Short Communication

Sonographic Assessment of Ovarian Follicular Dynamics during Breeding and Non-Breeding Season in Gaddi Goats

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Abstract | The present study evaluated the seasonal differences in ovarian follicular dynamics in Gaddi goats. Transrectal ultrasonographic ovarian examinations were carried out during a full estrous cycle in breeding (B; January-February, n=7) and 21 days in non-breeding (NB; May-June, n=11) seasons. Blood sampling on alternate days was done during both the seasons for serum progesterone (P4) estimations. Follicular growth was characterized by presence of three to five numbers of follicular waves in both seasons. Significantly higher (P<0.01) number of follicular waves (4.0±0.21 *versus* 3.18±0.12), number of follicles at wave emergence during second (2.85±0.26 *versus* 1.77±0.14; P<0.01) and third wave (2.57±0.2 *versus* 2.0±0.16; P<0.05), along with shorter persistence of dominant follicle (DF, 9.25±0.45 *versus* 12.02±0.44, P<0.001) and early attainment of DF during first three waves was observed during B compared to the NB season. Whereas, significantly higher maximum diameter of DF (7.66±0.10 *versus* 7.11±0.14 mm, P<0.05) and longer Inter wave interval (IWI) between 2nd and 3rd wave (5.81±0.46 *versus* 4.28±0.28 days) were observed during NB season. Average mean P4 concentration was 0.30±0.04 ng/ml throughout the period of observation during NB whereas, peak P4 concentration of 11.89±1.55 ng/ml was observed at Day 14 of estrous cycle during B season. Significant difference between average daily counts of small, medium along with daily total number of visible follicles were observed during B season. In conclusion, ovarian follicular growth in Gaddi goats during B and NB season is characterized by wave like pattern and exhibited the seasonal differences.

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Introduction

Goats are important livestock species of India. Goat husbandry plays an important role in the livelihood of a large proportion of small/ marginal farmers, landless labourers and sustain the rural economy, with multi-facet utility for chevon, milk, skins and manure. There is rich biodiversity among the indigenous goat in India, evidenced by more than 20 listed breeds of goat. The goat contributes around 27.8% of total livestock population in India (148.88 million; 20th Livestock census, 2019). Gaddi is well recognized breed of goat found in Himachal Pradesh, India. A progressively declining cattle population in the state accentuates goat rearing under the small household system.

Goat is considered to be seasonally polyestrous and

short day breeder. The annual change in the day length is the main environmental factor that affects seasonal breeding in goats (Fatet *et al.*, 2011), although temperature and breed differences has also been associated with reproductive activity (Farsi *et al.*, 2018).

The ovarian follicular dynamics and endocrine investigation are important yardsticks delineating the effects of environment on breeding and conception. Ultrasonography is simple, non-invasive and a reliable technique widely utilized in small ruminants (Gonzalez de Bulnes *et al.*, 1999). Ultrasound guided follicular dynamics have been studied by various researchers (Cruz *et al.*, 2005; Medan *et al.*, 2005; Simoes *et al.*, 2006; Nogueira *et al.*, 2015; Sharma and Sood, 2019) during B and NB season at different latitudes and in different breeds of goats. Ultrasonographic studies revealed that the follicle grows to a diameter of ≥5mm with average growth rate of 0.8-1.1 mm per day throughout the estrous cycle (Medan *et al.*, 2005; Simoes *et al.*, 2006) and anestrous periods (Cruz *et al.*, 2005; Nogueira *et al.*, 2015). Follicular growth occurs in a wave like manner throughout the estrous cycle irrespective of season which is characterized by sequence of three gonadotropin-dependent events in follicular growth viz. recruitment, selection and dominance (Ginther and Kot, 1994; Mohammadi *et al.*, 2010; Nogueira *et al.*, 2015). Also, temporal relationships between follicular dynamics and hormonal profiles are well established in goats (Medan *et al.*, 2005; Vasquez *et al.*, 2010).

