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Effect of seasonality on testicular blood flow as determined by color Doppler ultrasonography and hormonal profiles in Shiba goats

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ABSTRACT

The objectives of this study were to determine if there is a seasonal pattern of testicular blood flow in male Shiba goats, and ascertaining whether or not there is a relationship among changes in testicular hemodynamics and circulating testosterone (T) and estradiol (E2). Twelve sexually mature male Shiba goats (*Capra hircus*), aged 19.5 ± 2.5 months were used in this study. Monitoring of the changes in testicular blood flow was performed once a week for 1 year, using color spectral Doppler ultrasonography. Plasma concentrations of testosterone (T) and estradiol (E2) were determined by radioimmunoassay. Among the four seasons, the greatest increases ($P \leq 0.001$) in the values for the resistive index (RI) and pulsatility index (PI) of the testicular artery were recorded in the summer. Also, PI and RI values recorded in spring were greater than those recorded in autumn, while for winter there were the least values for these indices. Likewise, concentrations of T and E2 showed seasonal changes. Concentrations of T and E2 were greater during the autumn and winter compared to those in spring and summer. Interestingly, E2 concentrations were negatively associated with Doppler indices of the testicular artery. In conclusion, although Shiba goats are considered to be non-seasonal breeding animals, there were distinct seasonal patterns of testicular blood flow as measured by color Doppler ultrasonography, especially in Doppler indices. These changes were coincident with the changes in the circulating E2 and may be attributable to the seasonal changes in ambient temperature or daylight length.

1. Introduction

Ultrasonography is a diagnostic technique frequently used to monitor male reproductive health. This technique is useful in both clinical and research aspects, and provides many benefits because it allows for repeated and non-invasive scanning assessment of the scrotal tissues in the same individuals (Evans et al., 1996; Arteaga et al., 2005). Compared to B-mode ultrasonography, the advent of color Doppler ultrasonography has improved the accuracy of diagnoses by adding physiological information from the organ (Bollwein et al., 2016), and has allowed for re-evaluating conceptions that have been previously been considered definitive regarding the physiology of reproduction (Ferrereira et al., 2011). In veterinary practice, Doppler ultrasonography has become an important

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method for the assessment of male fertility in farm animals (Ginther and Utt, 2004; Ginther, 2014). It was used as an alternative diagnostic technique in camels to compare the differences in testicular blood flow between fertile and infertile males (Kutzler et al., 2011). Doppler ultrasonography was also used to characterize blood flow in the testicular artery and evaluate some pathological problems such as testicular varicocele in the stallion (Pozor et al., 2006; Pozor, 2007), canine testicular tumors and benign prostatic hyperplasia (Gunzel-Apel et al., 2001; Polisca et al., 2013). Furthermore, some Doppler parameters such as the resistive index (RI) and pulsatility index (PI) of the testicular artery are considered by some to be potential markers of seminal quality (Zelli et al., 2013) in dogs, and in stallions (Ortiz-Rodriguez et al., 2017). When considering goat reproduction, real time ultrasonography is thought to be a practical technique for accurate detection of early pregnancy and embryonic mortality (Karen et al., 2014; Samir et al., 2016). The available data on the importance of color Doppler ultrasonography in small ruminants, however, is scarce, especially with regard to male reproduction (Panarace et al., 2008; Serine et al., 2010; Gonzalez-Bulnes et al., 2010; Samir et al., 2015).

Testicular blood flow is an important process in the testis because it is the main pathway for the transport of nutrients, oxygen, and other regulatory hormones to and from the testis. As in all other organs, control of blood flow to and from the testis is important, and may be particularly so for the testis because of the relatively low concentration of oxygen in the seminiferous tubules compared with most other tissues of the body (Setchell, 1990). Any reduction in blood flow to the testis may cause ischemic damage that leads to impaired spermatogenesis (Kay et al., 1992), because the testis requires a stable blood supply for its function and maturation.

