



Ovarian follicular dynamics during the interovulatory interval in Gaddi goats

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ABSTRACT

Goat husbandry is an important component of the agrarian society of Himachal Pradesh, India. Adult, healthy, non-pregnant, Gaddi does (7), aged 4.52 ± 0.39 years, weighing 27.07 ± 1.03 kg were investigated during breeding season (January–February 2017) at University Farm for follicular wave characteristics. Cyclic Gaddi goats during breeding season mainly manifested four wave pattern of follicular growth (5; 71.4%), while the remaining animals had either 3 or 5 wave pattern (1 each; 14.3%). The last wave in all these animals was ovulatory, with interovulatory and interoestrus interval of 20.85 ± 0.63 and 21.0 ± 0.53 days, respectively. Average (Mean \pm SEM) diameter of ovulatory follicle was 6.85 ± 0.24 mm. The average progesterone concentration peaked to 11.89 ± 1.55 ng/ml at day 14 of estrus cycle. Mean luteal and follicular phase durations were 17.57 ± 0.36 and 3.14 ± 0.26 days, respectively. Overall daily total number of follicles during the breeding season were 5.50 ± 0.55 which suggests pronounced ovarian activity.

Key words: Estrous cycle, Follicular dynamics, Gaddi goats, Ultrasonography

Goats are important livestock species of India. Gaddi breed of goat is the mainstay of a large portion of nomadic and hilly tribe of Himachal Pradesh. They are reared by approximately 35% traditional ‘Gaddi’ families as migratory flocks on sub-mountainous pastures during winter and high altitude alpine pastures during summers (Dogra and Thakur 2010). Considering the advantages of goat rearing along with a declining cattle population in HP, goats may assume a more important role under small household system. Goats are spontaneously ovulating, polyestrous animals. They are bred in a wide range of production systems (Devendra and Coop 1982). A Non-invasive, real-time ultrasonographic observation permits the successive evaluation of ovarian structures in small ruminants. Ultrasonography guided follicular dynamics studies have been done by various workers in exotic breeds, but meager data is available in literature for Indian goats in general and Gaddi goats in particular. In light of above facts, a systematic study was planned to investigate follicular dynamics and progesterone profiling in Gaddi goats of Himachal Pradesh during the breeding season (short days).

MATERIALS AND METHODS

The experiment was carried out during conventional breeding season of short days (January–February 2017) at

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University Livestock Farm of CSK Himachal Pradesh Krishi Vishvavidyalya, Palampur (32.6°N, 76.3°E, altitude 1290.8 m).

The Gaddi goats were subjected to grazing (G) condition for 5 h 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 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 behavioral 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 (7), aged 4.52 ± 0.39 years, weighing 27.07 ± 1.03 kg, tag numbered (03, 04, 128, 1301, 1304, 1305 and 1306), respectively, were included for investigation on follicular dynamics in present study. The average of light:dark hours during January and February 2017 (breeding season) were 10.27 ± 0.01 : 13.73 ± 0.001 and 10.88 ± 0.004 : 13.12 ± 0.004 , respectively (Department of Agronomy, College of Agriculture, CSKHPKV, Palampur). Significant difference ($P < 0.01$) between the light and dark hours during breeding season were recorded in present study.

The cyclicity was ascertained on basis of transrectal ovarian ultrasonography and progesterone estimation. Gaddi goats had their estrous cycles synchronized with two injections of $187.5 \mu\text{g}$ of Pragma® (Cloprostenol $250 \mu\text{g}$ /

ml; Intas Pharmaceuticals Limited, India) 11 days apart. Ultrasonography was initiated once daily after administration of the last dose of Cloprostenol to detect ovulation with examinations continuing till the next ovulation.

Ultrasonography was done using 7.5 MHz linear array transducer (Exago, ECM France) as per defined technique (Kahn 1994, Ginther and Kot 1994, Medan *et al.* 2003). Different follicular wave parameters were studied during breeding season in Gaddi goats as per earlier observations (Ginther and Kot 1994, de Castro *et al.* 1999, Schwarz and Wierzchos 2000, Rubianes and Menchaca 2003, Medan *et al.* 2003, Simoes *et al.* 2006, 2008, Nogueira *et al.* 2015).

