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SEMEN QUALITY, TESTICULAR CHARACTERISTIC, BIOCHEMICAL PROFILE AND HISTOPATHOLOGY OF TESTES OF GOATS UNDER HEAT STRESS CONDITIONS

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ABSTRACT

High temperature during hot summer months is associated with poor animal reproductive performance. The study aimed to explore the seasonal thermal impacts on the bucks fertility. Twenty healthy mature bucks were used in this study. Depending on environmental temperature, bucks were equally divided into two groups: Winter season (n=10) and Summer season (n=10). Scrotal width, circumstances, length and diameter of right and left testicles, as well as length of right and left testicles were evaluated. From each goat, the semen samples were collected once a month during Summer and Winter seasons. Immediately after collection of semen, volume of semen, pH, concentration, motility, live sperm percentage, sperm morphology (1^{st} abnormalities and 2^{nd} abnormalities), sperm viability and intact acrosome were evaluated. Plasma triglyceride, cholesterol, glucose and testosterone concentrations were monitored. The results revealed that scrotal circumstances, length, diameter of right and left testicles and length of right and left testicles were lower ($p<0.05$) in Summer season than winter. Scrotal width was not different between the seasons. The Semen volume, concentration, motility, percentage of live sperm and intact acrosome in the Summer season were lower ($p<0.05$) compared to winter. Semen pH, 1^{st} abnormalities and 2^{nd} abnormalities in Summer season were higher ($p<0.05$) compared to winter. Plasma triglyceride, cholesterol, glucose and testosterone concentrations were decreased ($p<0.05$) during summer than in winter. Histopathological lesions were found in Summer group in comparison to winter. In conclusion, the findings confirm that a high temperature during summer conditions negatively affects bucks semen quality.

Keywords*:* Heat stress, season, subtropical climate, male goats, fertility.

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INTRODUCTION

Recognizing the effects of environmental changes, including temperature, humidity and daylight, is critical for successful livestock health and production. Heat stress is one of the major serious environmental stresses that threaten animal and poultry production (Belhadj Slimen *et al.,* 2016, Auboghaba *et al.,* 2021). The rise in ambient temperature harmed livestock welfare, production and reproduction (Jamal *et al.,* 2021). Many studies have been reported the negative impacts of heat stress on male reproductive functions like sperm quality, steroid hormone production, sexual desire and fertility (Al-Kanaan *et al.,* 2015). Heat stress has an immediate impact on fertility by impairing the function of reproductive cells and can indirectly alert reproduction because heat-stressed animals typically have lower nutrient intake (Sejian *et al.,* 2018). Also, it has a direct deleterious impact on the morphology of sperm and fertility (Shahat, *et al.,* 2020). Additionally, heat stress decreased the flow of blood to the testicles (Samir *et al.,* 2018; Hedia *et al.,* 2020).

Goat is thought to be more resistant to fluctuations of temperature compared to other farm animals because they reduce their metabolic rate while increasing their respiratory and cardiac rates (El-Sherbiny *et al.,* 2022). Moreover, goats respond differently to handling stress depending on individual responses, breed and province (Sharma *et al.,* 2013; Salama *et al.,* 2014). Baladi goat is sensitive to thermal stress compared to crossbred goat in subtropical conditions (Teama and El-Tarabany 2016; El-Tarabany *et al.,* 2017). The environmental factors affecting animal reproduction are temperature, humidity and daylight. Due to seasonal variables, the quality of bucks semen might fluctuate over time (Dias *et al.,* 2017; Isnaini *et al.,* 2020). The main indicator used to monitor the thermal stress condition for animal is the temperature humidity index (THI) (Ribeiro *et al.,* 2018a). Elevation of THI to more than 75 was considered a heat stress status for animals (Sejian *et al.,* 2018). Additionally, heat stress influences sperm quality in bucks semen, although decreased semen quality was collected during high THI (Isnaini *et al.,* 2020).