Reproduction in goats is commonly described as seasonal with variations in reproductive activity, and this seasonality is dependent on breed and geographical location (Fatet et al., 2011). Information on the normal reference values for testicular blood flow in farm animals during different seasons in a year is scarce. Studies that have involvement assessment of the effect of seasons on testicular blood flow are very few and have only been conducted in horses (Boyd et al., 2006). To the best of our knowledge, there has been no study where there has been evaluation of whether or not there are seasonal changes in testicular blood flow in goats. With these previously described considerations, and considering the potential need, the objective of the current study was to investigate the effect of the different seasons of the year, on the testicular blood flow. Blood flow was assessed using color-spectral Doppler ultrasonography in mature male goats. Also, another aim of this study was ascertaining whether or not there is an existing relationship among the changes in testicular hemodynamics and circulating testosterone (T) and estradiol (E2).

2. Materials and methods

This study was conducted in male Shiba goats (*Capra hircus*), a Japanese miniature goat, considered to be a non-seasonal breeder. Shiba goats attain puberty at 3.5 months of age, and are considered a useful model animal for studying the physiology of ruminants (Kano et al., 1977).

All procedures in the present study were conducted in accordance with guidelines established by the Tokyo University of Agriculture and Technology, Japan, for the use of animals.

2.1. Animals and management

Twelve sexually mature adult male Shiba goats (*Capra hircus*), 19.5 ± 2.5 months of age and weighing 24.30 ± 4.17 kg were used in this study. The goats were housed in natural daylight conditions and fed a maintenance diet of 400 gm of hay cubes per animal, twice a day. Clean water and mineralized salt licks were available *ad libitum*. Goats were maintained using a normal management program in a building belonging to the Laboratory of Reproductive Physiology, Department of Veterinary Medicine, Tokyo University of Agriculture and Technology, Japan. All goats were regularly vaccinated against important infectious diseases such as enterotoxaemia, and foot and mouth disease. In addition, there were other immunoprophylaxis treatments such as deworming, and in none of the goats was there any evidence of disease upon physical examination prior to the study. The animals were clinically healthy and were considered to have typical characteristics of libido before the experiment was initiated. Furthermore, to ensure the absence of any reproductive tract abnormalities before the research was initiated, each goat was assessed with a general ultrasonographic examination of the testes and epididymis by means of B-mode ultrasonography. Furthermore, female goats were maintained in a separate facility throughout the period of the study.

2.2. Ultrasonographic examinations

Ultrasonographic examinations were performed during an experimental period that lasted 12 months (from September 2014 to September 2015), covering four distinct seasons of the year with varying temperatures and weather conditions especially as related to season of the year. September to November is autumn in the region where the goats for this study were maintained; and December to February is winter; March to May spring; June to August summer. During each season, there were different temperatures and weather conditions. According to data from Japan Meteorological Agency, spring in Tokyo has a climate in which the goats would be in their thermo-neutral zone most of the time, and the average temperature (minimum to maximum) is between (9 and 18.5 °C). The temperature in summer is between 21 and 29.5 °C but the heat index is greater during this time of the year due to the high humidity. The hot summer is followed by a period when the temperatures in autumn are again in the typical thermo-neutral zone for goats (the temperature is between 14 and 21.5 °C). The humidity is minimal during the autumn, and the climate is cool and dry. In winter, the temperature decreases (2.5 to 10.5 °C) and it is very cold by the end of December until the beginning of March.

All ultrasonographic measurements were conducted once every week throughout the period of the study by the same technicians just after blood sampling. All examinations were performed using an ultrasonic scanner (EUB-7500, Hitachi Medical Corporation,

