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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Seasonal Changes of FSH, LH, Total and Free Testosterone Hormones in Saanen Bucks*

Saanen Tekelerde FSH, LH, Total ve Serbest Testosteron Hormonlarının Mevsimsel Değişimleri

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Abstract

In the study, seasonal changes in follicle-stimulating hormone (FSH), lutein hormone (LH), total testosterone (TTH), and Free Testosterone Hormone (FTH) of Saanen goats were investigated. In order to determine the hormone levels between February 2019 and December 2019, blood samples were taken from bucks (n=6) twice a week during the entire trial. FSH level started to decrease relatively after August and the lowest value was determined as 173.01 mlU/ml in January. The difference between the months in the mean FSH level was found to be significant ($p < 0.05$). The highest LH level was obtained in July (13.12 mIU/mL) and the lowest level (8.02 mIU/mL) was obtained in May. However, during the mating season, the increase in LH level was found to be similar to the FSH level. The difference in LH levels by months was found to be statistically significant ($p<0.05$). Total testosterone level was similar to FSH and LH levels. The highest and lowest total testosterone hormone (TTH) levels in March and January, respectively; 3.03 ng-/mL and 0.62 ng-/-mL. The highest free testosterone hormone (FTH) level was 119.69 pg/ml in August and the lowest was 29.09 pg/ml in December. The difference between FTH by months was significant ($p<0.05$). The effect of day length on seasonal variation of reproductive hormones in Saanen bucks is statistically significant ($p<0.05$). As a result, it was determined that reproductive hormone levels in Saanen bucks depend on the season and the release levels are significantly affected by climatic factors such as day length. To summarize, there is a need for more different studies including sampling frequency, sampling time as well as sample size, and other environmental factors in order to examine the effects of male reproductive characteristics and other important hormones in bucks in more detail to interpret the results more accurately.

Keywords: Saanen buck, Reproductive hormone, Seasonal change, Free testosterone hormone, Annual cycle

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Öz

Araştırmada, Saanen tekelerine ait folikül uyarıcı hormon (FSH), lutein hormon (LH), toplam testosteron (TTH) ve Serbest Testosteron Hormonunun (FTH) mevsimsel değişiklikleri incelenmiştir. Şubat 2019 ile Aralık 2019 arasındaki aylarda hormon düzeylerinin belirlenmesi için tekelerden (n=6) haftada iki kez olmak üzere tüm deneme süresince kan örnekleri alındı. FSH düzeyi, ağustos ayından sonra göreli olarak düşmeye başlamış ve en düşük değer ocak ayında 173.01 mlU/ml olarak belirlenmiştir. FSH düzeyi ortalamalarında aylar arasındaki fark anlamlı bulunmuştur ($p < 0.05$). En yüksek LH düzeyi Temmuz (13.12 mIU/mL) ve en düşük düzey ise (8.02 mIU/mL) mayıs ayında elde edilmiştir. Ancak çiftleşme mevsiminde, LH düzeyindeki artışın FSH düzeyiyle benzer olduğu saptanmıştır. Aylara göre LH düzeylerindeki fark istatistiksel olarak önemli bulunmuştur ($p<0.05$). Toplam testosteron düzeyi, FSH ve LH düzeyi ile benzerlik göstermiştir. Mart ve ocak aylarında en yüksek ve en düşük toplam testosteron hormonu (TTH) düzeyi sırasıyla; 3.03 ng-/mL ve 0.62 ng-/-mL dir. En yüksek Serbest testosteron hormonu (FTH) düzeyi ağustos ayında 119.69 pg/ml, en düşük ise aralık ayında 29.09 pg/ml olarak belirlenmiştir. Aylara göre FTH arasındaki fark anlamlıdır ($p<0.05$). Saanen tekelerinde üreme hormonlarının mevsimsel değişimi üzerine gün uzunluğunun etkisi istatistiki olarak önemlidir ($p<0.05$). Sonuç olarak, Saanen tekelerinde üreme hormonu düzeylerinin mevsime bağlı olduğu ve salım düzeylerinin gün uzunluğu gibi iklimsel faktörlerden önemli ölçüde etkilendiği belirlenmiştir. Özetlemek gerekirse, tekelerde erkek üreme özellikleri ve diğer önemli hormonların etkilerini daha ayrıntılı incelemek ve sonuçların daha sağlıklı yorumlanabilmesi için örnek alma sıklığı, örnek alma zamanının yanı sıra örnek büyüklüğü ve diğer çevresel faktörlerin de içinde yer aldığı daha farklı çalışmalara da ihtiyaç vardır.

