granulosa cells *(arrows)* and theca interna and externa cells (arrowheads), ABC-P method, x40, **e-** Moderate positiveness with GATA-4 in both cytoplasms of neoplastic cells *(arrows)* and some thecal cell *(arrowheads)*, ABC-P method x40, **f-** Mild positiveness with GATA-6 in cytoplasms of neoplastic thecal cells *(arrows)*, ABC-P method x40

Şekil 1. AMH, Inhibin-α, IGF-1, IGF-2, GATA-4 ve GATA-6 ile immunohistokimyasal boyamalar, **a-** Neoplastik granuloza hücre sitoplazmasında AMH ile orta derecede pozitiflik (oklar), ABC-P method, x400, **b-** Neoplastik granuloza hücre sitoplazmasında inhibin-α ile orta derecede pozitiflik *(oklar)*, ABC-P method, x400, **c-** Neoplastik granuloza hücre sitoplazmasında IGF-1 ile hafif derecede pozitiflik *(oklar)* ve iğ şekilli hücreler *(ok başları)* ABC-P method, x40, **d-** Neoplastik granuloza hücre sitoplazmasında IGF-2 ile orta derecede pozitiflik *(oklar)*, teka interna ve teka eksterna hücrelerinde *(ok başları)*, ABC-P method, x40, **e-** GATA-4 ile neoplastic hücre sitoplazmasında *(oklar)* ve bazı teka hücrelerinde orta derecede pozitiflik *(ok başları)*, ABC-P method x40, **f-** Neoplastic teka hücre sitoplazmasında GATA-6 ile hafif derecede pozitiflik *(oklar)*, ABC-P method x40

of serum AMH concentrations are also specific for GTCT, therefore serum AMH analysis may be used to monitor progression of tumor and to assess postoperative recurrence in mares as well as in women.

Inhibins play important roles in folliculogenesis by influencing granulosa cell maturation and proliferation, oocyte maturation and steroid hormone production. Lack of inhibin production is associated with several ovarian diseases, furthermore increased inhibin-α levels are detected in ovarian cancers [23].

The moderate positivity with inhibin-α versus undetermined positivity with GDF-9 and BMP-6 may be conceived as the attenuating effect of inhibin-α on GDF-9 and BMP-6 in this case of GTCT. Inhibins are capable of binding type-2 activin and BMP receptors through their β-subunits acted via type-3 TGF-β receptor. Therefore, high inhibin levels can antagonize the actions of activins, BMPs and potentially other TGF-β superfamily members [13].

Inhibin-α is a tumor suppressing gene in mice and deleting of this gene results in aggressive granulosa/sertoli cell tumors, but in contrast with mice, granulosa cell tumors in women are associated with high levels of inhibin in circulation which is thought to occur due to a loss in responsiveness to inhibins by tumor cells [13]. Inhibin-α is also present with high concentrations in both serum and ovarian tissue samples of mares with GTCT. Thus, inhibin-α is used as a marker for GTCT in mares as well as in women. Additionally, Ball et al.[18] found AMH expression was correlated with serum inhibin concentration in a study of equine GTCT. This knowledge supports the immunohistochemical findings of intensive positivity on neoplastic granulosa cells with AMH and inhibin-α markers in our case.

GATA-4 is associated with gonadal sex differentiation in embryo, and both GATA-4 and -6 play role in gonadal development. In adult gonads, GATA-4 and GATA-6 get involved in steroidogenesis [15]. Overexpressed GATA-4 may activate gene promoter of AMH [15] and inhibin-α [24]. In adult mice ovary, GATA-4 is expressed by healthy granulosa cells, some thecal cells, and interstitial cells. GATA-6 is detected in granulosa cells, and strongly expressed in corpora lutea [15]. GATA-4 expression is found to be high in human GTCTs [14]. Therefore, this characteristic expression conceives GATA-4 to be a potential prognostic tool for GTCTs. It is suggested that GATA-4 plays a role in granulosa cell differentiation via Cyclin D2 promoter [14]. In our case, the obtained reactions were also in this manner. Especially, GATA-4 was expressed moderately in granulosa and theca cells while mild reaction with GATA-6 was encountered. Accordingly, GATA-4 is found to have more prognostic value for GTCT diagnosis in mares when compared to GATA-6.

As well as intracellular GATA-4 phosphorylation is achieved by protein kinase A (PKA) pathway under effects of gonadotropins, it can occur by mitogen activated protein kinase (MAPK) signaling pathway under influences of stress and mitogenic effects [25]. Additionally, adrenal gland tumors were observed in mice due to high gonadotropic effects following gonadectomy [26]. High simultaneous expressions of LH receptors, GATA-4, and inhibin-α were detected in these tumors [24,26].