The climate seasons (summer and winter) in Shalateen, Halayib have variables ambient temperatures, relative humidity and THI. Shalateen, Halayib province located in southeastern Egypt, has the hottest dry season (summer). The objective of the current study is to characterize the degree of testicular damage on bucks in Shalateen, Halayib caused by summer thermal stress.

MATERIALS AND METHODS

Animals

Twenty healthy mature bucks, 4 to 5 years of age and weighed 41 ± 4.3 kg were used in the present study. Bucks were reared in the experimental animal Farm, Shalateen, Halayib, Egypt. Shalateen, Halayib province is located in the southeastern region of Egypt where the climate is subtropical. Depending on the environmental temperatures (Table 1 & Figure 1), bucks were divided into two groups: Winter season (n = 10) from December 2021 to February 2022 and Summer season from June to August, 2022 (N = 10). The climate data including the temperature, relative humidity percentage and daylight hours were obtained from the local meteorological center (Meteorological authority, Egypt, Shalateen, Halayib province). To calculate THI, the following equation was used: $THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$ (Kendall and Webster 2009).

The protocol was approved by the Animal Care Committee, South Valley University, Qena Governorate with ethical approval number (No.71/27.09.2022). Bucks husbandry and experimental procedures were performed under the South Valley University, Veterinary Medicine Faculty Animal Care Guidelines.

Testicular characteristics measurement

Testicular characteristics in bucks including scrotum circumference, length and width, right testicular length (TD) and diameter (TD), as well as left testicular length and diameter were measured monthly before semen collection. Metal calipers were used to measure TD and TL. A measuring tape was used to measure scrotum circumference and width.

Collection of semen and evaluation

Before the study, bucks were trained to collect semen samples by use of an artificial vagina. Ejaculates used in the study were selected according to the basic sperm parameters as follows; semen volume (≥ 0.2 mL), sperm concentration (10^6/ml) and spermatozoa motility (60 %). From bucks, semen was collected monthly (last Thursday of each month) during 8.30 am -12.30 pm. After semen collection, Semen volume was determined in the spermatic fraction using a graduated plastic tube. Sperm concentration was estimated using a Neubauer hemocytometer (Komsky-Elbaz and Roth, 2018). Sperm morphology was examined using eosin-nigrosin staining. Semen drop (5µl) was smeared on a slide and stained with eosin-nigrosin staining. The sperm abnormalities percentage (1^{st} and 2^{nd} abnormalities) was recorded after the examination of sperms (around 200 sperms) using bright field microscopy (magnification1000x, oil immersion objective).

Sperm viability and acrosome integrity

Sperm viability and acrosome integrity were evaluated as described previously (Nur *et al.,* 2005). Briefly, 50 µl of diluted semen mixed with 50 µl of sodium citrate 2.9%. From the mixture a smear was prepared. The smears were fixed with 10% formal solution (15 min), washed with water (15 min) and then immersed in Giemsa stain (3 hrs). The stained slides were rinsed with tap water for 15 minutes before drying. Under a microscope (x 100 magnification), about 100 spermatozoa were randomly selected per slide and examined for live sperm with intact acrosomes to determine viability and acrosome reaction.

Hormonal and biochemical analyses

Jugular vein puncture was used to collect plasma into vacutainer tubes without anticoagulant (Essawi *et al.,* 2021; Mohamed *et al.,* 2021a). Tube was centrifuged at 3000 g/15 minutes. The plasma was collected, gathered and kept with -20°c till analysis. Testosterone concentrations in plasma were determined by ELISA technique using the commercial kits (Monobind USA Cod: 3725-300). The Coefficients of variation of intra assay was 4.8% and inter assay was 9.7%. Utilizing a commercial kit, glucose levels were measured spectrophotometrically (Spectrum Egypt, Cod: 250- 001) (Mohamed *et al.,* 2021b). Commercially available kits were used to estimate cholesterol concentrations (Cod; CHOL230001, Spectrum, Egypt). Utilizing commercially available kits, triglyceride levels were calculated (Cod; M4200, Spectrum, Egypt).

