

# Serum $\beta$ -carotene and Vitamin A Levels in Spontaneous Premature Calves with Respiratory Distress Syndrome

Yusuf GÜL <sup>1</sup>  Burcu GÜL BAYKALIR <sup>2</sup>

<sup>1</sup> University of Firat, Faculty of Veterinary Medicine, Department of Internal Disease, TR-23119 Elazığ - TURKEY

<sup>2</sup> University of Firat, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, TR-23119 Elazığ - TURKEY

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## Summary

The aim of this study was to investigate serum  $\beta$ -carotene and vitamin A levels in spontaneous premature calves with respiratory distress syndrome. As study material, 25 spontaneously newborn premature calves with respiratory distress syndrome brought to Firat University, Faculty of Veterinary Internal Medicine Clinic for examination and treatment were used. After clinical examination, blood samples received from calves analyzed spectrometrically for serum  $\beta$ -carotene and vitamin A levels. Average values of serum  $\beta$ -carotene and vitamin A were found as  $5.22 \pm 0.72$   $\mu\text{g/dL}$  and  $16.63 \pm 0.96$   $\mu\text{g/dL}$  respectively. As a result; serum  $\beta$ -carotene levels in the animals examined here were found to be lower when compared to normal values thus, lack of  $\beta$ -carotene was considered to increase incidence of premature birth of calf with a multi-faceted etiology.

**Keywords:** *Premature, Calves,  $\beta$ -carotene, Vitamin A*

## Respiratorik Distres Sendromlu Spontan Prematüre Buzağlarda Serum $\beta$ -Karoten ve A Vitamini Düzeyleri

### Özet

Bu çalışmada, respiratorik distres sendromlu spontan prematüre buzağlarda serum  $\beta$ -karoten ve A vitamini düzeylerinin araştırılması amaçlanmıştır. Çalışma materyali olarak, Firat Üniversitesi Veteriner Fakültesi İç Hastalıkları Kliniğine muayene ve tedavi için getirilen spontan olarak doğmuş 25 respiratorik distres sendromlu spontan prematüre buzağı kullanılmıştır. Buzağların klinik muayeneleri yapıldıktan sonra alınan kan örneklerinde serum  $\beta$ -karoten ve A vitamini analizleri spektrometrik olarak yapılmıştır. Kan serumu ortalama  $\beta$ -karoten değeri  $5.22 \pm 0.72$   $\mu\text{g/dL}$  ve A vitamini değeri ise  $16.63 \pm 0.96$   $\mu\text{g/dL}$  olarak bulunmuştur. Sonuç olarak; çalışmaya alınan hayvanlarda  $\beta$ -Karoten düzeylerinin sağlıklı buzağlar için bildirilen normal değerlerin altında olması nedeniyle çok yönlü etiyojolojiye sahip prematüre buzağı doğum insidansının  $\beta$ -karoten yetersizliği ile artabileceği kanısına varılmıştır.

**Anahtar sözcükler:** *Prematüre, Buzağı,  $\beta$ -Karoten, A Vitamini*

## INTRODUCTION

Term of born-dead or removal of offspring from uterus that no longer live in a state in external environment due to underdevelopment takes its name as immaturus abortion (abortion). The term of birth of underdeveloped or can-continue-to-live-in-community-with-special-care offsprings defined as abortus prematurus (premature birth) <sup>[1-3]</sup> and offspring called as premature (immature) <sup>[2,3]</sup>.

Mean duration of normal pregnancy reported as 285 (284-286 days) days in culture bred cows and in general, discarded fetuses and offspring within 42 and 260 days

of pregnancy were considered as abortion <sup>[4]</sup> and born offspring after 260 days of pregnancy but those born before normal time were considered as premature birth <sup>[5-11]</sup>.

Causes of abortions in the animals may be infectious or non-infectious. Non-infectious causes may have nutritional, hormonal, genetic or congenital origin, as well as traumas, poisoning, vaccines and pharmaceutical applications, allergic and anaphylactic reactions, maternal febrile diseases, stress and psychic events, twin pregnancies also can be effective <sup>[1,4,5,11,12]</sup>.



### İletişim (Correspondence)



+90 424 2370000



ygul@firat.edu.tr

Although, Vitamin A deficiency (lack of  $\beta$ -carotene), iodine and selenium deficiency, dietary rations poor from protein and energy are nutritional factors that cause premature births [1,5], none of them referred as effective as Vitamin A deficiency [13].

Especially, Vitamin A deficiency can cause fetal deaths, stillbirths and premature births [4,5,13-16].

Different values of  $\beta$ -carotene and vitamin A have been reported in various sources for healthy calves [11,16-19].

In this study, we aimed to investigate serum  $\beta$ -carotene and vitamin A levels at spontaneous premature calves with respiratory distress syndrome.