The follicular dynamics alongwith endocrine profiling in goats during both the seasons have been reported in limited number of studies (Boer *et al.*, 2015; Saanen *et al.*, 2020). Hence, present study reports the comparative follicular growth characteristics along with progesterone profiling during NB and subsequent B season in Gaddi goats.

Materials and Methods

Selection of animals and period of investigation

Ultrasonography guided follicular dynamics study was done in total eighteen adult, healthy and non pregnant Gaddi does. Average (mean±SEM) age and bodyweight of the does during the period of investigation were 3.74±0.28 years, 24.27±0.70 kg and 4.52±0.39 years, 27.07±1.03 kg, respectively during NB (n=11) and B (n=7) season. Significant difference ($P<0.05$) in body weights during NB and B were observed in the Gaddi goats.

Location, housing and feeding of animals

All the does were maintained at University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur (32.6°N, 76.3°E, altitude 1290.8m). All animals were subjected to grazing for five hours and remained under confinement in a shed where they were fed as per the standards of Indian Council of Agricultural Research (ICAR, 2013) with round the clock access to the clean drinking water.

Ultrasonography

Ultrasonography was performed using 7.5 MHz linear array transducer (Exago, ECM France) as per defined technique (Ginther and Kot, 1994; Medan *et al.*, 2003; Nogueira *et al.*, 2015). All ultrasound examinations of the ovaries were done by a single operator. All follicles ≥3mm in diameter and corpora lutea were measured using electronic callipers and ovarian sketches were drawn. Different follicular wave parameters were studied and defined as per standard definitions (Ginther and Kot, 1994). A follicular wave was defined as one or more antral follicles growing from 3 to ≥5mm in diameter before subsequently regressing and being no longer detectable. The follicles were classified as small (3.0 to <3.5mm), medium (≥3.5mm to <5mm) and large (≥5mm). The first wave at the beginning was defined as Wave 1 and the following waves were numbered sequentially. The day of emergence (DE) of follicles was identified as the day on which the dominant follicle within a given follicular wave was retrospectively first observed to be ≥3mm in diameter. The end of a follicular wave was recorded when dominant follicle(s) associated with a follicular wave could no longer be identified. Individual follicles emerging within a 48h period of the day of emergence of the dominant follicle were regarded as belonging to the same follicular wave. The duration of a follicular wave was defined as the interval between the day of emergence and the day the follicular components of the said follicular wave could no longer be identified. The inter wave interval (IWI) was recorded as the number of days in between the start of two sequential follicular waves. Follicular growth rate (mm/day) was the maximum diameter after emergence divided by number of days taken to achieve it. The day of maximum follicular diameter (DM) was the first day in each wave when dominant follicles attained maximum diameter. The regression

rate was calculated by diameter of follicle at last day of static phase by number of days taken to its disappearance.

Synchronization

Reproductive cyclicity was ascertained on basis of transrectal ovarian ultrasonography and progesterone (P4) estimation. Estrus was synchronized with double injections of Cloprostenol (187.5µg) eleven days apart during the B season. Ultrasonography was carried out once daily once after administration of the last dose of Cloprostenol to detect ovulation with examinations continuing till the next ovulation. However, during NB season ultrasonography was initiated randomly and lasted for one month so that the cut-off period of 21 days was retrospectively analyzed to consider the initiation of a follicular wave and further evaluated for wave characteristics.

Ethical considerations and hormonal analysis

All the experiments were carried out after the approval of ethical committee of the institute and the principles under Declaration of Helsinki were also taken into consideration. Blood samples were collected every alternate day in the B and NB season. The samples were obtained by jugular venipuncture in a 5.0 ml sterile syringe. Serum was isolated and frozen (–20˚C) until assayed for P4 by Chemiluminiscence analyzer using Acculite Progesterone kit (Lilac Medicare Ltd., India). Progesterone concentration and ovarian status were correlated at different day of observation or estrous cycle depending upon the presence or absence of estrous cycle (Menchacha and Rubianes, 2002).