Histopathological examination

Specimens of the bucks testes were taken and they were then dipped into 10% neutral buffered formal saline. The samples were cleaned and embedded in paraffin wax after being dehydrated in different alcohol grades. For general histological investigation, paraffin sections (3-5 µm) were cut by a microtome (Leica RM2235, Leica Biosystems) and immersed in hematoxylin and eosin stains (Suvarna *et al.;* 2018).

Data analysis

Statistical comparison was made using Graph Pad Prism 5 statistics. Climate parameters such as temperature, humidity, THI and daytime were compared between seasons by analysis of variance (ANOVA) and a post-hoc test with Bonferroni. The differences in semen characteristics, testicular characteristics and biochemical parameters between seasons were analyzed using a student t-test. $P < 0.05$ was considered statistically significant. The data was expressed as mean ± SEM

RESULTS

Figure (1) and Table (1) showed the seasonal and monthly changes in ambient temperature, relative humidity as well as THI in Shalateen, Halayib region. Summer had the highest mean ambient temperature, which was higher ($p<0.05$) compared with winter. Winter had the highest mean relative humidity and was higher ($p<0.05$) compared with summer. Summer had the highest THI and was higher ($p<0.05$) compared with winter. Summer had a longer daytime and was higher ($p<0.05$) compared with winter.

Testicular measurements variation across seasons

Testicular measurements varied by season as shown in Table (2). The SC, SL, RTL, LTL, RTD, and LTD were smaller ($p<0.05$) during summer compared with winter. SW was not significantly different across seasons.

Effect of season on semen parameters

Parameters of semen quality varied by season Table (3). Semen volume, spermatozoa concentration, spermatozoa motility, live sperm % and intact acrosome% in the summer season were lower ($p<0.05$) compared with the winter. However, semen pH value, 1st abnormalities percentage, and 2nd abnormalities percentage in the summer season were higher ($p<0.05$) compared with the winter.

Impact of season on biochemical and hormonal parameters

The study showed that in summer when the temperature was high, plasma triglyceride, cholesterol, glucose and testosterone concentrations were lower ($p<0.05$) compared with the winter Table (4).

Effect of season on histology of testes

The current result investigated the histomorphological changes of the bucks testes under the seasonal variation. The normal testicular histological appearance was demonstrated within a moderate temperature season as Winter (F. 2A). On the other hand, in the high-temperature season as Summer season, the testicular histology showed some changes which influence the spermatogenesis process. These changes included degeneration of the seminiferous tubules, the appearance of excessive fragmented and desquamated epithelial cells within the lumen of the tubules, and edema within the interstitial tissues besides hemorrhage. Furthermore, vacuoles were observed within the lining of spermatogonic cells. The heat stress might be inducing shrinkage of the wall of the seminiferous tubules and decreasing the number of the sperms and spermatocytes (F. 2,B to H).

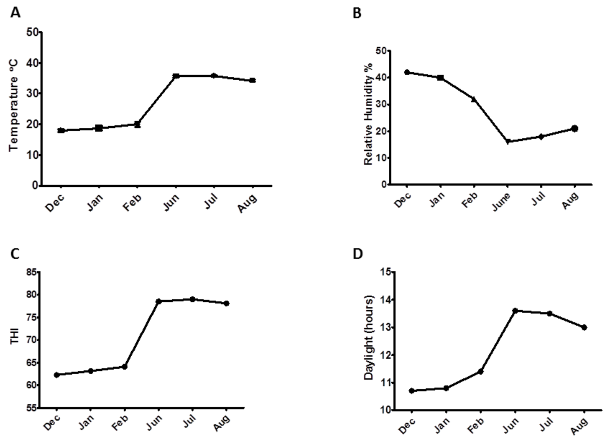

Figure (1). Environmental temperature (1A), Percentage of humidity (1B), Temperature and humidity index or THI (1C) and Daylight (1D) during Winter and Summer seasons were obtained from the Egyptian Meteorological Authority for Shalateen, Halayib province. Values are expressed as mean± SEM.