IGF-1 has significant tasks in antral follicles such as granulosa cell proliferation, estradiol, progesteron production and inhibin-A, activin-A, follistatin secretion by granulosa cells, oocyte viability, oocyte maturation, follicle dominance, multiple ovulation, and also increase in follicle sensitivity to gonadotrophin and to LH-R in granulosa and theca cells in mammals [27]. Mild positivity with IGF-1 in neoplastic ovarian tissue in our case may be a sign of IGF-1 inhibition that results in follicular development failure which is followed by formation of small cystic structures.

IGF-2 plays important roles in initiating primordial follicle growth, granulosa cell proliferation in secondary and antral follicles, progesteron and estradiol production by granulosa cells, and stimulating steroidogenesis by thecal cells in antral follicles [27]. Predictably, IGF-2 concentrations may remain constant or be increased when androgen synthesis from thecal cells, and aggressive and/or stallion-like behaviours in mares with GTCT are taken into account. The obtained moderate positivity with IGF-2 may be expressed in this context.

In the case, it was thought that there is a close relationship between inhibin-α and IGF-2 (due to their moderate staining) when evaluated as regards to initiation of follicle growth and granulosa cell proliferation. In this context, these two factors may trigger possible primary effect in the way of neoplastic changes as to be understood from literature knowledge.

In conclusion, AMH and inhibin-α showed the highest immunopositivity in neoplastic granulosa cells among other markers and proved their characteristic expression in equine GTCT. GATA-4 also showed high positivity characteristically in both neoplastic granulosa and thecal cells whereas any positivities could not be observed with BMP-6 and GDF-9. According to our results AMH, inhibin-α, and GATA-4 are all have a role as diagnostic tools for equine GTCT. When considering all these findings, the markers play a role in granulosa theca cell tumorigenesis in the case. It may be usefull for diagnosis and evaluating prognosis if any positivities were obtained from these markers in this type of GTCT cases. Then, these markers can properly use as diagnostic tool. Further study on these factors will have important implications for our understanding the tumorigenesis of the most common ovarian neoplasia in mares.

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REVIEW

Tendon Healing and Repair: A Review of Current Approaches

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Summary

The treatment of tendon diseases increasing as the result of longer life expectancy and further taking place of re-creative activities in life maintains its importance in orthopedic surgery. Significant data's have been obtained with numerous conducted studies and many developed surgical methods in this field, however the ongoing challenges and some complications are still lasting today. Therefore, a number of studies were conducted in order to obtain the ideal tendon repair method and are still conducted. For reaching the desired goal of all of these studies, to have a good knowledge about the biological and biomechanical structure of the tendon, the healing stages, and the factors affecting the repair mechanisms and healing has an undeniable place. In this review it has been aimed to help the researchers by reviewing the studies about tendon repair and healing in the literature.

Keywords: *Tendon healing, Tendon repair, Tendon surgery, bFGF, PRP, Hyaluronic acid, Growth factors, Gliding mechanism, Suture techniques*

Tendon İyileşmesi ve Tamirinde Güncel Yaklaşımların Gözden Geçirilmesi

Özet

Yaşam süresinin uzaması ve rekreatif aktivitelerin yaşamda daha fazla yer alması sonucunda artan tendon hastalıklarının tedavisi ortopedik cerrahideki önemini korumaktadır. Bu alanda yapılan çok sayıda çalışmalar ve geliştirilen birçok cerrahi metotlarla önemli kazanımlar elde edilmiş olmasına karşın öteden beri devam eden zorluklar ve komplikasyonların bir kısmı günümüzde de hala devam etmektedir. Dolayısı ile ideal tendon tamir metodu elde etmek için çok sayıda çalışma yapılmış ve yapılmaktadır. Bütün bu çalışmaların istenen hedefe ulaşabilmesinde, tendonun biyolojik ve biyomekanik yapısının, iyileşme evrelerinin, tamir mekanizmaları ve iyileşmesini etkileyen faktörlerin iyi bilinmesi yadsınamaz bir gerçektir. Bu çalışma, tendon tamiri ve iyileşmesi konusunda son yıllarda yapılan çalışmalar ve klasik bilgilerin harmanlanarak güncel yaklaşımların aktarılması amacıyla rapor edilmiştir.

Anahtar sözcükler: *Tendon iyileşmesi, Tendon tamiri, Tendon cerrahisi, bFGF, PRP, Hyaluronik asit, Büyüme faktörü, Kayma mekanizması, Sütür*

INTRODUCTION

Tendon is one of the most important part of musculo-skeletal system. It provides mobilization of the skeletal system by transfer of the forces obtained from the muscles to the bone. For this reason, the disorders occurred in the structure of tendon may directly affect the mobility of the organism. Tendon injury may occur as a result of acute direct injury or by chronic process which some of inflammatory diseases accompanied or ongoing with excessive use. According to their function and anatomical locations, tendon disorders have characteristic features in their selves. Increased life expectancy and recreational activities, lead to an increase in the incidence of tendon disorders. As a result of tendon injuries, appropriate treatment and the good management of recovery process, has a great importance in re-obtaining of the functions.