Statistical analysis

The data obtained were analysed using package R (ver 3.4.3). Interactions included in the Analysis of Variance (ANOVA) model were between the effects of the breeding season and the order in which follicular waves (first, second, third, fourth or fifth wave) recorded during the period of observation were tested using General Linear Model of one way ANOVA based on Fisher's Least Significant Difference method. The significant values in the ANOVA were further tested through Duncan analysis. Results are presented as mean±SEM and differences were considered significant when P<0.05.

Results and Discussions

Average (mean±SEM) light: dark h in a day during the month of May and June (NB season) and January and February (B season) were 13.88±0.006: 10.12±0.006, 13.76±0.004: 10.24±0.004, 10.27±0.01: 13.73±0.001 and 10.88±0.004: 13.12±0.004, respectively. Significant difference (P<0.01) between the light and dark hours during NB and B season were recorded in present study. Ultrasonography revealed that follicular development occurred in wave like manner irrespective of the season. Majority of goats (9/11; 81.8 per cent) exhibited 3 follicular waves during NB season whereas 71.4 per cent (5/7) goats exhibited 4 wave pattern during the B season. Overall data during NB and B season, the average number of follicular waves were significantly higher (P<0.01) during B season compared to NB season (4.0±0.21 *versus* 3.18±0.12). Our findings are in contrast to earlier observations (Nogueira *et al*., 2015, 4.8±0.1 *versus* 4.1±0.1) and Dogan *et al*., 2020 (4.95±0.27 *versus* 4.84±1.58) who reported higher number of waves during NB than breeding season. Various researchers' have examined follicular dynamics during the estrous cycle of goats and reported the occurrence of two to six follicular waves during an estrous cycle, with an average of four waves per cycle (Ginther and Kot, 1994; de Castro *et al*., 1999; Menchaca and Rubianes, 2002; Medan *et al*., 2005; Simoes *et al*., 2006; Mohammadi *et al*., 2010; Nogueira *et al*., 2015; Dogan *et al*., 2020). Variations in the number of waves could be related to breed, age, environment and nutrition as well as seasonal differences in the frequency and amplitiude of the gonadotropic hormones Hence, it could be concluded from the present study that Gaddi goats have wave-like pattern of ovarian follicular growth irrespective of season, as reported earlier in cyclic (Ginther and Kot, 1994; de Castro *et al*., 1999; Menchaca and Rubianes, 2002; Medan *et al*., 2005; Simoes *et al*., 2006; Mohammadi *et al*., 2010; Nogueira *et al*., 2015; Dogan *et al*., 2020) and anestrus (Cruz *et al*., 2005; Nogueira *et al*., 2015; Dogan *et al*., 2020) goats. During B season last wave of the estrous cycle was ovulatory as depicted by disappearance of preovulatory follicle and visualization of increased heterogenous echogenicity in its site characterizing corpus haemorrhagicum (Gonzalez-Bulnes *et al*., 2004).

Perusal of the Table 1 suggests insignificant variation in the day of emergence (DE) of respective waves during B and NB season except the third wave of B *versus* NB season (9.28±0.47 *versus* 11.36±0.50; P<0.05). Significantly higher number of follicles at wave emergence were recorded in second (2.85±0.26

Table 1: *Comparison of average (mean±SEM) follicular waves characteristics in Gaddi goats during non-breeding and breeding season.*