Anahtar Kelimeler: Saanen teke, Üreme hormonu, Mevsimsel değişim, Serbest testosteron hormonu, Yıllık döngü

1. Introduction

The seasonality of reproduction is influenced mainly by annual variations in the photoperiod. It means that increase the proportionally to latitude such that reproductive and non-reproductive seasons for some farm animals (Sharpe, 2003; Abecia et al., 2012; Vasantha, 2016). However, in animals raised in tropical regions, other environmental factors other than day length also influence the mating season (Rosa and Bryant, 2003; Nakao et al., 2008). The most obvious effects of seasonal variation in reproduction are seen in female mammalian farm animals, cattle may be excluded (Koluman Darcan et al., 2013). These animals have a mating and anestrus period, unlike poultry (Goodman and Inskeep, 2006; Cattanach et al., 1977). Males have a mating season similar to females. (Chemineau et al., 1988; Cheng et al., 1981). Characteristics in male animals that change depending on the season are respectively; sexual activity, semen production, testicular characteristics, and accordingly changes in the level of reproductive hormones (Kafi et al., 2004; Zamiri and Khodaei, 2005; Todini et al., 2007; Zarazaga et al., 2009).

FSH and LH hormones play an important role in spermatogenesis in male farm animals (Kumar et al., 1997; Araki et al., 2000). However, studies in male mice suggest that a mutation occurring during FSH production adversely affects both testicular and semen characteristics. In addition, growth retardation in reproductive organs negatively affected Gn - RH production of spermatozoa capable of fertilization in mice (Cattanach et al.,1977; Abdelrahman et al., 2019). FSH and LH hormones regulate the function of reproductive organs by a feedback mechanism. The issue of how the inhibin hormone regulates FSH secretion in domestic mammal female animals is still a research topic. Immunoneutralization of inhibin hormone in females has been studied in many animal species such as the rat (Culler and Negro-Vilar, 1988), cattle (Kaneko et al., 1995), sheep (Mann et al., 1990), and hamster (Kishi et al., 1997) and was observed to cause a certain increase in FSH secretion in most of the animals.

FSH and LH are important hormones in the reproductive physiology of both male and female organisms (Şenok et al., 2020). However, in male animals, testosterone is the most important gonadal hormone regulating both FSH and LH secretion. As known, the role of the inhibin hormone, which acts as a regulator of FSH secretion, is largely dependent on the species and age of the animal. However, the importance of inhibin hormone in the regulation of FSH secretion in adult male animals varies between species (Martin et al., 1991; McKeown et al., 1997; Dias et al., 2012). In this study, it was aimed to investigate the seasonal variations of some reproductive hormones in Saanen bucks. For this purpose, the experiment was carried out to determine the annual cycles of FSH, LH, total testosterone and free testosterone hormones of bucks.

2. Material and Method

This research was conducted under the supervision of the Ege University Animal Experiments Local Animal Ethics Committee (*number: 2017-0/90*). This study was carried out on province, Turkey. The average annual precipitation is 742 mm. Bornova district coordinates are latitude 38.4710 and longitude 27.2177. The highest and lowest ambient temperature the in Bornova district in June and January months is 31°C and 5.3°C, respectively. Relative humidity in the study area changes between 48 % and 79 %. The experiment animals consist of 2-year-old 6 head Saanen bucks.

The barn where the goats were stabled has a total floor area of 72 m2 (12 individual pens × 6 m2). In addition, there was a open-top area of 10 m2 in front of each pen. The energy content was 1.2 times the energy requirement in flushing rations for bucks (NRC, 2007). Alfalfa hay (89.44% DM, 18.59% CP), and corn silage (87.21% DM, 9.88% CP) were fed concentrated feed (90.44% DM, 17.99% CP) 700 g was given to the animals individually in the male units. There is at least one double and 8-liter capacity floated waterer in each pen in the facility. The staff veterinarian of Ege University routinely performed health protection practices for all animals.