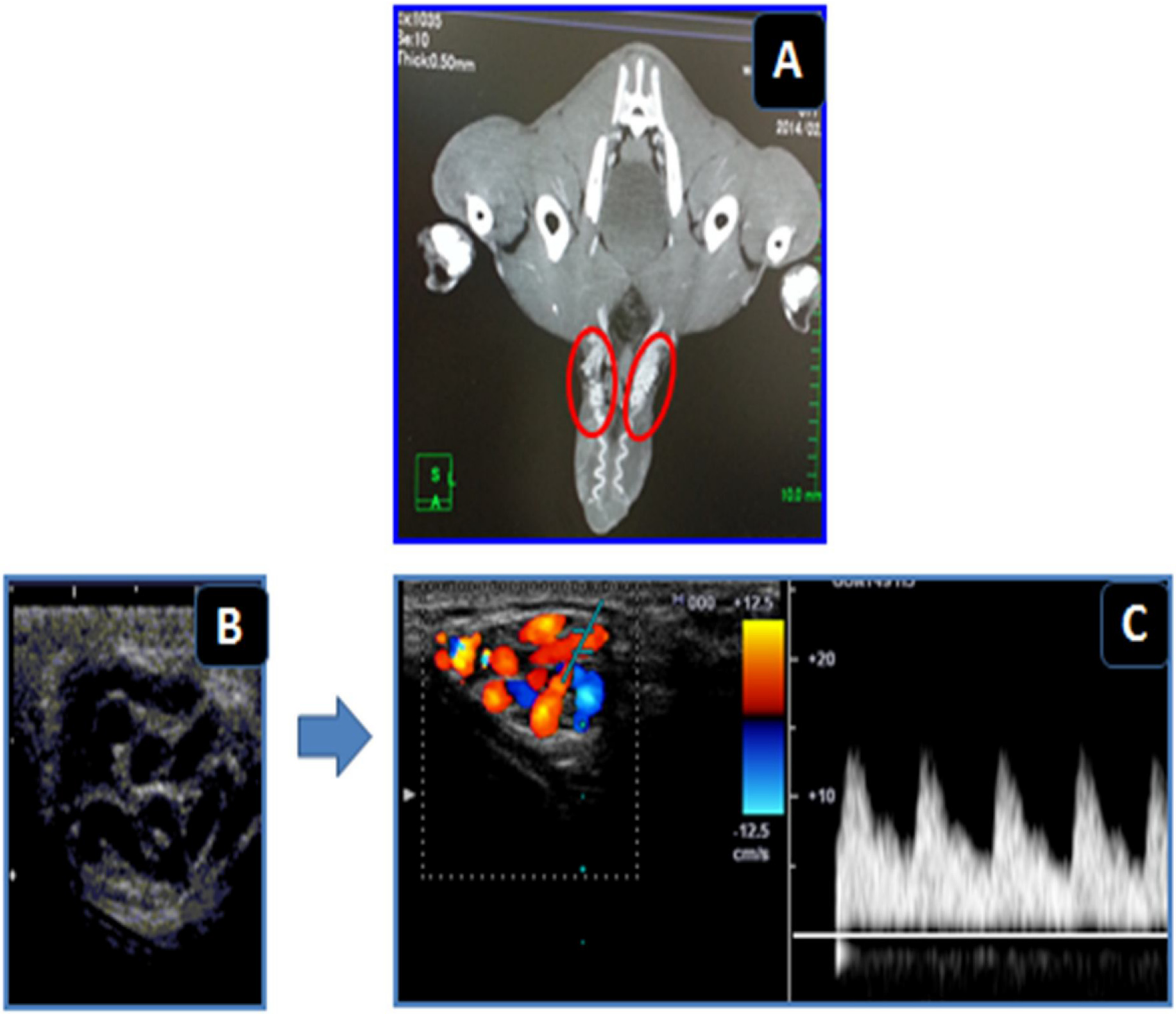

Fig. 1. (A) Imaging the location of the supratesticular artery (red circles) in a male Shiba goat using computerized tomography of the testis; (B) identification of the coiled appearance of the supratesticular artery using B-mode ultrasonography; (C) an assessment of testicular blood flow (within the supratesticular artery) using color spectral Doppler ultrasonography (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Tokyo, Japan) equipped with a linear multi frequency array transducer (6–14 MHz; Model EUP-L65; Hitachi Medical Corporation). For Doppler examinations, the bucks were restrained without being tranquilized or sedated to avoid its effect on testicular blood flow. To avoid the presence of air spaces, the hair was removed from both sides of the scrotum by shaving the areas, and the transducer was covered with a copious amount of gel to facilitate ultrasonographic imaging. Immediately before its entry into the testes, the testicular artery in goats convolutes to form a coiled or convoluted section, known as the supratesticular artery (STA) (Fig. 1). The testicular veins intertwine with the artery causing some problems with differentiation assessment between a testicular artery and vein by Doppler analysis. An artery, for example, will typically have a waveform on the spectral graph corresponding to the arterial pulse in each cardiac cycle (systole and diastole), while the flow in the veins is relatively constant without a distinct pulse.

Based on previous studies (Gunzel-Apel et al., 2001; Pozor and McDonnell, 2004; Samir et al., 2015), Doppler analysis was performed by identifying all the vascular structures using B-mode ultrasonography and locating the largest and, if possible, a longitudinal or oblique section of the STA. The angle between the long axis of the vessel and the Doppler beam was never positioned greater than 60 degrees in the direction of blood flow. The high-pass filter was set at 50 Hz, and the Doppler gate was kept constant at 1.5 mm.