Wave Parameters	Wave 1		Wave 2		Wave 3		Wave 4		Wave 5	
	NB Season (n=11)	B Season (n=7)	NB Season (n=11)	B Season (n=7)	NB Season (n=11)	B Season (n=7)	NB Season (n=2)	B Season (n=6)	NB Season (n=0)	B Season (n=1)
Day of emergence (DE)	- 0.09±0.25	0.28±0.18	5.54±0.31	5.0±0.61	11.36±0.50*	9.28±0.47*	14.0±1.0	15.33±0.49	0.0	19±0.0
No. of follicles at wave emergence	3.22± 0.22	3.14±0.34	1.77±0.14**	2.85±0.26**	2.0±0.16*	2.57±0.2*	2.0±0.0	2.66±0.21	0.0	2.0
Day of maximum diameter (DM)	6.18±0.48*	4.28±0.47*	12.27±0.57**	9.28±0.52**	17.8±0.75**	14.43±0.6**	19.0±1.0	20.67±0.49	0.0	23±0.0
Dominant follicle diameter (mm)	8.25±0.21**	6.90±0.36**	7.51±0.14	7.01±0.22	7.33±0.08	7.6±0.28	7.12±0.01	7.01±0.26	0.0	6.5
Growth rate of dominant follicle (mm/day)	0.74±0.04	0.87±0.12	0.60±0.03*	0.75±0.07*	0.61±0.03	0.78±0.10	0.72±0.11	0.71±0.08	0.0	0.70±0.31
Regression rate of dominant follicle (mm/day)	0.82±0.06	0.77±0.08	0.75±0.04	0.84±0.1	0.69±0.04	0.71±0.07	0.91±0.13	1.07±0.31	0.0	0.0
Persistence of dominant follicle (days)	13.36±0.65**	9.28±0.83**	13.09±0.76**	9.71±0.35**	10.36±0.47	11.2±0.74	8.0±1.0	7.0±0.77	0.0	5.0
Largest subordinate follicle diameter (mm)	5.23±0.35	5.08±0.31	5.22±0.25	5.28±0.29	5.21±0.22	5.34±0.24	4.65±0.05	5.3±0.23	0.0	4.8
Inter wave interval (IWI)	5.63±0.38	4.71±0.52	5.81±0.46*	4.28±0.28*	5.0±1.0	6.16±0.70	0.0	5.0±0.0	-	-

*NB season: Non Breeding season; B season: Breeding season. *: Significant (P<0.05); **: Highly Significant (P<0.01) between Non breeding and Breeding season within each wave.*

versus 1.77±0.14; P<0.01) and third wave (2.57±0.2 *versus* 2.0±0.16; P<0.05), respectively during B compared to the NB season. Researchers (de Castro *et al*., 1999; Menchaca and Rubianes, 2002) have established the relationship of mid luteal phase estradiol, P4 concentration and the number of follicular waves. They suggested that estradiol concentration declined earlier in the four wave than three wave goats and this early decrease of estradiol is hypothesized to cause early FSH rebound yielding in the emergence of second follicular wave. Whereas, in three wave animals the wave 2 emergence was delayed until the decrease of estradiol concentration, which occurred later (Rubianes and Menchaca, 2003). During B season endocrine and paracrine regulation of gonadotropins is well established justifying their role in follicular development (Hunter *et al*., 2004). In brief, the variation in the gonadotropin secretion would explain the differences in follicular dynamics between B and NB season (Bartlewski *et al*., 1999).

Significantly shorter persistence of dominant follicle in wave 1 (DF; 9.28±0.83 *versus* 13.36±0.6, P<0.01), wave 2 DF (9.71±0.35 *versus* 13.09±0.76, P<0.01) were observed during B than NB season (Table 1). Data combined separately for all the waves revealed significantly shorter persistence of DF during B than NB season (9.25±0.45 *versus* 12.02±0.44, P<0.001). Similarly, shorter persistence of DF was observed in wave 4 (71.4 %) and wave 5 (14.2 %), respectively during breeding season in Gaddi goats. Both of these waves were ovulatory as indicated by onset of luteolysis and LH surge which spurs the growth of follicles (Ginther and Kot, 1994; de Castro *et al*., 1999; Nogueira *et al*., 2015).

Barring the average diameter of wave 1 DF (6.90±0.36 mm) during B season that was significantly smaller than average diameter of corresponding wave (8.25±0.21 mm) during NB season, the average diameter of DF of wave 2 and 3 (considering the presence of at least 3 waves during both seasons) were similar during NB and B season. However, combining the data for all waves, though separately for NB and B season, the diameter of DF was smaller during B than NB season (7.11±0.14 *versus* 7.66±0.10 mm; P<0.05). The latter justifies the lower average size of DF in B than NB season. The maximum diameter of DF during the B season was attained earlier compared to NB season during first three waves which could be explained by the fact that majority of animals were following four wave pattern (71.4 per cent) in contrary to three waves pattern (81.6 per cent) during NB season.