Tendon healing is a very slow process. Surgical and conservative treatment methods have many challenges and complications. Some of them are re-ruptures, adhesions, loss or reduction of function in related extremity as a result of disorder at tendon slip mechanism. Today, all these challenges continue to be one of the most important issues

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that the orthopedic surgery is trying to manage. Thus, the innovations about the management of tendon healing and repair never ends. This review aimed to present these innovations at the field [1-3].

TENDON BIOLOGY and FUNCTIONS

Normal tendon is composed of the organization of soft fibrous connective tissues [4].

Tendon forms as the result of composition of collagen fiber bundles packaged by connective tissues. Fiber bundles show a parallel arrangement to the tendon axis. These tendon bundles are surrounded by the tendon sheath including also the extracellular matrix components (ECM) *(Fig. 1)* [5,6].

Seventy five percent of the dry weight of the tendon is formed by type I collagen. Type I collagen is the component which has the most important function in transmission of the force along axis of the tendon tissue. Tendon structure has been well organized to provide the transport of force, which is resulting from the muscle contractions, to the bone tissue [4-6].

Muscle-tendon units are composed of collagen fibers and rod or spindle-like fibroblasts embedded in the ECM [5-7].

Collagen is synthesized by 'tenocyte' and the most basic building block. Tenocytes have the ability to respond to mechanical loads. Collagen polypeptides, formed by cross-linking of the collagen fibrils in themselves, are triple helix structure. The parallel placement of these fibrils, which are triple helix structure, in fibers, is the most important feature which provides tensile strength of the tendon. Collagen fibrils which are at helix structure are synthesized within the cell and secreted into the ECM and formed collagen fibers by connecting to each other in the micro-fibril unit. Type I collagen is the major component forming tendon. However, there is a small amount of type III collagen. Type III collagen mostly involved in the structure endotenon and epitenon. Diameter of the collagen fibers is important for the durability of tendon. Due to bundles of collagen fibers is very thin at diameter in the early stages of repair, tensile strength is low in the early stage of healing. At this stage, the synthesis of type III collagen increases [7]. Type V collagen provides to regulate other collagen types in fibriller structure of tendon by cross-linking [6,8].

ECM also includes other regulatory proteoglycans such as agregant and decorin. It has been thought that, water consists approximately 55% of the ECM and proteoglycans have a friction-reducing effect in ECM. Also the proteins with the structure of elastin and glycopeptide in ECM play a role in collagen fibril stability [9,10].

Collagen fibrils tied in bundles in wide fibers with 100-500 nm in diameter. When they were investigated under light microscopy, according to their lengths, they were observed to elongated in 1-3%. It has been thought that, this state of elongation inhibited tendon damage with sudden loads [11].

Spindle - shaped tendon fibroblasts are the responsible cells for the secretion and the continuity of the contents in the ECM. Collagen fibers are wrapped by endotenon and formed fascicles [6,12,13]. Endotenon a thin layer of connective tissue that containing the vascular, lymphatic, and neural structures [14]. Tendons are wrapped by tendon sheath and pulleys which are located close to the regions of joints that tendons pass through. The tendon sheath called paratenon surrounds the tendon across all regions. Paratenon composed of loose tissue supplying the entry of blood vessels to epitenon and endotenon. These sheaths includes also synovial cells, thus provide lubrication and

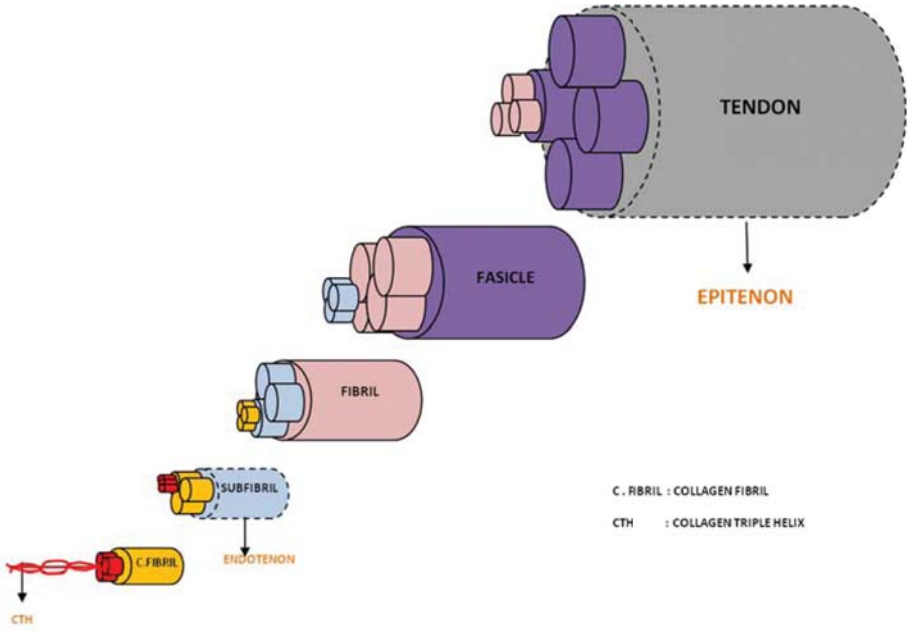

Fig 1. Structural schema of the tendon

Şekil 1. Tendonun yapısal şeması

help to tendon for sliding in the sheath [12-14].