In this study, five-milliliter blood samples were taken (*AYSET TUBE 8,5 mL Serum Sep Clot Activator*) twice a week from bucks (96 times/1 buck) to determine hormones level (Yarney and Sanford, 1983). Blood samples were centrifuged at 5000 rpm for 10 minutes and then stored at -20 degrees Celsius. The blood serum was performed by radioimmunoassay method. Levels of the free testosterone were measured by RIA kit (Immunotech Beckman Coulter Company, RIA Testosterone: REF DSL4900), the sensitivity of this assay was 0.13 pg/mL of serum. Levels of the testosterone were measured by RIA kit (Immunotech Beckman Coulter Company, RIA Testosterone: REF IM1087), the sensitivity of this assay was 0.05 ng/mL of serum. Levels of the FSH were measured by RIA kit (Immunotech Beckman Coulter Company, RIA Testosterone: REF IM3301), the sensitivity of this assay was 0.17 IU/L of serum. Levels of the LH were measured by RIA kit (Immunotech Beckman Coulter

Company, RIA Testosterone: REF IM1381), the sensitivity of this assay was 0.16 IU/L of serum. Analyzes on hormones took place in the pharmaceutical laboratory of the nuclear center research at Ege University. Serum FSH, LH, total and free testosterone hormone levels were analyzed using the SAS. Analysis was done on the monthly individual average of the samples taken. The experiment (differences between months investigated for each hormone parameter) were analyzed repeated measures analysis of variance (ANOVA). The difference between the months was observed with Duncan Test (Gürbüz et al., 2003).

3. Results

FSH hormone level has higher values in between August and November in bucks compared to other months due to the mating season. In all bucks, the highest value was obtained in August during the mating season. In August, the mean value of FSH is 411.65 mlU/ml. FSH hormone level started to decrease relatively after August and the lowest value was determined as 173.01 mlU/ml in January.

As expected, bucks first increased during the breeding season (between August and November), peaked in August, and then decreased with the autumn season. The difference in FSH level among months was found to be statistically significant. When the male goats were examined individually, the difference in FSH levels was significant in August ($p < 0.05$). Follicle Stimulating Hormone means were given in *Table 1*. Before the mating season (January – July), the LH analysis revealed that the individual data of LH were very close to each other. The figures are similar to FSH values. The highest LH levels were in July (13.12 mIU/mL) and the lowest in May (8.02 mIU/mL). However, by entering the mating season, it was found that LH values also increased as much as FSH values. When the means were examined for a mating period, the highest and lowest LH values were 34.24 mIU/mL, 12.00 mIU/mL, respectively. LH values of the Saanen bucks were given in *Table 1*. The difference in LH levels by months was statistically significant ($p<0.05$).

The TTH values (January - July) were found to be very close before the breeding season. The total testosterone values showed a resemblance to the FSH and LH values. The highest and lowest TTH levels were 3.03 ng/mL and 0.62 ng/mL in March and January, respectively. However, the TTH value, at the start of the breeding season, increased very rapidly similar to those of FSH and LH values. The highest TTH level was 14.85 ng/mL in August and the lowest value was 3.99 ng/mL in December. The difference between the TTH values in terms of months was significant ($p<0.05$). The total testosterone hormone (TTH) were given in *Table 1*.

Before the breeding season (January – July), the free testosterone hormone (FTH) level showed that the individual values of FTH were very close to each other. The values of free testosterone (FTH) show a similarity to FSH, LH, and TTH values. The highest FTH level in March was 10.76 pg/mL and the lowest was 0.82 pg/mL in January. However, with the emergence of the breeding season, similar to that of FSH, LH, TTH, and FTH values were also found to increase very rapidly. The highest FTH level in August was found to be 119.69 pg/mL and the lowest was 29.09 pg/mL in December. The difference between the FTH by months was significant ($p<0.05$). FTH values of Saanen bucks were given in *Table 1*.

Table 1. Hormones level changes by months (mIU/ml)