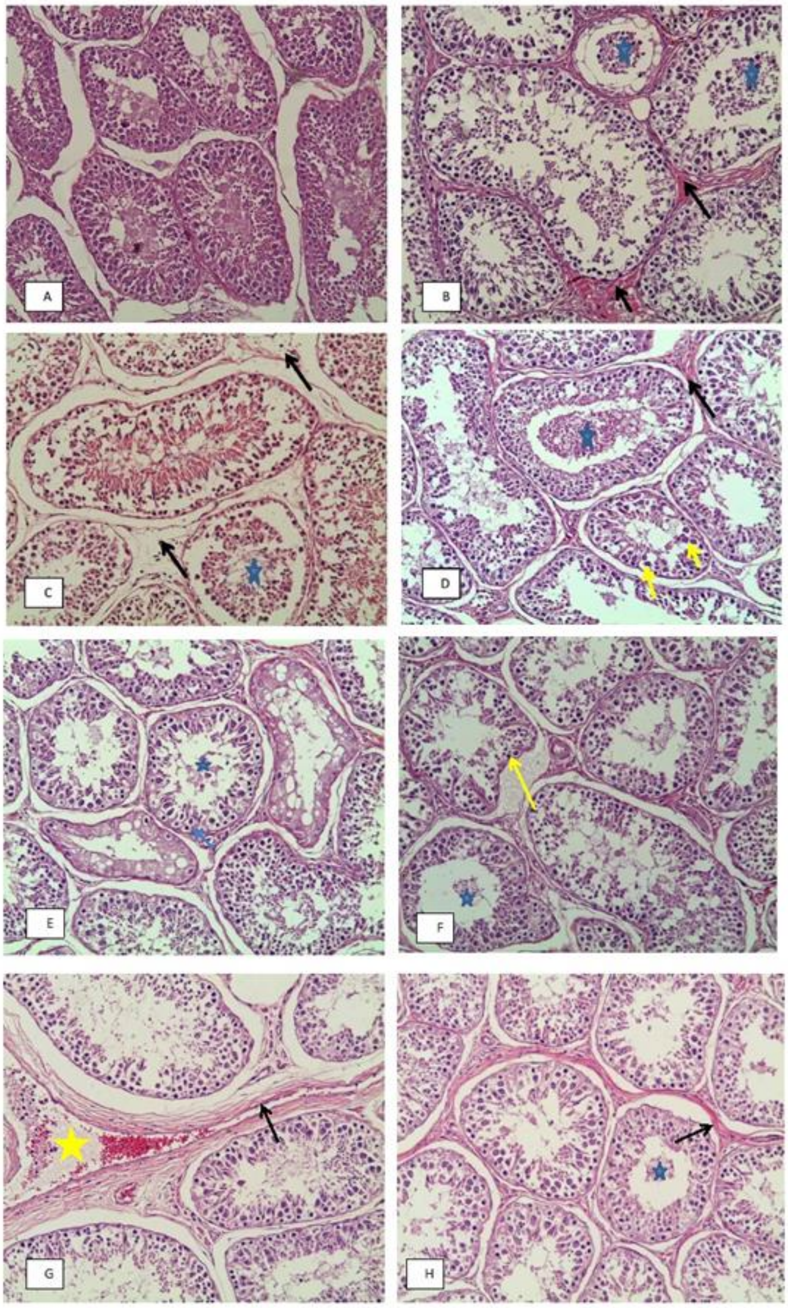

Figure (2): A: Testes showing normal appearance. B, C &D: Showing degeneration of seminiferous tubules, desquamated epithelial cells inside the lumen of tubules (star), interstitial edema (black arrow) and vacuolation of spermatogonic cells (yellow arrow). E, F, G&H: Testes showing degenerated seminiferous tubules, desquamated epithelial cells inside the lumen of seminiferous tubule (blue star), vacuolation of spermatogonic cells (blue arrow), severe interstitial edema (black arrow), G: desquamated epithelial cells and hemorrhage (yellow star), F: shrinkage of the wall of seminiferous tubule (yellow arrow). H&E stain, X10 magnification.