Despite tendon has so unique and strong structure. As of its structure, does not have a large security area [15]. Sometimes, in the transfer of the force generated by the muscles, the maximum resistance level for tendons can be exceeded. In this case, tendon can rupture and sometimes tears and degeneration can occur in the structure of tendon [16]. Although, tensile forces are within the borders of resistance of tendon, in repetitive overuse, as a result of deterioration of function of gliding mechanism and sheath defined as a functional medium of tendon, damage may occur [15,16].

Among other things, tendon injury may occur direct penetrating and cutting factors. The rate of loss of function resulting from tendon injury and clinical findings are the factors that determine the need and method for treatment [17].

TENDON HEALING PROCESS

Tendon does not rupture with the normal borders of physiological loads. However, tendon which has a deterioration of in its structure particularly as a result of aging and external injuries, can rupture without sudden and extreme loads [12]. Beside the ruptures, the exposure of tendon to the degenerative process sometimes may result in malfunction which can need medical treatment [18].

For the returning of tendon to its normal function after injury, the integration of tendon fibers parallel to the tendon axis and re-obtaining the gliding relationship between tendon and its environment are very important [13,19].

As in other tissues, with tendon injury, a repair cascade begins that cellular and biochemical events play a role. Tendon healing process consists of three main phases. In the first section, known as the inflammatory phase, hematoma caused by the injury, provides the release of many chemo-active factors. The pro-inflammatory agents and vaso-dilators, play an important role for providing the building blocks of repair. The fibroblasts collecting in the region, provide the synthesis of many components in ECM [20]. The angiogenic agents stimulate the formation of new blood vessels in the region [21].

With the proliferation of fibroblasts collected in the region and beginning of synthesis of ECM, proliferative phase begins. In this phase, abundant synthesis of collagen is performed. However, in general, the type of collagen synthesized in the inflammatory and proliferative phase is Type III collagen.

In the process which begins evolve towards the scar phase. Dense network of blood vessels formation is remarkable in scar phases [22,23].

At this stage, the density of type III collagen and water in the ECM is quite high. In the scar phase, the complete connection between ruptured end of tendon is established literally. Approximately in 6-8 weeks after the injury, scar begins to fall in. The ECM in the cell density decreases significantly, Type I collagen the rate of water and collagen type III begins to decrease. The cell density in ECM decreases significantly, Type I collagen begins to appear [24].

Then, Type I collagen begins to settle in parallel with the tendon axis. With the organization of type I collagen and resorbtion of scar tissue, it has been thought that, mechanical stimuli played a role in the initiation of transforming of tendon to its anatomic structure [25].

Biomechanics of Tendon in Healing Process

Tendons have more stronger structure than muscles, in terms of the resistance that they show against to tensile forces, are equal to bone tissue but more flexible and more extensible than the bone [26].

The biomechanical properties of tendons in healing process, have been the subject of extensive studies and the data obtained were compared with the normal tendon.

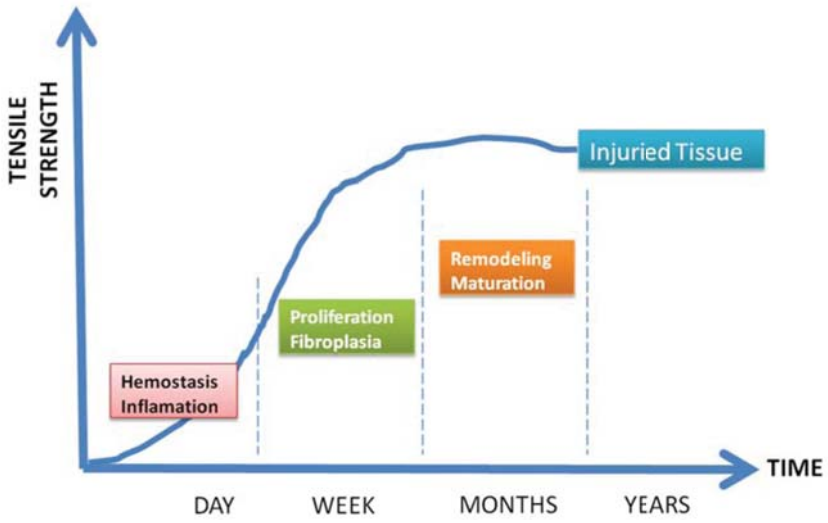

Fig 2. Biomechanic process of tendon healing [27]
Şekil 2. Tendon iyileşmesinin biyomekanik süreci [27]

It has been seen that in these studies, repaired or healed tendon tissue never reached the normal biomechanical properties of the tendon *(Fig. 2)* [27].