## MATERIAL and METHODS

Study material includes a total of 25 premature calves from both sexes (11 female, 14 male) and different species (16 Simmental, 5 Holstein, 4 Montofon) brought to Firat University, Faculty of Veterinary Internal Medicine clinic for examination and treatment at 1-5 days age and approximately at 30-40 kg weights and born spontaneously. In the study, due to risk of infection, materials here selected from enterprises with no abortion history. Also, the mothers of premature calves were said to be healthy before the events.

Information about the insemination date of mothers of premature calves was obtained from owners and pregnancy times were calculated. Calves that were born between 260-275 days during period of pregnancy were defined as premature.

During general clinical examination of calves [20], general status, birth weight, hair cover, incisive teeth, ears, nails, and sucking reflex examinations were especially performed. Also presence of several symptoms such as difficulty of breathing and cyanosis were noted.

After systematic clinical examination [20] of all the calves, for analysis of  $\beta$ -carotene and Vitamin A, blood samples collected from V. jugularis were transferred into glass tubes. Then, blood sera were tested by using Shimadzu UV-1208, UV-VIS 240 spectrophotometer as described Suzuki and Katoh [21].

The statistical evaluation of data was carried out by using SPSS MS Windows Release 10.0 software.

## RESULTS

According to the anamnesis, the premature calves were brought to clinic 1-5 after parturition with the complaints to reluctance rise, difficulty in sucking or with no sucking ability in some of them. The owners expressed that some colostrum was given to the calves which do not

suck from the mother with feeding bottle. Also, it was said that none of these calves received Vitamin A injection, but their mothers usually received vitamin A injection once a month, and in general mothers of the calves were given straw, bran, masoor (Lathyrus cicera), barley (broken), dry sugar beet pulp with molasses (some of them was humid), hay, dairy farming (industrial food) and rock salt kept in front of animals, but no additional food additives were added to ration.

Average duration of pregnancy of premature calves included in the study were determined as  $268.56 \pm 0.95$  days (260-275 days).

In general examination of premature calves, body temperature (T), heart (P), and respiratory (R) average (min-max) values determined as  $38.31 \pm 0.24^\circ\text{C}$  (35.3 to 41.0),  $136 \pm 6.46$  pcs/min (100 to 164), and  $55.20 \pm 2.75$  pcs/min (36 to 100) respectively.

Premature calves that brought to clinic were observed to show general weaknesses, most of them were distressed and some were comatose and in lateral decubitus position. Calves in lateral decubitus position, were determined to have difficulty to rise, can not cope up, most of them could not lie in sterno-abdominal position, and their sucking reflex was weak or not-exist.

Several findings, such as inadequate incisive teeth development, hyperemia of gums, short and glossy appearance of hair (fur appearance), easily bending ears and soft tabs were found in all premature calves.

Premature calves with respiratory problem history, were observed to have significant difficulty in breathing (very significantly type of intercostal and abdominal breathing, pulling their nose wings backward during inspiration), developing tachypnea and cyanosis (at nasal mucosa), hyperemic conjunctivae and evident episcleral veins during clinical examination.

In most patients, harsh vesicular sounds were heard during lung auscultation. Also diarrhea in six calves, inflammation of umbilical cord in four calves and both diarrhea and inflammation of umbilical cord in two calves were noted.

Mean serum levels of  $\beta$ -carotene and vitamin A of animals included in the study were presented in *Table 1*.

**Table 1.** Mean serum levels of  $\beta$ -carotene and vitamin A in premature calves (n = 25)

**Tablo 1.** Prematüre buzağılarda ortalama serum  $\beta$ -karoten ve A vitamini düzeyleri (n = 25)

Parameter	X $\pm$ Sx	Min. - Max.
$\beta$ -carotene ( $\mu\text{g/dL}$ )	5.22 $\pm$ 0.72	2.71-19.77
Vitamin A ( $\mu\text{g/dL}$ )	16.63 $\pm$ 0.96	6.78-23.24

## DISCUSSION

Fetal death, stillbirth, premature birth, and calve births with poor chance of survival, which cause should be seen as a complex. The causes of these are not only infectious. In most cases, metabolic disorders which causes weak immunity and defensive power, care and feeding problems should also be considered. In general, there is a combination indicated between microorganisms, immunity weakness and lack of maintenance and nutrition [5].

Average 268 days gestation period of premature calves included in this study was found to be lower when compared to normal gestation periods (286 days) of normal calves and average birth weights of these calves were also found to be lower than normal Holstein, Simmental and Montofon calves that have average of 38, 38, 40 kg live weight, respectively [4]. In addition to lower gestation period and lower birth weight of premature calves, findings such as, short and glossy appearance of hair (fur appearance), soft tabs, inadequate incisive teeth development, easily bending ears, general weakness, weak or non-existent sucking reflex, could not cope up and grogginess shows full compliance to premature calves reported in literature [6-10,22,23].