After the appearance of the spectral pattern of the STA, the parameters studied in the testicular arteries were: the maximum blood flow velocity, known as peak systolic velocity (PSV, cm/s), minimum blood flow velocity, known as end diastolic velocity (EDV, cm/s), and time-averaged maximum velocity (TAMAX, cm/s). Also, some Doppler indices were studied; such as the resistive index (RI = (PSV - EDV) / PSV) and pulsatility index (PI = (PSV - EDV)/mean velocity) (Gumbsch et al., 2002). To minimize the variations in recording, the ultrasonic settings (gains, focus, brightness and contrast) were standardized, fixed and used for all examinations. In this study, all examinations were performed at fixed times (10.00 A.M.), and recorded digitally for subsequent analysis. In this vein, the ambient temperature (°C) was always measured immediately before the ultrasonic scanning, using a digital thermometer which was held at half the height of the animals.

2.3. Blood sampling

On the day of ultrasonographic scanning, a venous blood sample (5 ml) was collected from the jugular vein into an evacuated heparinized tube (Venoject II, Terumo, Tokyo, Japan). The blood samples were placed on ice for no longer than 1 h, and then centrifuged at 3200 rpm for 15 min at 4 °C. Plasma was separated and stored at −20 °C until assessment of plasma testosterone (T) and estradiol (E2) by radioimmunoassay.

2.4. Hormonal analysis

All hormonal assays were performed in triplicate using a double antibody radioimmunoassay system with I^{125} labeled radioligands. Concentrations of testosterone (T) and estradiol (E2) were quantified in plasma as described by Taya et al. (1985) using antisera against T (GDN 250), and E2 (GDN 244). The intra- and inter-assay coefficients of variation were 8.4% and 9.5% for T, and 6.1% and 7.8% for E2, respectively. All hormonal assays were performed in triplicate and conducted in the same laboratory at Tokyo University of Agriculture and Technology.

2.5. Statistical analysis

In this study, there were no significant differences among the goats within each season; thus, the data for each season were pooled and comparisons made among all four seasons. In addition, all data, whether for Doppler parameters or hormonal results, was presented as means ± standard error of the mean (SEM). Statistical analysis of the data was performed using GraphPad Prism version 5. The statistical significance of differences in hormonal and ultrasonographic results was evaluated by ANOVA, and the Bonferroni *post hoc* test was used to detect the significant differences among the four distinct seasons. For all analyses, values of $P < 0.05$ were considered significant.

3. Results

3.1. Results of spectral Doppler ultrasonography

The data for effect of seasons on testicular blood flow in male Shiba goats in the current study are presented in Table 1. There were changes ($P < 0.05$) in all parameters of spectral Doppler ultrasonography of the testicular artery. The greatest values of both RI and PI of the testicular artery were detected in summer. There was no differences in values for all Doppler parameters of the testicular artery between the autumn and either winter or spring. Greater values for both RI and PI and slightly lesser values for EDV and TAMAX, however, were detected in the spring compared with the winter. In addition, the PSV was slightly less in the spring than in the summer. Interestingly, in the current study, changes of the RI and PI of the STA and changes in ambient temperatures in the different seasons were positively associated.

3.2. Results of circulating hormones

There was an effect of seasons on circulating T and E2 (Table 2). In autumn and winter, T concentrations were greater compared

Table 1
Changes in testicular blood flow as measured by color spectral Doppler ultrasonography of the supratesticular artery in male Shiba goats in different seasons of the year.

Parameters	Autumn (n = 156)	Winter (n = 156)	Spring (n = 156)	Summer (n = 168)	P-Value
A. Temp.(°C)*	(16.83 ± 1.50)	(10.38 ± 1.02)	(20.46 ± 1.34)	(29.43 ± 1.45)	
PSV (cm/s)	15.44 ± 0.96$^{a, b}$	16.01 ± 0.49$^{a, b}$	15.50 ± 0.22^{a}	16.59 ± 0.28^{b}	0.025
EDV (cm/s)	7.94 ± 0.37^{a,b,c}	8.98 ± 0.31^{a}	7.77 ± 0.13^{b}	7.33 ± 0.16^{c}	0.001
TAMAX (cm/s)	11.69 ± 0.62$^{a, b}$	12.49 ± 0.38^{a}	10.99 ± 0.15^{b}	11.07 ± 0.20^{b}	0.009
RI	0.47 ± 0.018$^{a, b}$	0.43 ± 0.011^{a}	0.49 ± 0.006^{b}	0.56 ± 0.007^{c}	0.0001
PI	0.63 ± 0.032$^{a, b}$	0.57 ± 0.019^{a}	0.71 ± 0.012^{b}	0.85 ± 0.016^{c}	0.0001