	Jan	***Feb***	***Mar***	***Apr***	***May***	***Jun***	***Jul***	***Aug***	***Sep***	***Oct***	***Nov***	***Dec***
FSH	173.01	176.58	176.47	173.37	180.50	182.74	193.73	411.65	282.41	278.77	274.34	239.98
(mIU/ml)	±0.71 [a]	±1.34 [a]	±1.40 [a]	±1.58 [a]	±0.71 [b]	±0.74 [b]	±1.08 [b]	±7.12 [d]	±5.45 [c]	±7.99 [c]	±4.23 [c]	±1.99 [c]
LH	9.06	8.51	9.74	8.80	8.02	8.82	13.12	34.24	25.78	20.70	18.33	12.00
(mIU/ml)	±0.47 [b]	±0.61 [a]	±0.36 [b]	±0.54 [a]	±0.40 [a]	±0.53 [a]	±0.34 [c]	±0.20 [e]	±0.38 [d]	±0.81 [d]	±0.40 [d]	±0.36 [c]
TTH	0.62	0.93	3.03	1.73	1.55	0.98	1.23	14.85	10.71	11.89	7.08	3.99
(mIU/ml)	±0.11 [a]	±0.34 [a]	±0.72 [c]	±0.46 [b]	±0.46 [b]	±0.21 [a]	±0.26 [b]	±0.91 [f]	±2.03 [e]	±0.90 [e]	±0.70 [d]	±1.31 [c]
FTH	0.82	1.50	10.76	4.30	5.03	1.83	3.41	119.69	63.48	69.62	57.39	29.09
(mIU/ml)	±0.29 [a]	±0.58 [a]	±3.15 [c]	±2.16 [b]	±2.14 [b]	±0.44 [a]	±1.12 [b]	±15.11 [f]	±15.0 [c]	±8.08 [e]	±6.20 [e]	±8.92 [d]

a,b,c,d :different lower cases in the same line represent statistically significant differences

Reproduction hormones analysis revealed in examined that the individual data of all hormones were very close to each other (*Figure 1*). The highest hormones levels were in August. However, this peak continued to decline after a month.

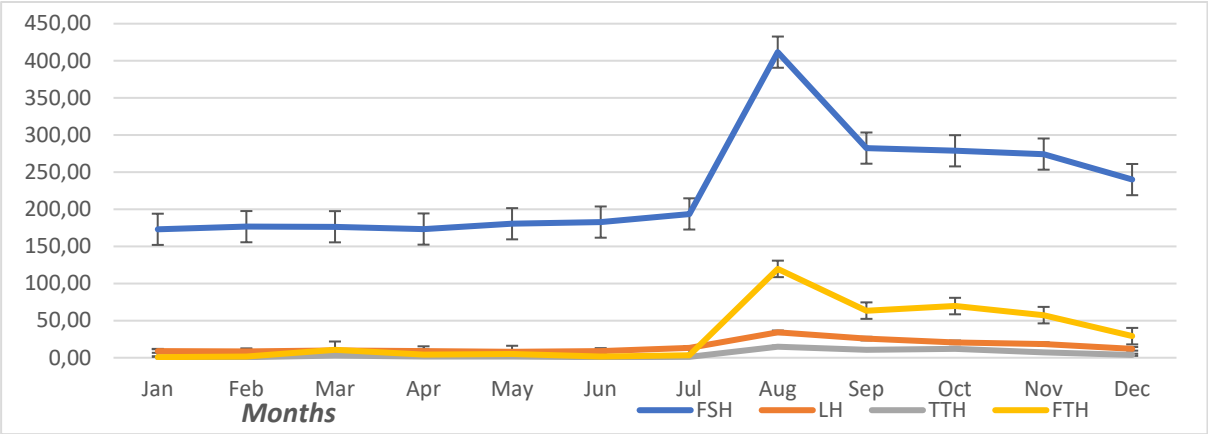

Figure 1. Change of hormones throughout the year

4. Discussion and Conclusion

This study was conducted in order to determine the year-round variability of some reproductive hormones levels in Saanen bucks vary during the whole year. In addition to the determining factor of day length, incommercial goat farms, the length of the breeding season is varying among different genotypes also depending on different environmental factors and especially day length (Yılmaz, 1999; Karadağ and Soysal, 2018; Nikbin et al., 2018). The effect of day length on bucks is proportionally less than on does. However, this effect can sometimes be negative on testicular development and sperm production (Chemineau et al., 1988; Marai et al., 2007; Coloma et al., 2011; Fatet et al., 2011). Reproductive activity on bucks raised in temperate climates is not wholly dependent on the season such as goats, but they generally show a reproductive cycle stimulated by reduced day length (Todini et al., 2007; Muduuli et al., 1979; Dellal and Cedden, 2012). In small ruminants, ovulation/mating generally occurs in autumn. The breeding season length of Saanen bucks in İzmir province where the study was conducted continues from September until the end of November (Kandemir et al., 2018). As it is known, the control of reproduction depending on the length of the day is controlled by increasing regular secretions of melatonin hormone produced from the pineal gland in the period when the days begin to decrease (Eldon, 1993; Gomez-Brunet et al., 2009).