Taking into consideration of biomechanical properties of tendon in the healing process is critical for the management of tendon repair and healing. The scar tissue in the first stages of the repair process is in insufficient state for stretching. For this reason, immobilization of the joints related to tendon in the process of repair and recovery is necessary for recovery. It has been mentioned that, in tendon healing process, the mechanical stimuli play an important role in the formation of scar tissue and collagen fibrils [28]. To achieve this depends on the strength of the repaired or healing tendon tissue. Therefore, to obtain the strength of healing tissue was considered as main part in the development of tendon repair methods [29].

SURGICAL REPAIR APPROACHES OF THE TENDON

In orthopedic surgery, tendon repair plays an important role. Spontaneous recovery after the tendon injuries is possible in extensor tendons and partial tendon injuries. However, in the events that tendon is injured directly and resulted in complete rupture of the tendon, surgery is almost the only option.

In surgical repair of the tendon, many tendon repair methods, which are able to show resistance against the tension occuring in the repair region and is able to show resistance against the 'gap' formation, were developed [28,30,31].

Double core suture stands technique and the early methods such as Kessler, Bunnel, Tajiama etc. which tendon ends matched mutually were applied together with post-operative immobilization. Many subsequent studies on these techniques have showed that, tendon tissue has failed to reach the baseline values biomechanically in tendon repair region even after 3-4 weeks [32]. For this reason, the multi-stand techniques were developed and thus more resistant tissue was obtained in the early period and the continuity of this resistance could be maintained and thus the opportunity of early mobilization was obtained despite it was a limited situation. Winters method was obtained with the eight-strand loop shaped modification of double parallel Kessler suture technique and superior values were obtained than the modified Kessler method applying as four-strand suture. Double loop techniques, without increasing the number of knots, by providing the passage of more number of suture, provides more resistant repair mechanically [32-34].

All of these methodological improvements have not provided exactly becoming secure of repaired tendon in the level of desirable active exercise [35]. Therefore, as well as the development of surgical methods, many researches were needed for the materials used in repair [35]. Barie et al.[37] compared in cadaveric digitorum profundus tendons repaired with USP 4/0 dacron with the tendons repaired with USP 3/0 dacron, they seen that, with USP 4/0 dacron, they obtained more durable repair.

Taras et al.[36] obtained 167%, 391% more durable repaired human cadaveric digitorum profundus tendons in with the modified Kessler technique which they applied USP 2/0 diameters of suture instead of USP 5/0 and with double grasping method, respectively.

On the other hand, it has been proposed that, the location and number of knot points of core sutures increased the endurance [39].

The distance of the knots of core sutures from the the repair area increases durability. Adding peripheral sutures to the core sutures is also contributes to a stronger repair [40].

Despite the modification of tendon repair techniques and improvement of the properties of materials used, complications were not fully eliminated. These complications may be listed as re-rupture, scar tissue hypertrophy, suture pullout, and adhesions [15,41,42]. Because, extrinsic and intrinsic biological repair mechanisms of tissues play a role with surgical applications in wound healing. These mechanisms allow for the healing of tendon, but on the other hand, by causing complications such as scar tissue hypertrophy and adhesion, may also result in deterioration of the gliding mechanism [15].

BIOLOGICAL MODIFICATIONS OF THE TENDON

Researchers, in order to shorten the duration of repair, achieve more resistant repair tissue and gliding ability, continue to perform the studies which may ensure to modify the repair mechanisms [41]. These studies aimed at biological modifications and revived a different approach for the repair. Some studies conducted for this purpose, showed that the use of hyaluronic acid (HA) had an effect on tendon healing [43-45].

Mora-oka et al.[46] reported that, they reduced the adhesion with the combination of HA and phosphatidylcolin.

Moreover, in another study, lubricin was used with the aim of improving tendon gliding mechanism and it was found successful [47].

In addition to all of these developments, it has been thought that, the growth factors whose effects in tendon healing were known, may be used in order to modification of the biological environment, and many studies have been conducted [48-51].

These studies seems to offer significant opportunities

for accelerating the repair event, decreasing the duration of immobilization and obtaining more durable repair tissue. For this purpose, in the studies the agents such as bFGF (fibroblast growth factor beta), TGF-beta (transforming growth factor beta), PDGF (platelet derived growth factor) and PRP (platelet rich plasma) are used, usually good results have been reported. All of these factors increase mitotic activity of fibroblasts and collagen synthesis, in specific experimental conditions [52-55].

However, in practice, the problems such as to reach sufficient concentration of these agents in repair area and maintenance the concentration, have not been resolved [56,57].

The huge advances in genetic engineering and the complications and deficiencies which still continue in tendon repair, pointed the researches to this direction. Tang et al. cloned bFGF (basic fibroblast growth factor) gene by transferring adeno associated virus-2 vector to tendon fibroblasts [58]. In this study, the obtained genes were incorporated the ends of the cut tendon, expression of local bFGF was increased and thereby, it has been shown that, the strength of tendon repair tissue was higher in 2nd and 4th weeks.