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 breeding season. The samples were obtained by jugular venipuncture in a 5 ml sterile syringe. Serum was isolated and frozen (-20°C) until assayed for progesterone 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 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 \pm SEM and differences were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Investigation during the present study revealed presence of three to five waves with four number of waves in majority (5, 71.4%) of Gaddi goats during breeding season. Various workers (Ginther and Kot 1994, de Castro *et al.* 1999, Schwarz and Wierzchos 2000, Menchaca and Rubianes 2002, Rubianes and Menchaca 2003, Medan *et al.* 2003, Simoes *et al.* 2006, 2008, Nogueira *et al.* 2015), suggests that majority of animals exhibit four follicular wave pattern in animals with interovulatory period ranging between 19–21 days. Combined average (Mean \pm SEM) no. of follicular waves were 4.0 ± 0.21 in present study, which were similar to Serrana goats (4.1 ± 0.6 , Simoes *et al.* 2006), lesser than Boer goats (4.8 ± 0.01 , Nogueira *et al.* 2015). Whereas, Bartlewski *et al.* (1999) reported 2.8 ± 0.1 waves in Western White-faced crossbred ewes. Researchers (de Castro *et al.* 1999, Menchaca and Rubianes 2002, Rubianes and Menchaca 2003) established the relationship of mid luteal phase estradiol, progesterone concentration and the number of follicular waves and suggested that estradiol concentration declined earlier in the four wave than three

wave goats and this early decrease of estradiol is speculated to cause early FSH rebound yielding 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).

In present study, individual wave parameters were not comparable (small sample size) between the animals exhibiting 3, 4 and 5 waves, respectively. Therefore, comparisons between different waves in goats exhibiting 4 waves were only analysed (Table 1). Significant differences between the persistence of DF (9.4 ± 0.40 vs 6.4 ± 0.6 ; wave 2 vs 4; $P < 0.05$) and (12.0 ± 0.83 vs 6.4 ± 0.6 ; wave 3 vs 4; $P < 0.01$) were observed in goats with 4 waves. Shorter persistence of DF of fourth wave compared to other waves was probably due to fourth wave being the ovulatory wave in 71.4% of goats which was in agreement to the findings of Simões *et al.* (2006, 61.3%) and Nogueira *et al.* (2015, 71%), respectively. The onset of luteolysis and an LH surge coincident with the development of the fourth follicular wave in majority of goats would contribute to a shorter duration of the fourth follicular wave compared to earlier waves (Ginther and Kot 1994, de Castro *et al.* 1999, Nogueira *et al.* 2015).

The mean diameter of DF of the third, fourth and fifth waves was 7.5, 6.8 ± 0.31 and 6.5 mm, respectively (Table 1). Combined average (Mean \pm SEM) diameter of ovulatory follicle was 6.85 ± 0.24 mm, which was higher than Najdi goats (6.3 ± 0.13 mm, Mohammadi *et al.* 2010), Saanen goats (6 and 7 mm, de Castro *et al.* 1999), Serrana goats (6.3 ± 1.0 mm, Simoes *et al.* 2006), similar to White Polish goats (7 mm, Schwarz and Wierzchos 2000), and lower than Boer goats (7.8 ± 0.1 mm, Nogueira *et al.* 2015), Shiba goats (8.2–8.3 mm, Medan *et al.* 2003 2005), respectively. A single ovulation was recorded in all the goats in present study (Fig. 1). Contrarily, higher ovulation rates of Gaddi goats (1.1, Sharma 2015), Jakhrana goats (1.33 ± 0.10 , Goel and Aggrawal 2003), Maradi goats (1.55, Akusu *et al.* 1986), West African dwarf goat (1.57, Akusu *et al.* 1986), Shiba goats (1.8 ± 0.2 , Medan *et al.* 2005), Najdi goats (1.8, Mohammadi *et al.* 2010) and Boer goats (1.8,

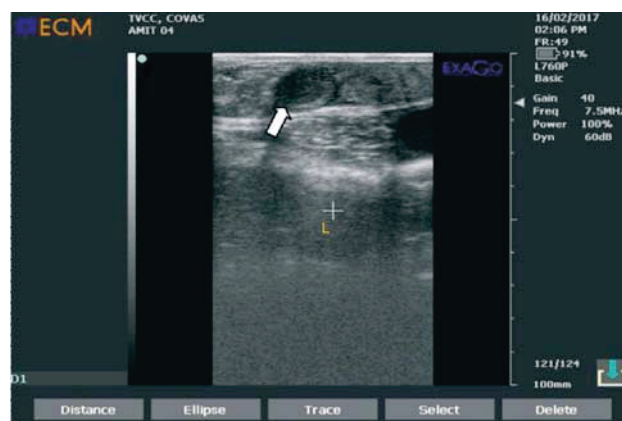


Fig. 1. Ultrasonographic image of ovulation marked by overall increased echogenicity of corpus haemorrhagicum (arrow head).