[a, b, c]Values with different superscripts in the same row are different $P < 0.05$ (among the four seasons).
Notes: A. Temp. (°C) is the average of ambient temperature that recorded just before ultrasonographic evaluations.
n = total number of ultrasonographic analyses for all goats within each season.
Parameters and indices of spectral Doppler ultrasonography that used were: PSV = Peak systolic velocity (cm/s); EDV = End diastolic velocity (cm/s); TAMAX = Time average maximum velocity (cm/s); RI = Resistive index; PI = Pulsatility index.

Table 2
Changes in circulating testosterone (T; ng/ml) and estradiol (E2; pg/ml) in male Shiba goats in different seasons of the year.

Parameters	Autumn	Winter	Spring	Summer	*P*-Value
Testosterone	2.64 ± 0.106[a]	2.45 ± 0.124[a]	1.76 ± 0.107[b]	1.53 ± 0.090[b]	0.0001
Estradiol	20.21 ± 0.526[a]	24.33 ± 0.792[b]	16.77 ± 0.600[c]	13.30 ± 1.032[d]	0. 0001

[a,b,c,d]Values with different superscripts in the same row are different $P < 0.05$ (among the four seasons).

to the values during spring or winter. Furthermore, there were changes in T concentrations between the winter and autumn, and also between spring and summer. Furthermore, there were greater E2 concentrations ($P \leq 0.001$) in the winter compared with the autumn, spring, and summer. The least values for E2 were detected in the summer season compared to other seasons of the year.

4. Discussion

The effect of different seasons on the reproductive capacity of farm animals (fertility parameters, seminal quality, and mating activity) is obvious in seasonal breeding animals, such as stallions (Roser and Hughes, 1992; Boyd et al., 2006), and certain breeds of goats (Fatet et al., 2011). In Shiba goats, which are continuous breeders throughout the year, seasonal variations in fertility have not been observed (Kano and Mori, 1982; Mori et al., 1984). Studying the seasonal changes in testicular blood flow, however, is of great importance to fully understand the reproductive physiology in seasonal and non-seasonal breeding animals. Testicular vascularization is important for normal physiological functions related to spermatogenesis and hormone production (Ortiz-Rodriguez et al., 2017). An assessment of testicular hemodynamics by color Doppler ultrasonography, therefore, could be valuable for providing information about the normal reference values for testicular blood flow during different seasons of the year in goats. Providing such data is important for further elucidation and enhancing diagnoses of different problems associated with the fertility in goats. The results of the current study supported the hypothesis that seasons influence testicular hemodynamics in Shiba goats. In addition, the seasonal changes in testicular blood flow were concomitant with changes in circulating concentrations of both T and E2.

Owing to difficulties encountered with the volumetric blood flow, blood perfusion in individual vessels is typically evaluated semi-quantitatively using the so-called Doppler indices such as resistance index (RI; known as the Pourcelot ratio) or pulsatility index (PI). These indices are not a direct measure of blood flow, but rather describe the resistance to blood flow in vessels peripheral to the vessel being examined. These indices are angle independent, and indicate the flow condition downstream (Blanco, 2008; Serine et al., 2010). It was reported that RI and PI have negative correlations with vascular perfusion of tissue downstream from the sample gate (Ginther, 2007). As the values increase, the blood flow resistance increases, and *vice versa* (Dickey, 1997; Bollwein et al., 2016). Decreased values for RI and PI are considered to have resulted from increased blood flow and these changes in values are important if there is to be a continuous supply of oxygen and nutrients to the respective organ (Jaffe, 1995; Varughese et al., 2013). In the present study, winter and autumn represent the periods of the greatest blood perfusion (least RI and PI values) in comparison to spring and summer. In stallions, increases in testicular perfusion during the breeding season in spring in comparison to the non-breeding season in the winter months, has been reported (Boyd et al., 2006). Seasonal changes in testicular capillary blood flow have been reported to be associated with the variations of endocrine and spermatogenic functions of the testis (Joffre and Joffre, 1973).