The overall average growth rate of DF was significantly increased during B than NB season (0.79±0.04 *versus* 0.65±0.03, P<0.05). Insignificant differences (P>0.05) between the growth and regression rates of the DF during different waves, except for wave 2 (growth rate; 0.75±0.07 *versus* 0.60±0.03, P<0.05) during B and NB season were observed in present study. Both, maximum diameter and growth rate of DF are governed by the varying concentration of LH (Ginther and Kot, 1994; Gonzalez de Bulnes *et al*., 1999; de Castro *et al*., 1999; Schwarz and Wierzchos, 2000; Rubianes and Menchaca, 2003; Medan *et al*., 2003; Simoes *et al*., 2006; Nogueira *et al*., 2015). In cycling animals, simulating to goats during B and NB season, increasing concentration of P4 after ovulation is one reason which limits the LH pulse frequency (Nogueira *et al*., 2015). Our observation pertaining to higher growth rates (around 1 mm/day) during B are in consonance to earlier reports (Ginther and Kot, 1994; Gonzalez de Bulnes *et al*., 1999; de Castro *et al*., 1999; Schwarz and Wierzchos, 2000; Rubianes and Menchaca, 2003; Medan *et al*., 2003; Simoes *et al*., 2006; Nogueira *et al*., 2015; Dogan *et al*., 2020), than NB season (Nogueira *et al*., 2015; Dogan *et al*., 2020). Increased secretion of the LH during B season could be hypothesized for faster follicular growth rates compared to during NB season (Ginther and Kot, 1994; Nogueira *et al*., 2015).

Inter wave interval (IWI) between wave 2 and 3 was significantly longer (5.81±0.46 days) in NB compared to B (4.28±0.28 days) season (Table 1). Combining the IWI for different waves revealed marginally longer IWI during NB (5.66±0.2 days) than B (4.90±0.31 days) season. Insignificant differences in IWI between wave 3 and wave 4 during the B season have also been observed earlier (de Castro *et al*., 1999; Menchaca and Rubianes, 2002; Simoes *et al*., 2006). Such differences have been proposed to occur due to endocrine variations during the estrous cycle, especially effect of P4 concentration on the follicular development. High P4 concentration terminates a wave earlier causing an emergence of next wave that ultimately reduces the follicular wave emergence interval. Progesterone inhibits follicular development by suppressing the LH pulse frequency. Longer IWI between ovulatory and its immediately preceding wave (6.16±0.70 days) during B season than any other two successive waves in NB season in

present study, corroborates to observations of Simoes *et al.* (2006). This supports the concept of indirect P4 action on follicular turnover and the fundamental role of natural luteolysis in providing an opportunity for final follicular maturation and ovulation of the DF of the ovulatory wave.

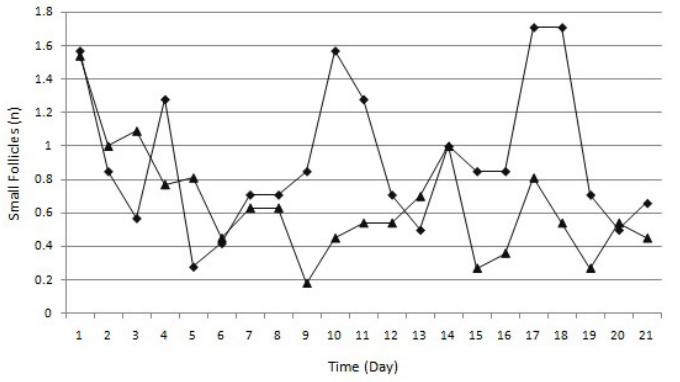

Figure 1a: *Daily changes in the number of small follicles (3 mm-<3.5mm diameter) for 21 days in Gaddi goats during the breeding (■) and non-breeding seasons (▲).*

