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Ultrasonographic guided follicular dynamics in Gaddi goats during non-breeding season

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ABSTRACT

Goat rearing is an important component of the agrarian society of Himachal Pradesh, India. Gaddi goats constitute about three-fourth of the total goat population of the state and are reared by approximately 35% traditional Gaddi families as migratory flocks on sub-mountainous pastures during winter and high altitude alpine pastures during summers. Out of season breeding is practiced by shepherds which however, needs to be validated with follicular dynamics and endocrine estimations. Adult, healthy, non-pregnant Gaddi does (11), aged 3.74±0.28 years, weighing 24.27±0.70 kg were investigated during conventional non-breeding season of long days (May-June 2016) at University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur for follicular wave characteristics. The follicular wave pattern comprised either three (81.8%, 9) or four (18.2%, 2) waves, the average being 3.18±0.12 during non-breeding seasons with none of these being ovulatory. The data combined for all waves during non-breeding season revealed an average inter wave interval of 5.66±0.28 days, the average growth rate and maximum size of dominant follicles was 0.65±0.03 mm per day and 7.66±0.10 mm, respectively. The dominant follicle (DF) persisted for lesser duration in majority of four wave animals than three wave animals with second wave exhibiting significantly lower (10.0±3.0 *vs* 13.78±0.57) duration of persistence of DF. Significant difference in the average daily count of small, medium and large follicles were observed in present study. The average daily progesterone concentration during the period of investigation was 0.30±0.04 ng/ml. In conclusion, out of seasonal breeding, could not be supported by repeated ovarian ultrasonography examination in Gaddi goats during long days revealing recurrent follicular waves without ovulation.

Key words: Gaddi goats, Follicular dynamics, Ultrasonography, Non-breeding season

Livestock is an integral component of socio-economic viability of India in general and Himachal Pradesh in particular with greater than 90% of farmers engaged in agriculture. Out of the total livestock population in the state, goats constitute more than 40% of total livestock with state capacity. Majority of goat population in Himachal Pradesh is of 'Gaddi' breed which is mainly confined to sub-temperate to sub-tropical zones of the state. It is reared in a migratory pattern moving from high hills to plains, during transition of summer to winter and vice-versa (Dogra and Thakur 2010). Goat serves as a multipurpose animal. Besides being a source of chevon, Gaddi goats are also a source of milk, fibre, skin and manure. Considering the advantages of goat rearing along with a declining cattle population in Himachal Pradesh, goats may assume a more important role under small household system. The kidding in goats is spread throughout the year which indicates that some of these breed successfully during long days. This contradicts well established fact of goat breeding exclusively during short days.

In light of above facts, a systematic investigation was required to justify 'out of season' breeding in Gaddi goats. The ovarian follicular dynamics and endocrine investigation are important yardsticks delineating the suitability of environment for breeding and conception. So the present study was planned to investigate the ultrasound guided follicular dynamics and progesterone profiling in Gaddi goats of Himachal Pradesh during the non-breeding season (long days).

MATERIALS AND METHODS

Firsthand information regarding the breeding/kidding pattern practiced by the Gaddi shepherds was collected from 25 shepherds of Palampur and surrounding areas of Kangra district, Himachal Pradesh, India (Annexure 1). The experiment was carried out during conventional non-breeding season of long days (May-June 2016) at University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur (32.6°N, 76.3°E, altitude 1290.8 m).

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The Gaddi goats were subjected to grazing (G) for five hours in a day (9:00 to 12:00 h and from 14:00 to 16:00 h). During the remaining period, the goats were housed under confined (C) conditions in a shed. The shed was half open (9.2×6.4 feet) and half closed (8.5×6.4 feet). Each shed housed 11 to 13 does.The Gaddi goats were fed as per the standards of Indian Council of Agricultural Research (ICAR 2013). All goats had round the clock access to the clean drinking water under C condition. The does were observed twice daily for behavioural signs of estrus by visual recording and parading/teasing of one mature buck for 30 min during the period of study. The does which allowed the buck to mount were classified as being in estrus. Intromission by the buck during mounting was prevented by manual withdrawal of the buck from the doe.