The results of this study on FSH concentrations in Saanen bucks during the breeding season were examined, the difference in FSH between months was statistically significant ($p<0.05$). These findings of FSH concentrations are similar to those obtained in other goat and sheep breeds (Araki et al., 2000; Gomez-Brunet et al., 2009, Findlay and Clarke, 1987). Increased levels of Gn-RH cause the expression and secretion of FSH and LH hormones for goats (Mori and Kano, 1984; Malpaux et al., 1994; Medan et al., 2003).

The differences between months in FSH and LH concentrations for Saanen bucks are similar to studies in Angora goats (Loubser et al., 1983), dwarf goats (Howland et al., 1985), Australian Kashmiri goats (Walkden-Brown et al., 1994), Zaraibi goats (Barkawi et al., 2006), and Angora goats (Pehlivan et al., 2017). Similar to our study, Todini et al. (2007) found that plasma testosterone concentrations for bucks were higher in summer than autumn. The study of Chentouf et al. (2011) confirms these results. The authors stated that plasma testosterone levels in Northern Moroccan bucks increased during the spring and summer. This indicates the photoperiod-related reproductive seasonality of Northern Moroccan bucks. On the other hand, seasonal changes in plasma testosterone levels for Akkeçi bucks in Turkey were determined in autumn with the highest values and the lowest values in spring (Polat et al., 2011). Todini et al. (2007) found that plasma testosterone concentrations for bucks were higher in summer than autumn. Similar results were found in this study. Plasma testosterone levels increased during the spring and summer (Chentouf et al., 2011). This indicates the photoperiod-related reproductive seasonality of northern Moroccan bucks. Barkawi et al. (2006) concluded that seasonal sexual activity has a different period in Zaraibi goats. In the northern hemisphere, especially in autumn and summer, gonadal hormones are working effectively above the standard level. Perez and Mateos (1995) reported that Maguena and Vareta bucks were at high levels in autumn and summer. Zarazaga et al. (2009) determined the effects of the season on the reproductive performance with intense sexual activity between August and November for Payoya bucks. Circumference of the

scrotum is positively related to sexual activity. Al-Ghalban et al. (2004), detected that circumference of the scrotum had high figures in August for Damascus bucks. In the same study, they reported that it started to increase for the spring months. Circumference of the scrotum is positively related to sexual activity

The plasma testosterone increase begins before or around the summer in subtropical regions in Mexico's local breeds (Delgadillo et al., 2002; Delgadillo et al., 2004). Similar effects were seen on testosterone hormone levels in Creole bucks from Mexico (Delgadillo et al., 2001). The testosterone hormone plays an important role breeding, regulation of secondary sexual characteristics and spermatogenesis in male goats (Perez and Mateos, 1995; Polat et al., 2011). The seasonal variation of testosterone hormone level is controlled by LH concentrations synthesized in the pituitary gland (Delgadillo et al., 1999; Delgadillo et al., 2001). According to the results, the levels of LH and testosterone hormones in Saanen bucks during the breeding season (September-October) show similar results. Mean testosterone hormone levels for Saanen male goats' mean in August were 119.69 ng/ml (*Table 1*). Similar results have been reported in other studies performed for Angora male goats, the testosterone values in breeding season were observed to be 15.86 ng/ml by Loubser et al. (1983), 9.21 ng/ml by Ritar (1991) and 13.80 ng/ml by Pehlivan et al. (2017). The results of the testosterone concentrations in the Saanen bucks were also consistent with those reported in the other goat breeds (Zarazaga et al., 2009; Muduuli et al., 1979; Howland et al., 1985; Polat et al., 2011). The effects of the seasons affect the sexual activity of females more than male goats. Throughout the year, sexual activity in male goats does not completely stop, and when the level decreases, it is not observed. Male reproductive cells production continues. Male reproductive cell production continues. It is not affected by the seasons. It is observed that the expression of sexual activity increases as the mating season approaches (Delgadillo et al., 2001, Quin et al., 2000; Zhang and Yang, 2006; Mayer, 2016).

In this study, it was determined that the reproduction hormone secretions in Saanen bucks were season dependent and their release levels were significantly affected by environmental factors such as day length. Additionally, the Saanen bucks studied were found to exhibit intense sexual activity in July and August.

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