Table 1: Observed average monthly ambient temperature, relative humidity percentage, temperature and humidity index (THI), and daylight hours during Winter and Summer months based on data from the Egyptian Meteorological Authority for Shalateen, Halayib province.

Parameters	**Temperature**(°c)	**Relative humidity** (%)	**THI** (%)	**Daylight** (hrs)
Winter	18.8± 0.2^{b}	38±0.8^{a}	63.2±0.1^{b}	10.9±0.1^{b}
Summer	35.2±0.1^{a}	18.3±0.6^{b}	78.5±0.05^{a}	13.4±0.08^{a}

Means±SEM in the same column with different superscripts differ significantly at ($p<0.05$).

Table 2: Effect of Winter and Summer seasons on bucks testicular characteristic.

Parameters		**Winter Mean ± SEM**	**Summer Mean ± SEM**	**P value**
Scrotal circumstances (SC)		24.6 ± 0.7	18.6 ± 0.4	0.002
Scrotal length (SL)		12.6 ± 0.1	11 ± 0.3	0.006
Scrotal width (SW)		9.8 ± 0.3	11 ± 0.3	0.23
Testicular diameter (TD)	**Right (R)**	16.8 ± 0.2	12 ± 0.5	0.0001
	Left (L)	15.2 ± 0.2	14 ± 0.2	0.02
Testicular length (TL)	**Right (R)**	14.8 ± 0.2	12.4 ± 0.3	0.002
	Left (L)	14 ± 0.3	12.2 ±0.2	0.003

Values are expressed as mean± SEM, means in the same row differ significantly at ($p<0.05$).

Table 3: Mean and standard error of the mean (SEM) of considered traits for bucks semen in the groups designated by climate condition (Winter and Summer).

Semen characteristic	**Winter Mean ± SEM**	**Summer Mean ± SEM**	**P value**
Semen volume (ml)	1.14± 0.1	1±0.1	0.02
PH	6.5 ± 0.1	6.9 ± 0.03	0.001
Concentration (10^6/ml)	1599.6 ± 1.5	1139.8 ± 2.4	0.0001
Motility (%)	85 ± 0.4	62 ± 1	0.0001
Live sperm (%)	81 ± 0.4	60 ± 0.9	0.0001
1st abnormalities (%)	2.8 ± 0.2	13.6 ± 1	0.0001
2nd abnormalities (%)	8.6 ± 0.5	16.8 ± 0.7	0.0001
Intact acrosome (%)	85.6 ± 0.1	56 ± 0.7	0.0001

Values are expressed as mean ±SEM, means in the same row differ significantly at ($p<0.05$).

Table 4: Impact of Winter and Summer seasons on blood biochemical parameters and testosterone concentration in bucks.

Parameters	Winter Mean ± SEM	Summer Mean ± SEM	P value
Testosterone (ng/ml)	5.4 ± 0.03	5 ± 0.08	0.001
Triglyceride (mg/ml)	51.8 ± 0.06	50.6 ± 0.07	0.004
Cholesterol (mg/ml)	82.9 ± 0.07	80.5 ± 0.09	0.001
Glucose (mg/ml)	52.5 ± 0.1	50.6± 0.1	0.005

Values are expressed as mean ± SEM. Means in the same row differ significantly at ($p<0.05$).

DISCUSSION

In this study, testes dimensions as well as histopathology, sperm quantity, and quality of bucks during the winter and summer in Shalateen, Halayib region, Egypt, were evaluated and differentiated according to the seasons. The findings suggested that from June to August, the captured amounts of bucks' semen were tiny with relatively low sperm concentration, motility, and viability. Between June and August, testosterone concentrations were relatively the lowest and testes were smaller in size. From December to February, these variable values started to rise. Temperature, humidity, THI, and the length of the day were all related to changes in reproductive and endocrine activities, with higher-quality semen being generated in December and February when temperatures were lower and daylight hours were shorter. The study assess the relationship between semen characteristics, testosterone concentrations, and testes morphology collected during summer and winter to a length of daylight and temperature, confirming that the best time for bucks to breed in Shalateen, Halayib region is December to March.

