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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

Duygu BAKİ ACAR [1] Muhammed Kürşad BİRDANE [1] Erhan ÖZENÇ [1]
Deniz YENİ [2] İsmet DOĞAN [3]

[1] Afyon Kocatepe Üniversitesi, Veteriner Fakültesi, Doğum ve Jinekoloji Anabilim Dalı, TR-03200 Afyonkarahisar - TÜRKİYE
[2] Afyon Kocatepe Üniversitesi, Veteriner Fakültesi, Dölerme ve Suni Tohumlama Anabilim Dalı, TR-03200 Afyonkarahisar - TÜRKİYE
[3] Afyon Kocatepe Üniversitesi, Tıp Fakültesi, Biyoistatistik ve Tıbbi Bilişim Anabilim Dalı, TR-03200 Afyonkarahisar - TÜRKİYE

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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

İletişim (Correspondence)
+90 272 2149309
dbakiacar@aku.edu.tr

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,

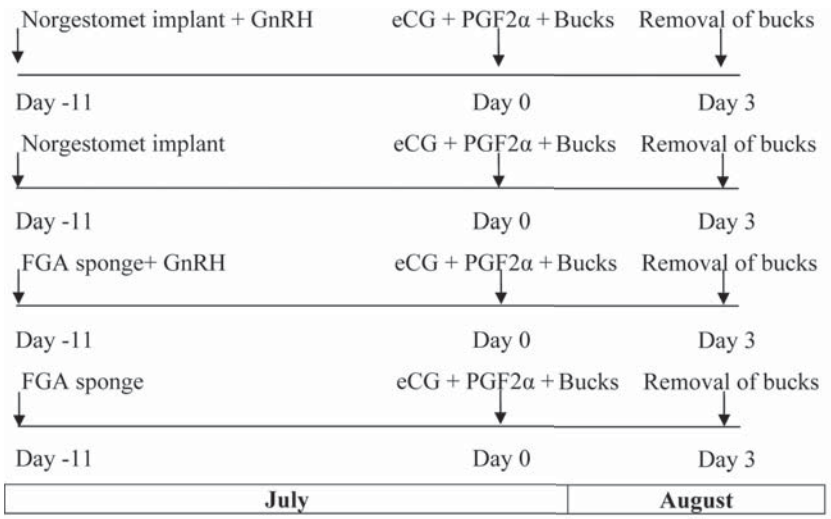

Fig 1. Schematic diagram of the overall experimental design

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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