It is proposed that these changes in testicular blood flow among the seasons, as observed in the current study, may be attributable to two possible mechanisms. Firstly, these changes may be related to the changes in ambient temperature between the four different seasons when the study was conducted. The least values in RI and PI of testicular artery were reported during the winter (low ambient temperature), while during the summer season there were the greatest values for the previously described Doppler indices concomitantly with the increases in environmental temperature. In the summer season, increases in ambient temperature may result in concurrent increases in testicular temperature, and in turn, affect the testicular blood flow. It is well known that testis must be 4–5 °C below the core body temperature to maintain normal spermatogenesis, and to ensure that production of sperm that have the capacity for motility and typical morphology for the species (Skinner and Louw, 1966; Setchell, 1978; Barros Adwell et al., 2018). Increases of the testicular temperature results in increases in metabolism and oxygen demand, and in turn, hypoxia, production of reactive oxygen species, and impairment of semen quality (Setchell, 1978, 1998; Kastelic et al., 2017). Clearly, there are many studies in different animal species where there have been changes in testicular blood flow in response to changes in testicular temperature. Recently, in bulls, it was found that testicular blood flow increased in response to increasing ambient temperature (Barros Adwell et al., 2018). In rams, the blood flow to the testis increased spontaneously 26% when testicular temperature was increased to 36 °C by applying heat directly to a testis (Mieusset et al., 1992; Setchell et al., 1995). Increases in RI and PI values of the STA (decreases in the testicular blood perfusion) in the current study during summer season may not be consistent with the findings in previous studies. The extent to which the increases of testicular temperature affect testicular blood flow should be considered, as these changes are dependent on whether the testicular temperature exceeded the core body temperature or not (Setchell, 1978). Compared to other species, an increase in average scrotal surface temperature during scrotal hyperthermia (> 3 °C) resulted in no changes in testicular blood flow in dogs, as assessed by pulsed Doppler ultrasonography (Henning et al., 2014). After artificial cooling of dog testicles, Glode et al. (1984) observed marked decreases in the blood flow within the scrotal skin without any effect on the testicular blood flow. Notwithstanding, variations in testicular blood flow in response to an increased testicular temperature may also be affected by breed type. For example, testicular blood flow did not change with increased scrotal temperature in rams selected for skin wrinkling, but increased in rams selected against skin wrinkling (Fowler and Setchell, 1971).

Recently, arterial blood flow was reported to be the main source of testicular heat in bulls (Barros Adwell et al., 2018). There should be consideration, however, of the adaptation of the testis to changes in ambient temperature by complex physiological mechanisms responsible for scrotal/testicular thermoregulation (Kastelic et al., 1996, 1997). Findings in the present study, indicate the coincidental variations in testicular blood flow with the changes in ambient temperature among the seasons may be a part of the compensatory mechanism of goat testes for regulation of testicular hemodynamics, for the purpose of overcoming the fluctuations in ambient temperature so that normal testicular functioning can be maintained.

Another possible explanation for the changes in the testicular perfusion with season of the year in the present study is that these changes may be attributable to the photoperiod or annual season, which is considered the principal factor influencing seasonality of reproduction in small ruminants (Delgadillo et al., 1993). Photoperiodic control of reproductive patterns is mediated through the pineal gland that is responsible for circadian rhythmic secretions of melatonin with increases in secretion occurring during darkness (Fatet et al., 2011). In tropical and equatorial regions, there is less change in photoperiod and temperature; thus, the animals in these regions have a longer breeding season than those in temperate and Polar Regions where there are more distinct seasonal effects (Fatet et al., 2011). Although Shiba goats are continuous breeders, the pattern and the magnitude of the nocturnal increase in plasma melatonin and the responses to the dark or light interruption were substantially congruous between the seasonal (Saanen), and continuous (Shiba) breeding goats (Maeda et al., 1984). Furthermore, the seasonal changes in the gonadal activity in Shiba goats are thought to be controlled by photoperiod; short day length is stimulatory whereas long day length has an inhibitory effect on gonadal functions (Mori et al., 1984, 1985).