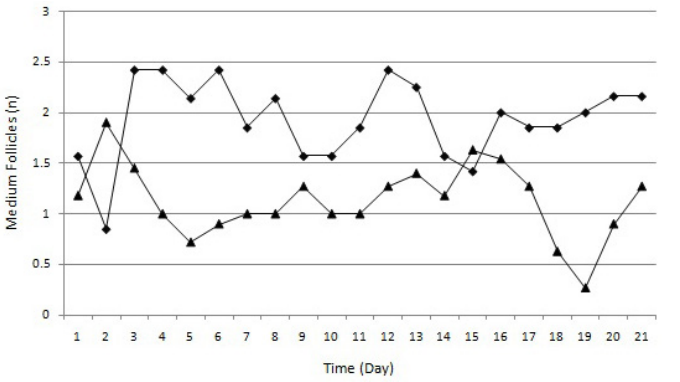

Figure 1b: *Daily changes in the number of medium follicles (≥3.5 mm to <5.0 mmdiameter) for 21 days in Gaddi goats during the breeding (■) and non-breeding seasons (▲).*

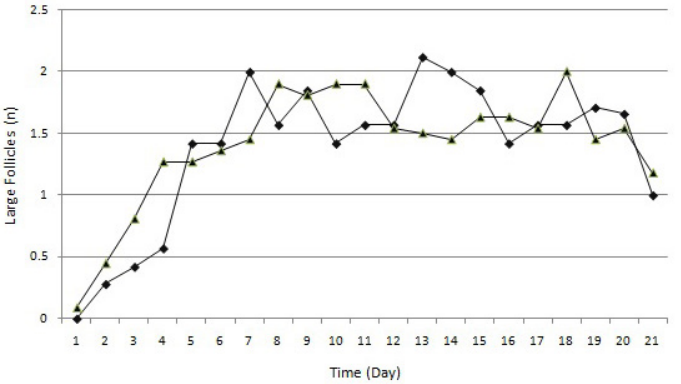

Figure 1c: *Daily changes in the number of large follicles (≥5.0 mm diameter) for 21 days in Gaddi goats during the breeding (■) and non-breeding seasons (▲).*

Perusal of (Figure 1a-d) indicates significantly higher daily number of small and medium sized follicles along with the average daily total number of visible follicles (1.12±0.20 *versus* 0.63±0.06, P<0.05; 2.59±0.69 *versus* 1.14±0.07, P<0.01 and 5.50±0.55 *versus* 3.15±0.67, P<0.05), respectively. Significantly higher daily number of small and medium sized follicles along with the average daily total number of visible follicles during B season (Figure 1a-d) is suggestive of pronounced ovarian activity during the B season in agreement to the earlier observations (Ginther and Kot, 1994; Medan *et al.*, 2003, 2005; Simoes *et al.*, 2006; Mohammadi *et al.*, 2010; Nogueira *et al.*, 2015; Dogan *et al.*, 2020). High plasma P4 concentrations during the estrous cycle suppress the basal secretion of LH, which is responsible for changes in antral follicle maturation until deviation. The dependence of the development of large antral follicles on FSH results in a decrease in number of follicles leading to a reduced number of follicles during NB season (Fatet *et al.*, 2011).

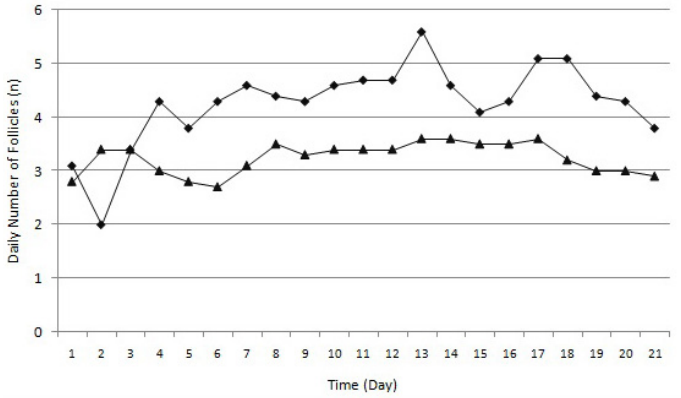

Figure 1d: *Daily changes in the number of total follicles (≥3.0 mm diameter) for 21 days in Gaddi goats during the breeding (■) and non-breeding seasons (▲).*