On the other hand, BMP-12 was used for tenogenesis and found to be effective. In the patients undergoing BMP-12, the resistance of repaired tendon was higher than control group [58-61].

Despite the developments in all of these exogenous modification methods, surgical techniques and the materials used, today, the basic inability and complications affecting the success of the repair of the tendon were not fully resolved. Maybe in the future the use of the combination of all of these developments in the tendon repair, a new era can be opened.

Some researchers thought the use of the implant in order to overcome the failure caused by tendon repair and complications. So, putting an extra support next to the suture materials, they aimed to overcome the problems developing due to both early active exercise and the limp use and immobilization-induced adhesion and gliding [62].

Aygün et al.[15] in their study implanted flexible polyethylene (PE) material for rabbit ruptured achilles tendons. In this way, through the use of plate, instead of putting the suture parallel to the tendon axis, they provided the facility for positioning les number of suture vertically to the tendon axis. In addition, by ensuring the transfer of the tendon tensile forces to the implant instead of repaired ends and suture materials, they made use of early active possible [15]. However, in order to able to take the place of these and similar studies in the practical application, production of implants which are able to resorb and have biological compatibility and able to adapt to the tendon functions and development of these implants with extensive researches are required.

CONCLUSION

Tendon injuries and repair are the main topics of orthopedic surgery and the complications and deficiencies which have continued since the beginning since are not fully resolved yet. Despite the development of surgical techniques and suture qualifications which are the main materials in surgery and the use of many exogenous biological modifiers, for the ideal method of tendon repair, there is still long way to be taken. In the future, these studies will progress rapidly, seems to be effective in the improvement and recovery of function of the injured tendon in the shortest time.

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YAZIM KURALLARI

1- Yılda 6 (Altı) sayı olarak yayımlanan Kafkas Üniversitesi Veteriner Fakültesi Dergisi'nde (Kısaltılmış adı: Kafkas Univ Vet Fak Derg) Veteriner Hekimlik ve Hayvancılıkla ilgili (klinik ve paraklinik bilimler, hayvancılıkla ilgili biyolojik ve temel bilimler, zoonozlar ve halk sağlığı, hayvan besleme ve beslenme hastalıkları, hayvan yetiştiriciliği ve genetik, hayvansal orijinli gıda hijyeni ve teknolojisi, egzotik hayvan bilimi) orijinal araştırma, kısa bildiri, ön rapor, gözlem, editöre mektup, derleme ve çeviri türünde yazılar yayımlanır. Dergide yayımlanmak üzere gönderilen makaleler Türkçe, İngilizce veya Almanca dillerinden biri ile yazılmış olmalıdır.

2- Dergide yayımlanması istenen yazılar Times New Roman yazı tipi ve 12 punto ile A4 formatında, 1.5 satır aralıklı ve sayfa kenar boşlukları 2.5 cm olacak şekilde hazırlanmalı ve resim, tablo, grafik gibi şekillerin metin içindeki yerlerine Türkçe ve yabancı dilde adları ve gerekli açıklamaları mutlaka yazılmalıdır.

Dergiye gönderilecek makale ve ekleri (şekil vs) *http://vetdergi.kafkas.edu.tr* adresindeki online makale gönderme sistemi kullanılarak yapılmalıdır.

Başvuru sırasında yazarlar yazıda yer alacak şekilleri (13 X 18 cm boyutlarından büyük olmamalı) online makale gönderme sistemine yüklemelidirler. Yazının kabul edilmesi durumunda tüm yazarlarca imzalanmış *Telif Hakkı Devir Sözleşmesi* editörlüğe gönderilmelidir.

3- Yazarlar yayınlamak istedikleri makale ile ilgili olarak gerekli olan etik kurulu onayı aldıkları kurumu ve onay numarasını Materyal ve Metot bölümünde belirtmelidirler. Yayın kurulu gerekli gördüğünde etik kurul onay belgesini ayrıca isteyebilir.

4- ***Makale Türleri***

Orijinal Araştırma Makaleleri, yeterli bilimsel inceleme, gözlem ve deneylere dayanarak bir sonuca ulaşan orijinal ve özgün çalışmalardır.

Türkçe yazılmış makaleler Türkçe başlık, Türkçe özet ve anahtar sözcükler, yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, giriş, materyal ve metot, bulgular, tartışma ve sonuç ile kaynaklar bölümlerinden oluşmalı ve metin, tablo, şekil vs dahil) 10 sayfayı aşmamalıdır. Yabancı dilde yazılmış makaleler yabancı dilde başlık, yabancı dilde özet ve anahtar sözcükler, Türkçe başlık, Türkçe özet ve anahtar sözcükler ile birlikte Türkçe makale yazım kurallarında belirtilen diğer bölümlerden oluşur. Türkçe ve yabancı dilde özetlerin her biri yaklaşık 200±20 sözcükten oluşmalıdır.