Table 1. Average (Mean±SEM) characteristics of follicular waves in Gaddi goats (n=7) during breeding season

Wave parameter	3 Wave (n=1)	4 Wave (n=5)	5 Wave (n=1)
<i>Wave 1</i>			
Day of emergence (DE)	0.0	0.2±0.20	1.0
No. of follicles at wave emergence	4.0	2.8±0.37	4.0
Day of maximum diameter (DM)	6.0	4.0±0.54	4.0
Dominant follicle diameter (mm)	8.2	6.83±0.50	5.95
Growth rate of dominant follicle (mm/day)	0.85	0.88±0.15	0.51
Regression rate of dominant follicle (mm/day)	0.52	0.81±0.11	0.91
Persistence of dominant follicle (days)	12.0	9.2±0.91 ^b	7.0
Largest subordinate follicle diameter (mm)	5.6	4.88±0.40	5.6
Inter wave interval (Wave 1-Wave 2; days)	7.0	4.0±0.31 ^x	6.0
<i>Wave 2</i>			
Day of emergence (DE)	7.0	4.2±0.48	7.0
No. of follicles at wave emergence	3.0	2.8±0.37	3.0
Day of maximum diameter (DM)	7.0	7.04±0.41	6.9
Dominant follicle diameter (mm)	7.0	7.04±.32	6.9
Growth rate of dominant follicle (mm/day)	0.72	0.75±0.09	0.83
Regression rate of dominant follicle (mm/day)	0.74	0.94±0.13	0.63
Persistence of dominant follicle (days)	11.0	9.4±0.40 ^b	10.0
Largest subordinate follicle diameter (mm)	4.2	5.32±0.27	6.2
Inter wave interval (Wave 2-Wave 3; days)	3	4.6±0.24 ^x	4.0
<i>Wave 3</i>			
Day of emergence (DE)	10.0	8.8±0.48	11.0
No. of follicles at wave emergence	2.0	2.8±0.2	2.0
Day of maximum diameter (DM)	18.0	13.6±0.40	15.0
Dominant follicle diameter (mm)	7.5	7.48±0.46	8.4
Growth rate of dominant follicle (mm/day)	0.54	0.84±0.13	0.95
Regression rate of dominant follicle (mm/day)	–	0.66±0.06	1.04
Persistence of dominant follicle (days)	9.0	12.0±0.83 ^d	10.0
Largest subordinate follicle diameter (mm)	4.2	5.64±0.18	5.0
Inter wave interval (Wave 3-Wave 4; days)	–	6.8±0.37 ^y	3.0
<i>Wave 4</i>			
Day of emergence (DE)	–	15.6±0.50	14.0
No. of follicles at wave emergence	–	2.6±0.24	3.0
Day of maximum diameter (DM)	–	21.0±0.44	19.0
Dominant follicle diameter (mm)	–	6.88±0.34	7.7
Growth rate of dominant follicle (mm/day)	–	0.68±0.08	0.86
Regression rate of dominant follicle (mm/day)	–	–	1.07
Persistence of dominant follicle (days)	–	6.4±0.6 ^{ac}	10.0
Largest subordinate follicle diameter (mm)	–	5.1±0.15	6.3
Inter wave interval (Wave 4-Wave 5; days)	–	–	5.0
<i>Wave 5</i>			
Day of emergence (DE)	–	–	19.0
No. of follicles at wave emergence	–	–	2.0
Day of maximum diameter (DM)	–	–	23.0
Dominant follicle diameter (mm)	–	–	6.5
Growth rate of dominant follicle (mm/day)	–	–	0.70±0.31
Regression rate of dominant follicle (mm/day)	–	–	–
Persistence of dominant follicle (days)	–	–	5.0
Largest subordinate follicle diameter (mm)	–	–	4.8

^{c-d, x-y}Values with different superscripts among same column differs (P<0.01). ^{a-b}Values with different superscripts among same column differs (P<0.05).

Nogueira *et al.* 2015), Olkuska ewes (3.75, Dorota *et al.* 2001), respectively were also observed.

Significantly higher (P<0.01) IWI of 6.8±0.37 days, between third and fourth wave (ovulatory) in 71.4% animals was evident in present study exhibiting 4 waves with a combined average IWI of 4.90±0.31 days during breeding

season (Table 1). Similar or slightly higher IWI were earlier reported in Boer goats (4.2±0.4, Nogueira *et al.* 2015), Shiba goats (5.6±0.3, Medan *et al.* 2005) and Olkuska ewes (4.4±1.6, Dorota *et al.* 2001), respectively. Present findings support the concept of indirect progesterone action on follicular turnover and the fundamental role of natural

Table 2. Average (Mean±SEM) daily number of follicles of different sizes in Gaddi goats (n=7) during breeding season

Follicle category	Follicle number Breeding Season	P value
Small follicle (3.0 to < 3.5 mm)	1.12±0.20 ^A	P<0.001
Medium follicle (≥ 3.5 to < 5 mm)	2.59±0.69 ^C	
Large follicle (≥ 5 mm)	1.79±0.45 ^B	
Total number of follicles	5.50±0.55	

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

luteolysis in ovulatory wave selection (Rubianes and Menchaca 2003).