Adult, healthy, non-pregnant Gaddi does (11), aged 3.74±0.28 years, weighing 24.27±0.70 kg, respectively were included for investigation on follicular dynamics in present study. Average light**:**dark (h) during the month of May and June 2016 (non-breeding season) were 13.88±0.006**:** 10.12±0.006 and 13.76±0.004:10.24±0.004 (h), respectively (Department of Agronomy, College of Agriculture, CSKHPKV, Palampur). Significant difference ($P<0.01$) between the light and dark hours during non-breeding season were recorded in present study.

Investigation during the non-breeding season (anestrous animals) comprised follicular dynamics lasting one month so that the cut-off period of 21 days was evaluated for wave characterstics. Ultrasonography was initiated randomly and a retrospective analysis was made to consider the initiation of a follicular wave. The seasonal anestrous or acyclicity was ascertained on basis of transrectal ovarian ultrasonography and progesterone estimation.

A 7.5 MHz linear array transducer (Exago, ECM France) was used for ultrasonography to facilitate the process. A Cervical dialator (steel rod; diameter, 15 mm; length, 24 cm) was fastened to the transducer with adhesive tape so that the probe could be manipulated with a hand external to the rectum. All ultrasound examinations of the ovaries were done by a single operator. Goats were restrained in the ventro-dorsal recumbancy on the fabricated wooden table. Faecal pellets were removed digitally. Ultrasound gel was inserted with the fingers into the rectum to act as a coupling medium between the rectal wall and transducer. The transducer was inserted and manipulated in the rectum by external control of the extension. The urinary bladder, cranial vagina, and cervix were viewed in the longitudinal planes while the transducer was being inserted. After the cervix and caudal uterus were viewed, the transducer was rotated 45 to 90 degrees clockwise and counter clockwise to locate the ovaries (Kahn 1994, Ginther and Kot 1994, Medan *et al.* 2003).

All follicles ≥ 3 mm 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 ≥ 5 mm in diameter before subsequently regressing and being no longer detectable. The follicles were classified as small (3.0 to <3.5 mm); medium (≥3.5 mm to <5 mm) and large (≥5 mm). 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 48 h 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 interwave interval (IWI) was recorded as the number of days 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 growing phase of a follicle was defined as the period between its emergence and the day on which it appeared to stop its progressive increase in diameter. The day of maximum follicular diameter (DM) was the first day in each wave when dominant follicles attained maximum diameter. The regression phase of a follicle was defined as the period between its progressive cessation of increase in diameter to the day it was last detected. The regression rate was calculated by diameter of follicle at last day of static phase by number of days taken to its disappearance. The static phase of a follicle was defined as the period between the end of the growing phase and the beginning of the regression phase. During the non-breeding season, Day 0 was the day of emergence of the first recorded follicular wave (retrospective analysis, Nogueira *et al.* 2015).

In brief, various follicular dynamics which were studied during non-breeding season in Gaddi goats included day of emergence of largest follicle (DE), day of maximum diameter attained by the largest follicle (DM), diameter of largest follicle, diameter of largest subordinate follicle, growth and regression rate of largest follicle, number of waves, duration of waves, interwave intervals, follicle size classifications, daily number of follicles of different categories, total number of follicles.

Proper ethical considerations related to animal handling and blood collections were observed and ensuring not to cause any injury during sampling. Blood samples were collected every alternate day in the non-breeding season. The samples were obtained by jugular venipuncture in a 5 ml sterile syringe. After the collection, blood was allowed to clot. Serum was isolated and frozen (–20°C) until assayed for progesterone. Progesterone was estimated by Chemiluminiscence analyzer using Acculite Progesterone kit (Lilac Medicare (P) 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 (Thimonier 2000).