Respiratory distress syndrome (RDS) is a progressive disorder characterized by abnormal respiration (tachypnea, apnea) and cyanosis at premature calves due to lack of surfactant [23,24]. Abnormal respiration and cyanosis symptoms determined at premature calves shows relevance that reported in RDS [7,23,24].

Although average values of overall clinical findings detected in premature calves (T, P, R) shows compliance with healthy calves [10,11,20,25] and premature calves reported in literature [10,23] in some cases higher body temperature, and higher cardiac and respiratory frequency can be explained possible complications due to infections detected in calves that were studied here (enteritis, omphalitis, pneumonia) [10,12,23] and respiratory distress syndrome [23]. Because of immune system weakness and problems with colostrum intake of premature calves, susceptibility to infectious agents such as, bacteria, protozoa and viruses also increased [11,23]. Premature calves have more susceptibility to respiratoric, metabolic and infectious agents compared to calves that were born in time [23].

In some cases, reported in literature [10,22,23], lower body temperatures were determined. Initially, there was no heat loss in premature newborn calves and body temperature was found around 39°C [10], but then premature calves were determined to have difficulty in maintaining body temperature [22] and reducing body temperatures [10,22,23].

Low body temperatures found in some calves, may be explained by premature calves born with lack of adipose

tissue, that develops towards to the end of fetal life and provide thermoregulation, exposed to cold [10], energy sources can not be used economically due to respiratory difficulty and organism that is unable to produce heat [10,23].

Although, sucking reflex stimulated by finger occurs in 2 to 20 min in healthy calves, weakness or absence of this reflex at premature calves are compatible with literature notifications [6-10,22,23].

Serum levels of vitamin A and  $\beta$ -carotene, which are one of precursors of this vitamin, considered as the most important and utilized parameters in diagnosis of vitamin A deficiency [12,14,16,27-34]. Also serum carotene levels were considered as sensitive indicators of carotene supply [13].

In a study conducted by Stöber [16], blood vitamin A levels were considered as normal, critical and pathological over 12  $\mu\text{g}/\text{dL}$ , between 7-12  $\mu\text{g}/\text{dL}$  and below 7  $\mu\text{g}/\text{dL}$ , respectively, and blood  $\beta$ -carotene levels were considered as normal, critical and pathological over 30  $\mu\text{g}/\text{dL}$ , between 10-30  $\mu\text{g}/\text{dL}$  and below 10  $\mu\text{g}/\text{dL}$ , respectively.

Mean serum vitamin A levels of animals in this study [16.63  $\mu\text{g}/\text{dL}$ ] were found to be compatible with expressed as normal values by other researchers [16-19]. These results were reported as close to mean 18.25  $\mu\text{g}/\text{dL}$  [12.60-31.40  $\mu\text{g}/\text{dL}$ ] of serum vitamin A levels of healthy calves from the region in a study conducted by Gazioglu and Gül [27]. But, average values of serum vitamin A were found to be significantly lower than 25 to 85  $\mu\text{g}/\text{dL}$  levels for healthy cows reported by Smith [11], also found to be lower than levels of 25  $\mu\text{g}/\text{dL}$ , which determined as complete security against hypovitaminosis [16,35].

Alcohol form of vitamin A does not exceed placental barrier, thus liver reserve do not occur in fetus of cows that graze in green grass, in contrast ester form of vitamin A (found as in fish oil) can exceed placental barrier [12,14,36]. In that case, because mothers that feed with green pasture during pregnancy, vitamin A levels in liver of calves are not affected [14] so vitamin A supply of fetuses and calves depends on blood ester vitamin A levels of mother [12,14,36]. At beginning, calves provide their vitamin A needs from colostrums [14]. Vitamin A levels in colostrum also depend on provision of vitamin A of mother during late period pregnancy and because it depends largely on liver reserves [14,16], vitamin A and carotene provision to mother before birth increases vitamin A and carotene levels of colostrums [12,36].

Reddy and Ganapathy [18], reported that vitamin A levels of calves were between 12-24  $\mu\text{g}/\text{dL}$  (mean 19  $\mu\text{g}/\text{dL}$ ) after birth and determined that these values increased within 3 days after supply of colostrums.

Burgstaller et al. [17], reported that blood vitamin A levels of hungry calves (10.3  $\mu\text{g}/\text{dL}$ ) were up to one-third of the mother's blood values, and it increases significantly within

5 days because of high levels of vitamin A in colostrums (22.4  $\mu\text{g}/\text{dL}$ ).