Kısa Bildiri, konu ile ilgili yeni bilgi ve bulguların bildirildiği fakat orijinal araştırma olarak sunulamayacak kadar kısa olan yazılardır. Kısa bildiriler, orijinal araştırma makalesi formatında olmalı, fakat özetlerin her biri 100 sözcüğü aşmamalı, referans sayısı 15'in altında olmalı ve 4 sayfayı aşmamalıdır. Ayrıca, en fazla 4 şekil veya tablo içermelidir.

Ön Rapor, kısmen tamamlanmış, yorumlanabilecek aşamaya gelmiş orijinal bir araştırmanın kısa (en çok 6 sayfa) anlatımıdır. Bunlar orijinal araştırma makalesi formatında yazılmalıdır.

Gözlem, uygulama, klinik veya laboratuar alanlarında ender olarak rastlanılan olguların sunulduğu makalelerdir. Bu yazıların başlık ve özetleri orijinal makale formatında yazılmalı, bundan sonraki bölümleri giriş, olgunun tanımı, tartışma ve sonuç ile kaynaklardan oluşmalı ve 4 sayfayı geçmemelidir.

Editöre Mektup, bilimsel veya pratik yararı olan bir konunun veya ilginç bir olgunun resimli ve kısa sunumudur ve 1 sayfayı geçmemelidir.

Derleme, güncel ve önemli bir konuyu, yazarın kendi görüş ve araştırmalarından elde ettiği bulguların da değerlendirildiği özgün yazılardır. Bu yazıların başlık ve özet bölümleri orijinal araştırma makalesi formatında yazılmalı, bundan sonraki bölümleri giriş, metin ve kaynaklardan oluşmalı ve 10 sayfayı geçmemelidir.

Çeviri, makalenin orijinal formatı dikkate alınarak hazırlanmalıdır.

Yazarla ilgili kişisel ve kuruma ait bilgiler ana metin dosyasına değil, on-line başvuru sırasında sistemdeki ilgili yerlere unvan belirtilmeksizin eklenmelidir.

5- Makale ile ilgili gerek görülen açıklayıcı bilgiler (tez, proje, destekleyen kuruluş vs) makale başlığının sonuna üst simge olarak işaret konularak makale başlığı altında italik yazıyla belirtilmelidir.

6- Kaynaklar, metin içinde ilk verilenden başlanarak numara almalı ve metin içindeki kaynağın atıf yapıldığı yerde parantez içinde yazılmalıdır.

Kaynak dergi ise, yazarların soyadları ve ilk adlarının başharfleri, makale adı, dergi adı (orijinal kısa ad), cilt ve sayı numarası, sayfa numarası ve yıl sıralamasına göre olmalı ve aşağıdaki örnekte belirtilen karakterler dikkate alınarak yazılmalıdır.

Örnek: **Gokce E, Erdogan HM:** An epidemiological study on neonatal lamb health. *Kafkas Univ Vet Fak Derg,* 15 (2): 225-236, 2009.

Kaynak kitap ise yazarların soyadları ile adlarının ilk harfleri, eserin adı, baskı sayısı, sayfa numarası, basımevi, basım yeri ve basım yılı olarak yazılmalıdır.

Editörlü ve çok yazarlı olarak yayınlanan kitaptan bir bölüm kaynak olarak kullanılmışsa, bölüm yazarları, bölüm adı, editör(ler), kitap adı, baskı sayısı, sayfa numarası, basımevi, basım yeri ve basım yılı sırası dikkate alınarak aşağıdaki örneğe göre yazılmalıdır.

Örnek: **Mcllwraith CW:** Disease of joints, tendons, ligaments, and related structures. **In,** Stashak TS (Ed): Adam's Lameness in Horses. 4th ed. 339-447, Lea and Febiger, Philadelphia, 1988.

Online olarak ulaşılan kaynaklarda web adresi ve erişim tarihi kaynak bilgilerinin sonuna eklenmelidir.

Diğer kaynakların yazımında bilimsel yayın ilkelerine uyulmalıdır.

Kaynak listesinde "et al." ve "ve ark." gibi kısaltmalar yapılmaz.

7- Bakteri, virus, parazit ve mantar tür isimleri ve anatomik terimler gibi latince ifadeler orijinal şekliyle ve italik karakterle yazılmalıdır.

8- Editörlük, dergiye gönderilen yazılar üzerinde gerekli görülen kısaltma ve düzeltmeleri yapabileceği gibi önerilerini yazarlara iletebilir. Yazarlar, düzeltilmek üzere yollanan yazıları online sistemde belirtilen sürede gerekli düzeltmeleri yaparak editörlüğe iade etmelidirler. Editörlükçe ön incelemesi yapılan ve değerlendirmeye alınması uygun görülen makaleler ilgili bilim dalından bir yayın danışmanı ve iki raportörün olumlu görüşü alındığı takdirde yayımlanır.

9- Yayınlanan yazılardan dolayı doğabilecek her türlü sorumluluk yazarlara aittir.