The data obtained were analysed using package R version 3.4.3. Interactions included in the ANOVA model were between the effects of the non-breeding season and the order in which follicular waves (1st, 2nd, 3rd or 4th waves) 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. Differences in means between the number of waves in non-breeding season were compared using independent t-test. Results are presented as mean±SEM and differences were considered significant when $P<0.05$.

RESULTS AND DISCUSSIONS

Appraisal of data collected as per questionnaire (Annexure 1), from shepherds (25) of different locations of Kangra district revealed an average herd size of 116.68±15.67 goats. Nearly 40% of the farmers followed male segregation. These males were reintroduced into the flock while migrating towards alpine pastures with the onset of long days (April onwards). Majority of females showed pronounced follicular activity within fortnight after the introduction of male which is marked by behavioural manifestation of estrus resulting in service by the running male. The presence of male and probably optimal grazing conditions results in onset of estrous cycle and breeding thereby culminating into nearly 80% kidding between September to December months during short days.

The follicular wave pattern observed in Gaddi goats during non-breeding season (May–June 2016) exhibited three (81.8%, 9) and four (18.2%; 2) waves, respectively. Nogueira *et al.* (2015) observed 4–6 follicular waves (4 wave: 28.6%; 5 wave: 64.3% and 6 wave: 7.1%) respectively during non-breeding season in Boer goats raised in tropics of Australia (September–October 2011). Similarly, Schwarz and Wierzchos (2000) observed 4 waves (Polish White goats, April-June); Cruz *et al.* (2005) (3) waves (Anglo-Nubian and Saanen goats) during anestrous period. Combined average mean (±SEM) number of follicular waves were 3.18±0.12 in the present study which was much lesser than Nogueira *et al.* (2015) (4.8±0.01; Boer goats). Whereas, Bartlewski *et al.* (1999) reported 2.8±0.1 waves in Western White-faced crossbred ewes (March–July).

Different follicular wave parameters comprised of day of emergence (DE), number of follicles at wave emergence, day of maximum diameter attained by the dominant follicle (DM), dominant follicle diameter, growth and regression rate of dominant follicle, persistence of dominant follicle, largest subordinate follicle diameter and inter wave interval (IWI) were studied and results are presented in Table 1.

The dominant follicle (DF) attained the maximum diameter significantly earlier in wave 2 (9.5±0.5 versus 12.88±0.48 day; ($P<0.01$)) and wave 3 (15.0±1.0 versus 18.44±0.64 day; ($P<0.05$)) in animals exhibiting four wave in comparison to three wave animals. The DF persisted for lesser duration in majority of four wave animals than three wave animals with second wave exhibiting significantly lower (10.0±3.0 versus 13.78±0.57; $P<0.05$) duration of persistence of DF. Similar observations with lower wave durations were earlier made by Nogueira *et al.* (2015) (Boer goats; 8.5±0.4, 8.3±0.3, 8.5±0.4 and 8.4±0.4; wave 1, 2, 3 and 4), respectively who observed 4–6 number of follicular waves, hence, as the number of waves increases the persistence of DF and the wave duration decreases. Overall mean persistence of the DF observed was 12.02±0.44 days in present study which was quite higher than reports of Nogueira *et al.* (2015) (Boer goats; 8.4±0.4 days).

Non-significant variations ($P>0.05$) with respect to maximum diameter of the DF and largest subordinate follicle (SF) were recorded in between the waves as well as within the wave (i.e. 3 and 4 wave animals) in present study. Combined average (Mean±SEM) diameter of DF (7.66±0.10 mm) and largest SF (5.19±0.15 mm) was observed in present study. Similar mean diameter of DF (7.3±0.16 mm) was observed in Saanen goats exhibiting 3 waves (Cruz *et al.* 2005). Whereas, other workers reported lower diameters of DF in Anglo-Nubian goats (6.5 mm, Cruz *et al.* 2005), Boer goats (6.7±0.1 mm, Nogueira *et al.* 2015) and Western White-faced crossbred ewes (5.9±0.3 mm, Bartlewski *et al.* 1999), respectively during non-breeding season.