The concentrations of reproductive hormones in the blood are most commonly used as an effective indicator for testicular function (Mandour *et al.;* 2022). In the current study, testosterone concentration was significantly reduced in summer compared to the winter, indicating that as the length of the day lengthened and ambient temperatures rose, testosterone level decreased. Previous studies on goats (Delgadillo *et al.*, 2004) and rams (Ntemka *et al.*, 2019) have well documented the effects of environmental conditions on testosterone concentrations. The length of the day is an important regulatory factor influencing buck sperm quality. There is an improvement in buck sperm quality during a short-day season (El Kadili *et al.*, 2019). Changes in photoperiod and semen characteristics in bucks show distinct seasonal variation (Hammoudi *et al.*, 2010; Talebi *et al.*, 2009). It has been observed in goats that photoperiod changes over time cause changes in testosterone concentration due to changes in the melatonin secretory pattern (FARSI *et al.*, 2018; Swelum *et al.*, 2018). Melatonin is a key regulator of GnRH secretion, regulating testosterone secretion from the testis via the release of luteinizing hormone (LH) pulses (Samir *et al.*, 2020). Furthermore, it was reported that using an eye mask and inserting implants of melatonin increased bucks and rams reproductive performance (Malpaux *et al.*, 2020; Zarazaga *et al.*, 2021).

From the current findings, elevation in ambient temperatures and daytime length during summer was associated with decreased values of testicular dimensions in bucks (SC, SL, SW, TD, and TL) compared to winter. Previous research found similar seasonal variations in the size and dimension of testes (El Kadili *et al.*, 2019). The increase in bucks testicular dimensions during the breeding season (winter) could be contributed to the high testosterone concentration during the breeding season which increases spermatogenesis (Shi *et al.,* 2018). The high environmental temperatures adversely impact the male reproductive activity (Boni and Development 2019), disrupt spermatogenesis, and sperm output, and raise the proportion of sperm with defective morphology (Abdelhamid *et al.,* 2019; Kanter *et al.,* 2013). Based on that, the current study showed that the high temperatures induce some histopathological alterations of the testicular tissue of the bucks. The findings are in line with those of the previous data that have shown that the changes in the testicular structure were noticed in mammals exposed to thermal stress (Setchell 2018). Some changes are represented in the appearance of excessive desquamated epithelial cells within the lumen of the seminiferous tubules, and shrinkage of the wall of the tubules. In this respect, Gao *et al.* (2012) reported that the testicular histology changes included; reduced epithelium thickness, presence of cellular debris, fragmented cells, and a lack of sperm and spermatocytes (Gao *et al.,* 2012). Additionally, the mitochondria degenerate, SER dilated, and functional disruption of Leydig cells. The former authors clarified that the rate of spermatogenesis is reduced by 14% for every 1°C increase in testicular temperature (Durairajanayagam *et al.,* 2015). Moreover, heat stress induces low testicular blood flow which impairs testicular functions (Hedia *et al.,* 2020).

Interestingly, these changes in testicular dimensions were linked to a reduction in the sperm quantity and quality produced during the summer. The current study found that values for bucks sperm variables varied between the Summer and Winter seasons. Furthermore, sperm volume, concentration, motility, and viability were reduced in Summer than in Winter. Several studies (Abdi-Benemar *et al.,* 2018; Kulaksız *et al.,* 2019; Samir *et al.,* 2020) reported seasonal differences in semen volume in other goat breeds. Particularly, in the Summer group semen volume significantly decreased in Summer group compared with Winter group. Similarly, bucks study in Nigeria (Maina *et al.,* 2006) found lower semen volume during the dry seasons (non-breeding season) when ambient temperatures were higher. Seasonal variation in semen quality has also been reported in rams (Goshme *et al.,* 2020), and bulls (Sinha *et al.,* 2021). In the current study, seasonality had an impact on sperm morphology, demonstrating that high temperatures can lead to the creation of sperm cell structures that are morphologically aberrant.