Average (Mean±SEM) interovulatory and interoestrus interval for Gaddi goats in present study was 20.85±0.63 and 21.0±0.53 days, respectively (Table 3). 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), 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 and West African dwarf goats (21.3±0.4, Medan *et al.* 2005; 23.8, Akusu *et al.* 1986), 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).

Nonsignificant differences between the number of follicles at wave emergence, DF diameter, largest subordinate follicle diameter were observed within four wave goats in present study. Average (Mean±SEM) daily number of follicles of different sizes in Gaddi goats (7) during breeding season were studied and their results are presented in Table 2.

Overall 636 follicles of different sizes were recorded on either of ovary in Gaddi goats (7) during the period of investigation. Statistically significant (P<0.01) differences between daily number of follicles of different sizes (small, 1.12±0.20 mm; medium, 2.59±0.69 mm and large, 1.79±0.45 mm) were observed in this study which were in agreement to earlier finding of Medan *et al.* (2003, 2005; Shiba goats), Nogueira *et al.* (2015; Boer goats). Overall daily total number of follicles during the breeding season were 5.50±0.55 which suggests pronounced ovarian activity during the breeding season.

Average maximum progesterone (P₄) concentration in the present study was 11.89±1.55 ng/ml which was recorded on day 14 of estrous cycle, and mean luteal phase was 17.57±0.36 days and follicular phase 3.14±0.26 days (Table 3). Similar or higher peak progesterone concentrations (12, 12.4±2.1; 13.3±1.7 ng/ml at day 15) to those recorded in present study were observed earlier (Levy-Ocariz *et al.* 1995, Sharma 2015, Nogueira *et al.* 2015). Contrarily, lower peak P₄ concentration of 8.35±2.60

Table 3. Average (Mean±SEM) characteristics of follicular dynamics in Gaddi goats (n=7) during the breeding season

Parameter	(Mean±SEM)
Interovulatory interval (days)	20.85±0.63
Interestrus interval (days)	21.0±0.53
Maximum diameter of preovulatory follicle (mm)	6.85±0.24
Growth rate of ovulatory wave (mm/day)	0.67±0.08
Diameter of corpus luteum haemorrhagicum (mm)	9.88±0.36
Largest follicle diameter (mm)	7.01±0.15
Interval between emergence to ovulation of the ovulatory follicle (days)	6.57±0.61
Maximum diameter of CL (mm)	10.21±1.06
Maximum concentration of P ₄ (ng/ml)	11.89±1.55
Day of maximum concentration of P ₄	13.71±0.99
Day of lowest concentration of P ₄	1.0±0.21
Mean luteal phase length (days) ^a	17.57±0.36
Mean follicular phase length (days) ^b	3.14±0.26

^awhen concentration of the progesterone in plasma were ≥ 1 ng/ml. ^bwhen concentration of the progesterone in plasma were ≤ 1 ng/ml.

ng/ml (Goel and Kharche 2012) and 9.16–10.95 ng/ml (Farshad *et al.* 2008) at day 14 of estrous cycle were also observed. Mean luteal phase (17.57±0.36 days) and follicular phase (3.14±0.26 days) lengths were similar to earlier observations of Nogueira *et al.* (2015; Boer goats; 16±0.4 and 5±0.3 days).

Follicular dominance was weakly expressed in present study as suggested earlier (Ginther and Kot 1994, Medan *et al.* 2003, 2005, Simões *et al.* 2006, Mohammadi *et al.* 2010, Nogueira *et al.* 2015) than that had been reported in cattle (Adams *et al.* 1993).

In conclusion, the Gaddi goats during breeding season were cyclic and manifested mainly 4 wave patterns of follicular growth (5, 71.4%), while the remaining animals had either 3 or 5 wave pattern (1 each; 14.3%). The last wave in all these animals was ovulatory, with inter-ovulatory and inter-estrus interval of 20.85±0.63 and 21.0±0.53 days, respectively. The average progesterone concentration peaked to 11.89±1.55 ng/ml at day 14 of estrus cycle. Mean luteal and follicular phase durations were 17.57±0.36 and 3.14±0.26 days, respectively.

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