Non-significant difference ($P>0.05$) were observed in between the waves in DE and IWI in animals exhibiting three and four waves in the present study. Similar observations with regard to DE, DM and IWI were made earlier in goats (Anglo-Nubian and Saanen breeds, Cruz *et al.* 2005; Boer, Nogueira *et al.* 2015), ewes (Bartelewski *et al.* 1999). Overall IWI was 5.66±0.28 days in present study which was higher than earlier observations in Boer goats (4.4±0.2 days; Nogueira *et al.* 2015) and Western White-faced crossbred ewes (4.6±0.5 days; Bartlewski *et al.* 1999).

During non-breeding season, circulating concentrations of progesterone were 0.30±0.04 ng/ml whereas, Nogueira *et al.* (2015) reported higher concentrations (0.65 ng/ml) in Boer goats. Progesterone concentration of <1ng/ml

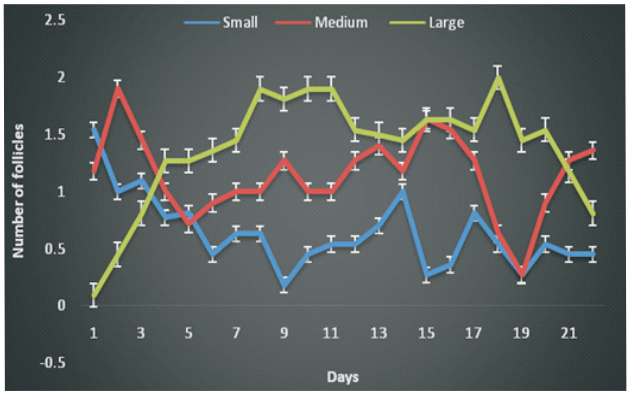

Fig. 1. Average (Mean±SEM) daily number of follicles of different sizes in Gaddi goats (11) during non-breeding season.

Table 1. Average (mean±SEM) characteristics of follicular waves in Gaddi goats (11) during non-breeding season

Wave parameter	3 Wave (9)	4 Wave (2)	P value
	Wave 1		
Day of emergence (DE)	0.0±0.28	–0.5±0.50	0.940
No. of follicles at wave emergence	3.22±0.22	3.5±0.5	0.603
Day of maximum diameter (DM)	6.22±0.59	6.0±0.0	-
Dominant follicle diameter (mm)	8.10±0.19	8.92±0.71	0.131
Growth rate of dominant follicle (mm/day)	0.73±0.05	0.80±0.09	0.550
Regression rate of dominant follicle (mm/day)	0.79±0.05	0.99±0.26	0.204
Persistence of dominant follicle	13.44±0.78	13.0±1.0	0.808
Largest subordinate follicle diameter (mm)	5.1±0.45	5.49±1.24	0.720
Inter wave interval (Wave 1-Wave 2; days)	5.66±0.47	5.5±0.50	0.882
	Wave 2		
Day of emergence (DE)	5.66±0.33	5.0±1.0	0.440
No. of follicles at wave emergence	1.77±0.14	2.0±1.0	0.644
Day of maximum diameter (DM)	12.88 ± 0.48^{B}	9.5 ± 0.5^{A}	0.001
Dominant follicle diameter (mm)	7.58±0.11	7.19±0.69	0.296
Growth rate of dominant follicle (mm/day)	0.58±0.03	0.69±0.14	0.223
Regression rate of dominant follicle (mm/day)	0.74±0.05	0.86±0.10	0.330
Persistence of dominant follicle	13.78 ± 0.57^{Y}	10.0 ± 3.0^{X}	0.050
Largest subordinate follicle diameter (mm)	5.14±0.20	4.83±1.38	0.656
Inter wave interval (Wave 2-Wave 3; days)	6.22±0.43	4.0±1.0	0.057
	Wave 3		
Day of emergence (DE)	11.88±0.45	9.0	–
No. of follicles at wave emergence	2.0±0.16	2.0±0.0	–
Day of maximum diameter (DM)	18.44 ± 0.64^{Y}	15.0 ± 1.0^{X}	0.043
Dominant follicle diameter (mm)	7.35±0.09	7.23±0.09	0.566
Growth rate of dominant follicle (mm/day)	0.60±0.03	0.65±0.09	0.517
Regression rate of dominant follicle (mm/day)	0.70±0.05	0.66±0.08	0.734
Persistence of dominant follicle	10.0±0.47	12.0±1.0	0.103
Largest subordinate follicle diameter (mm)	4.92±0.22	5.23±0.88	0.608
Inter wave interval (Wave 3-Wave 4; days)	–	5.0±1.0	–
	Wave 4		
Day of emergence (DE)	–	14.0±1.0	–
No. of follicles at wave emergence	–	2.0±0.0	
Day of maximum diameter (DM)	–	19.0±1.0	–
Dominant follicle diameter (mm)	–	7.12	–
Growth rate of dominant follicle (mm/day)	–	0.72±0.11	–
Regression rate of dominant follicle (mm/day)	–	0.91±0.13	–
Persistence of dominant follicle	–	8.0±1.0	–
Largest subordinate follicle diameter (mm)	–	4.65±0.05	–