10- Yazarlara telif ücreti ödenmez.

11- Resim ve baskı masrafları için yazarlardan ücret alınır. Ücret bilgileri *http://vetdergi.kafkas.edu.tr/* adresinden öğrenilebilir.

12- Yazarlara 50 adet ayrı baskı ücretsiz olarak yollanır.

INSTRUCTIONS FOR AUTHORS

1- Kafkas Üniversitesi Veteriner Fakültesi Dergisi (Journal of the Faculty of Veterinary Medicine, Kafkas University) (abbreviated title: Kafkas Univ Vet Fak Derg), published bi-monthly, covers original papers, short communication and preliminary scientific reports, case reports, observation, letter to the editor, review and translation on all aspects of veterinary medicine and animal science (clinical and paraclinical sciences, biological and basic sciences, zoonoses and public health, animal feeding and nutritional diseases, animal breeding and genetics, hygiene and technology of food from animal sources, exotic animal science). Manuscripts submitted for publication should be written in Turkish, English or German.

2- The manuscripts submitted for publication should be prepared in the format of Times New Roman style, font size 12, A4 paper size, 1.5 line spacing and 2.5 cm margins of all edges. The legend or caption of all illustrations such as figure, table and graphic must clearly be written in both Turkish and foreign language and their appropriate position should be indicated in the text.

The manuscript and its supplementary (figure etc.) should be submitted by using online manuscript submission system at the address of *http://vetdergi.kafkas.edu.tr/*

During the submission, the authors should upload the figures of the manuscript (the dimensions must not to exceed 13 X 18 cm) to the online manuscript submission system. If the manuscript is accepted for publication, the copyright transfer agreement form signed by all the authors should be send to the editorial office.

3- Authors should indicate the name of institute approves the necessary ethical commission report and the serial number of the approval in the material and methods section. If necessary, editorial board may also request the official document of the ethical commission report.

4- Original (full-length) manuscripts are original and proper scientific papers based on sufficient scientific investigations, observations and experiments.

Manuscripts written in Turkish consist of the title in Turkish, summary and keywords in Turkish, introduction, material and methods, results, discussion and references and it should not exceed 10 pages including text, tables and illustrations. Manuscripts written in a foreign language should follow the title in foreign language, summary and keywords in foreign language, title in Turkish, summary and keywords in Turkish and the remaining sections described above for the manuscripts written in Turkish.

Summaries written in Turkish or foreign language should contain 200±20 words.

Short communication manuscripts contain recent information and findings in the related topics; however, they are written with insufficient length to be a full-length original article. They should be prepared in the format of full-length original article but each of the summaries should not exceed 100 words, the reference numbers should not exceed 15 and the length of the text should be no longer than 4 pages. Additionally, they should not contain more than 4 figures or tables.

Preliminary scientific reports are short description (maximum 6 pages) of partially completed original research findings at interpretable level. These should be prepared in the format of full-length original articles.

Case reports describe rare significant findings encountered in the application, clinic and laboratory of related fields. The title and summary of these articles should be written in the format of full-length original articles and the remaining sections should follow introduction, case history, discussion and references without exceeding the total of 4 pages.

Letters to Editor are short and picture-documented presentations of subjects with scientific or practical benefits or interesting cases without exceeding 1 page.

Reviews are original manuscripts gather the literature on current and significant subject along with the commentary and findings of the author on the particular subject. The title and summary of this manuscript should be prepared as described for the full-length original articles and the remaining sections should follow introduction, text and references without exceeding 10 page.

Translations should be prepared based on the format of original document being translated.

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6- References should be listed with numerical order as they appear in the text and the reference number should be indicated inside the parentheses at the cited text place. References should have the order of surnames and initial letters of the authors, title of the article, title of the journal (original abbreviated title), volume and issue numbers, page numbers and the year of publication and the text formatting should be performed as shown in the example below.

Example: **Gokce E, Erdogan HM:** An epidemiological study on neonatal lamb health. *Kafkas Univ Vet Fak Derg,* 15 (2): 225-236, 2009.

If the reference is a book, it should follow surnames and initial letters of the authors, title of the book, edition number, page numbers, name and location of publisher and year of publication. If a chapter in book with an editor and several authors is used, names of chapter authors, name of chapter, editors, name of book, edition number, page numbers, name and location of publisher and year of publication and the formatting should be performed as shown in the example below.

Example: **McIlwraith CW:** Disease of joints, tendons, ligaments, and related structures. **In,** Stashak TS (Ed): Adam's Lameness in Horses. 4th ed. 339-447, Lea and Febiger, Philadelphia, 1988.

In the references can be reached online only, the web address and connection date should be added at the end of the reference information. The generally accepted scientific writing instructions must be complied with the other references.

Abbreviations, such as "et al" and "and friends" should not be used in the list of the references.

7- The Latin expression such as species names of bacterium, virus, parasite and fungus and anatomical terms must be written in italic character keeping their original forms.

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