Levels of vitamin A and  $\beta$ -carotene in blood and especially in liver of newborn offspring are very low [37,38]. It has been reported that these levels increase with colostrums intake [17-19,38,39] and especially there is a positive correlation between mother, colostrums and calf for  $\beta$ -carotene [40].

Because premature calves have weak or no sucking or swallowing reflex [6-10,23], their colostrums intake is low or zero. Therefore normal vitamin A levels of the animals studied here, may be explained by vitamin A injection to mother (placental absorption) and vitamin A supply via low or mandatory colostrum intake.

In this study, the values of serum  $\beta$ -carotene were not seen below 0.8  $\mu\text{g}/\text{dL}$  which was reported by Stöber [16] the time of birth for healthy calves. This can be explained slight sucking or colostrum intake necessarily by the owners to calves.

Although, serum  $\beta$ -carotene levels of premature calves (mean  $5.22 \pm 0.72$   $\mu\text{g}/\text{dL}$ ) found to be up to border levels reported by Stöber [16] for newly-born offspring (6.5  $\mu\text{g}/\text{dL}$ ), due to it is lower than reported values for calves which supplied with green fodder or colostrum (30-100  $\mu\text{g}/\text{dL}$ ) and it is compatible with values reported by Gül and İssi [31] for calves with amaurosis condition, this shows mothers of calves, which has not been feeded yet, has been feeded with rations without  $\beta$ -carotene and their colostrum has lower  $\beta$ -carotene levels [12,27,32].

Serum  $\beta$ -carotene levels of study offspring (mean  $5.22 \pm 0.72$   $\mu\text{g}/\text{dL}$ ) found to be lower than values that accepted [16] values for deficiency (10  $\mu\text{g}/\text{dL}$ ), and maximum measured serum  $\beta$ -carotene levels between study animals (19.77  $\mu\text{g}/\text{dL}$ ) also found to be between values that accepted values for critical level [16] (10-30  $\mu\text{g}/\text{dL}$ ). Mean  $\beta$ -carotene levels identified for study offspring found to be significantly lower than reported values for healthy calves by Reddy and Ganapathy [18] and Surynek et al. [19] which is 43  $\mu\text{g}/\text{dL}$  and 87.5  $\mu\text{g}/\text{dL}$ , respectively. Although these differences may be the result of different nutritional conditions, no or poor sucking reflex of premature calves believed to be effective enough for this results.

Due to ruminants only taking precursor  $\beta$ -carotene for vitamin A supply, vitamin A deficiency in cattle always shows a primary  $\beta$ -carotene deficiency. This also expressed that embryonic deaths and abortions occurred due to advanced degree  $\beta$ -carotene deficiency (despite vitamin A supply is high). Bostedt and Klein [5] determined that in 2003 to 2005, 30% of 38 enterprise with stillbirth complexity has  $\beta$ -carotene supply deficiency.

Serum  $\beta$ -carotene levels of premature calves in this study (mean  $5.22 \pm 0.72$   $\mu\text{g}/\text{dL}$ ), suggest that these calves

were affected from severe  $\beta$ -carotene deficiency.

In accordance with the literature notifications about catarrhal and haemorrhagic bowel inflammation can be seen due to vitamin A deficiency [15,29,41,42], diarrhea has been observed in some premature calves. It is thought that this might have occurred due to  $\beta$ -carotene deficiency [31] because compliance of reported serum vitamin A levels for healthy animals and in serum vitamin A levels of premature calves with observed diarrhea [16-18]. Because it has been reported as low levels of  $\beta$ -carotene results in breakage of intestinal epithelium resistance and helps formation of intestinal infections [15,41]. Also contrary to conventional opinion, it has been expressed as  $\beta$ -carotene deficiency is predisposing factor for colibacillosis rather than vitamin A deficiency [29]. Prohaszka [42] has determined that a significant relationship between frequency of diarrhea due to colibacillosis and seasonal patterns of  $\beta$ -carotene levels of cows.

Gazioglu ve Gül [27], reported that injection of vitamin A preparations may be useful due to the lower serum  $\beta$ -carotene and vitamin A levels of premature calves with diarrhea.

Gül [29] expressed that feeding cows with  $\beta$ -carotene-rich rations reduces morbidity and mortality of calf diseases (enteritis, neonatal, septicemia).

Lotthammer [43] investigated effects of  $\beta$ -carotene deficiency on health, and reported that despite being granted sufficient vitamin A, calves of cows that has undernourishment of  $\beta$ -carotene, have more cases observed diarrhea and died few weeks after birth.

Clinic observations, other studies conducted in region [27-34] and the results of this study showed that in general, cattle has been undernourished with  $\beta$ -carotene in this region.

As a result; serum  $\beta$ -carotene levels in animals examined here were found to be lower when compared to normal values thus, lack of  $\beta$ -carotene was considered to increase incidence of premature birth of calf with a multifaceted etiology.

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