$^{A–B}$Values with different superscripts within same row differs (P<0.01); $^{X–Y}$Values with different superscripts within same row differs (P<0.05).

Table 2. Average (Mean±SEM) daily number of follicles of different sizes in Gaddi goats (11) during non-breeding season

Follicle category	Follicle number non-breeding season
Small follicle (3.0 to < 3.5 mm)	0.63 ± 0.06^{A}
Medium follicle (≥3.5 to < 5 mm)	1.14 ± 0.07^{BD}
Large follicle (≥5 mm)	1.38 ± 0.10^{CD}
Total number of follicles	3.15±0.67

$^{A-D}$Values with different superscripts among same column differs (P<0.01).

throughout the period of study in present case indicated and supported the ultrasound investigations of not detecting corpus luteum in the group of goats during the non-breeding season. The findings in goats during the non-breeding season indicates that the gonadotrophic stimulation of follicular development would have been similar throughout the period of investigation contributing to similar wave dynamics between follicular waves (Nogueira *et al.* 2015). A longer day length in the non-breeding 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 a reduction in pulsatile LH secretion (Fatet *et al.* 2011).

Average (Mean±SEM) daily number of follicles of different sizes in Gaddi goats during non-breeding season were studied and their results presented in Table 2 and Fig. 1.

Overall 773 follicles of different sizes were recorded in Gaddi goats (11) during the period of investigation (Fig. 1). Statistically significant (P<0.01) differences between daily number of follicles of different sizes (small, 0.63±0.06 mm;

medium, 1.14±0.07 mm and large, 1.38±0.10 mm) were observed in present study which was in agreement to earlier finding of Cruz *et al.* (2005) (Anglo-Nubian and Saanen goats), Nogueira *et al.* (2015) (Boer goats) and Bartlewski *et al.* (1999) (Western White face crossbred sheep).

In conclusion, out of seasonal breeding, could not be supported by repeated ovarian ultrasonography examination in Gaddi goats during long days revealing recurrent follicular waves without ovulation suggesting the role of optimum grazing conditions supported by male effect which further needs to be investigated.

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ANNEXURE 1

Breeding pattern in Gaddi goats of Himachal Pradesh

- Name and Address of Owner .....
- Flock Strength: ..... (M) ..... (F) Total = ...
- For how long you are into this profession:
- Period of year (month) when estrus signs are more discernible? .....
- Are males and females reared separately ..... (Yes/No)
- If yes (Specify months) .....
- Method of male segregation followed (please specify): Use of leather apron method/Change of flock/Kept at home
- Kidding pattern (percentage): ..... (Sep–Nov); ..... (Dec–Feb); ..... (March–May) ..... (June–Aug)
- Advantages of kidding pattern in different seasons / periods?