The changes in the photoperiod or daylight length among the seasons may be mediated through steroid-dependent and independent mechanisms (Walkden-Brown et al., 1997). In the current study, there were significant changes in circulating T and E2 in male goats among the different seasons, and these changes may be associated with the seasonal changes in testicular blood flow. These results were consistent with those reported for other species (Peirce et al., 1987; Ritar, 1991; Boyd et al., 2006). In seasonal breeding animals, such as Angora goats, serum T concentrations during the non-breeding season are less than in the breeding season, indicating there is a seasonal rhythm in the reproductive cycle (Ritar, 1991). There, however, are seasonal changes in the reproductive activity of males of strains that are continuous breeders. Seasonal changes in circulating testosterone concentrations have been found in bulls (Peirce et al., 1987). Similarly, in Shiba goats, which are continuous breeders, the peripheral plasma testosterone concentration is greater in the autumn than spring (Sawada et al., 1992). The extent of changes in the mean concentrations of T was 7.0–9.1 fold greater in the autumn than those in spring in Saanen goats (seasonal breeder), whereas these values were 1.2–2.9 fold greater in Shiba goats (non-seasonal breeder) (Sawada et al., 1992). It was noted that circulating E2 is more important than T in the maintenance of this circannual cycle (Walkden-Brown et al., 1997).

In the present study, plasma E2 concentrations were negatively associated with the values of both RI and PI of the STA. Likewise, in the stallion, E2 concentration was correlated with PI ($r = 0.79$, $P < 0.05$), and RI ($r = 0.72$, $P < 0.05$) of the testicular artery among the different seasons (Boyd et al., 2006). In male Shiba goats, there were negative correlations between E2 and both the RI ($r = -0.610$, $P = 0.027$) and PI ($r = -0.763$, $P = 0.002$) of the STA following hCG administration (Samir et al., 2015). Also, hCG treatment in stallions induces an increase in testicular blood flow in parallel with an increase in the E2 concentration (Bollwein et al., 2008). Indeed, the significant correlations between E2 and Doppler indices in the current study might be related to the marked vasodilatory effect of estrogen and its role in testicular perfusion (Bollwein et al., 2008; Rosenfeld et al., 2002). Results of the present study indicated an associative relationship between E2 and Doppler indices, an observation deemed important, but there was no elucidation of the underlying mechanism for this E2 action. Also in females, the uterine and ovarian blood flow is regulated by the sex steroids and the exogenous administration of these hormones results in increases in the blood perfusion of the uterus and ovary (Dickey, 1997). The vasodilatory effect of E2 may be mediated through an intracellular signaling that involves decreases in the calcium uptake of potential sensory channels by the E2 receptors in the tunica media and E2 in the uterine arteries (Stice et al., 1987).

From a previous study (Setchell, 1970), it was reported that changes in testicular blood flow in different seasons are probably largely related to the effect of undernutrition, and subsequently a reduction of gonadotropins. In the present study, however, goats had been regularly and adequately fed and did not have marked seasonal variations in feeding management, suggesting that nutritional factors are not responsible.

In Shiba goats, there have been reports (Kano and Mori, 1982; Mori et al., 1984) that there are no seasonal variations in fertility. Furthermore, other results from a previous study (Medan et al., 2006) indicate that there are not fluctuations in scrotal circumference (cm), and sperm cell concentrations between the summer and autumn in Shiba goats.

5. Conclusion

Even though Shiba goats are considered non-seasonal breeding animals, the pattern of testicular blood flow as measured by color spectral Doppler ultrasonography indicated that there were seasonal variations especially in Doppler indices such as RI and PI. Interestingly, the values of RI and PI of the testicular artery tend to coincide with the changes in ambient temperatures, and the circulating concentrations of E2 among the seasons of the year. These fluctuations in the testicular blood flow may be considered as a part of the potential competence of the testicular hemodynamic in the thermoregulation or compensation in adapting to variations in the ambient temperature in Shiba goats. The changes in daylight length, however should be taken into consideration. Providing such data will be important in the future to elucidate or diagnose different problems of male fertility in goats and monitor the effect of different regimens for controlling animal fertility. Determining and explaining the actual mechanism for blood flow changes, however, was beyond the scope of the present study and needs to be investigated in future studies.

Conflict of interest

The authors declare that there is no conflict of interest

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