In addition, the triglyceride, cholesterol, and glucose concentrations in the Summer group were significantly lower compared to the Winter group. According to some studies, when goats are exposed to high temperature, glucose and cholesterol levels in the blood decrease, indicating a failure in the body homeostasis (Ribeiro *et al.,* 2016; Ribeiro *et al.,* 2018b; Sezen and GÜNEY 2010). Cholesterol, phospholipids, and triglycerides are the three types of lipids in plasma. Triglycerides are mobilized as an energy source when glucose requirements are not met (Ribeiro *et al.,* 2018a). Heat stress induce decrease on total cholesterol concentration, which may be attributed to the increased fatty acids use for production of energy in heat-stressed animals due to decreased glucose concentration (Mundim *et al.,* 2007).

In conclusion, the findings clearly showed that climate conditions affect the reproductive capacity of bucks in the Shalateen, Halayib region, Egypt. Small amounts of sperm with lower gross motility, concentration, and velocity were collected from June to August when the days were longer and the temperatures were high. Bucks produced less testosterone during these months due to an increase in photoperiodic stimuli, resulting in smaller testes. There was a stimulated reproductive axis as well as an increase in the volume of high-quality semen during the short daytime (winter). The results of the study clearly indicate the impacts of high summer temperatures on characteristics of the testicles and quality of sperm in goats.

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جودة السائل المنوي ، خصائص الخصية ، الخصائص الكيميائية الحيوية و التشريح المرضي للخصيتين فى الماعز تحت ظروف الإجهاد الحراري

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ترتبط درجات الحرارة المرتفعة خلال أشهر الصيف بضعف الأداء التناسلي للحيوانات. هدفت الدراسة إلى استكشاف التأثيرات الحرارية الموسمية على التيوس. تم استخدام عشرين من التيوس الصحية في هذه الدراسة اعتمادًا على درجة الحرارة البيئية ، تم تقسيم التيوس بالتساوي إلى مجموعتين: فصل الشتاء (ن = ١٠) وفصل الصيف (ن = ١٠). تم تقييم عرض الصفن وظروفها وطولها وقطرها للخصيتين اليمنى واليسرى وكذلك طول الخصيتين اليمنى واليسرى. تم جمع عينات السائل المنوي من كل تيس مرة واحدة في الشهر خلال فصلي الصيف والشتاء. مباشرة بعد جمع السائل المنوي ، تم تقييم حجم السائل المنوي ، درجة الحموضة ، التركيز ، الحركة ، نسبة الحيوانات المنوية الحية ،مورفولوجيا الحيوانات المنوية (التشوهات الأولية والثانوية) وحيوية الحيوانات المنوية والأكروسوم السليم. تم رصد تركيزات الدهون الثلاثية والكولسترول والجلوكوز والتستوستيرون في البلازما. أظهرت النتائج أن ظروف كيس الصفن وطول وقطر الخصيتين اليمنى واليسرى وطول الخصيتين اليمنى واليسرى كانت أقل ($p < 0.05$) في موسم الصيف عنها في الشتاء. عرض الصفن لا يختلف بين الفصول. كان حجم السائل المنوي وتركيزه وحركته ونسبة الحيوانات المنوية الحية والأكروسوم السليم في فصل الصيف أقل ($P < 0.05$) مقارنة بالشتاء. كان الرقم الهيدروجيني للسائل المنوي والتشوهات الأولى والثانية في موسم الصيف أعلى ($p < 0.05$) مقارنة بالشتاء. انخفضت تركيزات الدهون الثلاثية والكوليسترول والجلوكوز والتستوستيرون في البلازما ($p < 0.05$) خلال الصيف عنها في الشتاء. تم العثور على الآفات النسيجية المرضية في المجموعة الصيفية مقارنة بالشتاء. في الختام ، أكدت النتائج أن ارتفاع درجة الحرارة خلال ظروف الصيف يؤثر سلبًا على جودة السائل المنوي لذكور الماعز.