None of the does exhibited estrus behavior and ovulation during NB season. Whereas, all does exhibited estrus signs after the second injection of Cloprostenol during B season. Average circulating concentrations of P4 during NB season was 0.30±0.04 ng/mL whereas during B season average maximum concentration was much higher (11.89±1.55 ng/ml) which was recorded on Day 14 of estrous cycle. The last wave during B season was ovulatory, with interovulatory and interestrus interval of 20.85±0.63 and 21.0±0.53 days, respectively. Average (mean±SEM) diameter of ovulatory follicle (6.85±0.24 mm), mean luteal and follicular phase durations were 17.57±0.36 and 3.14±0.26 days, respectively. Similar pattern of lower progesterone concentration of <1ng/ml concentration were observed during NB season (0.65 ng/ml, Nogueira *et al.*, 2015; 0.79 ng/ml, Dogan

et al., 2020). During B season variable higher P4 concentration (12.4±2.1, 13.3±1.7 ng/ml) at Day 15 by (Sharma, 2015; Nogueira *et al*., 2015) and lower P4 concentration (7.68±0.46, Dogan *et al*., 2020; 8.35±2.60, Goel and Kharche, 2012; 9.16-10.95 ng/ml, Farshad *et al*., 2008) at Day 14 to 16 were also observed. Progesterone concentration of <1ng/ml throughout the NB season support our ultrasound investigations of non detection of corpus luteum in the present study. Progesterone concentrations are mediated by LH which plays an important role in follicular turnover during B season but not in the NB season. Increased day length during NB season is known to reduce the secretion of melatonin and to increase the negative feedback of oestradiol, which in turn inhibits the secretion of GnRH and results in reduced pulsatile LH secretion (Fatet *et al*., 2011). Similar observations (Farsi *et al*., 2018) suggesting the relationship of photoperiod and ambient temperature regarding the reproduction being activated in response to shorter photoperiod and lower ambient temperature, both of which were present during the B season in our study.

Average (mean±SEM) interovulatory interval recorded in present study was higher than Boer goats (19.7±0.2, Nogueira *et al*., 2015), White Polish goats (20.4±1.1; Schwarz and Wierzchos, 2000), Saanen goats (20.47±0.19; Dogan *et al*., 2020), Najdi goats (20.6±0.89; Mohammadi *et al*., 2010), similar to Saanen goats (20.8±0.89; Menchaca and Rubianes, 2002; 20.7±1.0; Simoes *et al*., 2006), lower than Shiba goats (21.3±0.4, Medan *et al*., 2005), respectively. Duration of estrous cycle observed in present study was slightly higher or similar to earlier reports of Boer goats (20.7±0.2, Nogueira *et al*., 2015), Anglo-Nubian goats (20.3±0.8 and 22.8±1.3 days), Serrana goats (20.4±1.1 days nulliparous; 21.0±0.7, multiparous goats; Simoes *et al*., 2006).

Conclusion and Recommendations

Significantly higher number of follicular waves, number of follicles at wave emergence during second and third wave, shorter persistence of dominant follicle and early attainment of DF during first three waves with higher growth rate were observed during B season. Whereas, significantly greater maximum diameter of DF and longer IWI between wave 2 and 3 was observed during NB season. Average mean P4 concentration was 0.30±0.04 ng/ml throughout the period of observation during NB whereas, peak P4 concentration of 11.89±1.55 ng/ml was observed at Day 14 of estrous cycle with interovulatory and interoestrus interval of 20.85±0.63 and 21.0±0.53 days during B season. Significant difference between average daily count of small and medium sized follicles along with average daily total number of visible follicles were observed during B season. Present study also supports the relationship of follicular development with progesterone concentration.

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Authors Contribution

Amit Sharma conducted the research trial, data collection, analysis and writing of manuscript. Dr. Pankaj Sood helped in conceptualizing the research. Both the authors contributed and coordinated in finalization of manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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