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Ovarian follicular dynamics in peripubertal Malabari goats#

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Abstract

The aim of this study was to characterise the follicular dynamics in first two oestrous cycles of peripubertal Malabari goats. Transrectal ultrasonography was carried out on alternate days of oestrous cycles after first observed oestrus. Follicular studies revealed presence of two to five wave cycles with predominant three and four wave patterns. The interwave interval for ovulatory wave was longer than preceding waves in three and five wave cycles. The diameter of largest follicles in wave 1 was larger than follicles of subsequent waves of three, four and five wave cycles. The largest follicles in each wave could be observed from day 3 to day 13 of oestrous cycle. Majority of ovulations occurred on day 20 of oestrous cycles and short cycles were more in first oestrous cycle. The overall mean per cent of single and double ovulations were 56.3 and 43.8 and mean ovulatory follicle size was 6.59 ± 0.11 mm in peripubertal goats. The mean length of first and second oestrous cycle was observed as 17.72± 0.79 and 20.19± 0.43 days, respectively and mean duration of first and second oestrus was 36.88 ± 2.44 and 40.13 ± 1.84 h, respectively. The study revealed that predominant wave pattern was three and four waves in peripubertal Malabari breeds. The first oestrous cycle length was shorter and characterised by higher percentage of single ovulations with smaller periovulatory follicle size. Larger ovulatory follicles and double ovulations were observed in second oestrous cycle.

Key words: *Follicular dynamics, peripubertal Malabari goats*

Ovarian follicular development occurs in wave like pattern in small ruminants. This involves various growth phases like recruitment, selection, dominance and atresia. These sequential events

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occurring during the interovulatory period is under the influence of gonadotropins *viz*. follicle stimulating hormone (FSH) and luteinizing hormone (LH). Various intraovarian factors like sex steroids, growth factors, regulatory proteins and cytokines also play a vital role in folliculogenesis. Follicular dynamics is characterised by numerous parameters such as number of waves, emergence of wave, interwave interval, day of largest follicles in wave, diameter of largest follicle in wave, number of ovulations and interovulatory interval (Simoes *et al*., 2006). Studies with regard to characterisation of follicular development in Malabari breed of goat are scanty, hence this study was undertaken to evaluate the follicular growth pattern in peripubertal Malabari doelings after first observed oestrus.

Materials and methods

The experiment was conducted in 16 peripubertal Malabari goats maintained under identical management conditions at University Goat and Sheep Farm, Mannuthy under Kerala Veterinary and Animal Sciences University. Follicular dynamics of two oestrous cycles were evaluated using transrectal ultrasonography (TRUS) on alternate days after the first exhibited oestrus. Animals were housed in well ventilated sheds and stall fed with complete ration in the form of pellets. Daily feeding allowance was according to ICAR (2013) standards and water was provided *ad libitum*.

Ultrasonography

Reproductive ultrasonography was carried out using a real time scanner (MyLab Gamma, EsaoteSpA, Italy) equipped with 5-10 MHz frequency linear array transrectal transducer (SV3513, EsaoteSpA, Genova, Italy). Alternate day examination of ovaries was carried out for two oestrous cycles. The TRUS examination was done with the animals in standing position. Rectum was evacuated and ultrasound gel was introduced to avoid damage to the mucosa and to improve the transmission of ultrasound waves. The transrectal probe was protected with a disposable plastic sleeve with ultrasound gel inside to avoid any air contact. Thereafter, probe was introduced to the rectum, orienting the transducer perpendicular to pelvic floor for locating the urinary bladder. The uterine horns and ovaries were then located on either side by rotating the probe laterally clockwise and counter clockwise as the bladder was surpassed (Gonzalez- Bulnes *et al*., 2010).

The following parameters were studied as described by Simoes *et al*. (2006):

Number of follicular waves per oestrous cycle

The onset of a wave was recognised by first observation of at least one follicle of size ≥ 3 mm diameter resulting in a follicle with a minimum diameter of 5 mm on following days.

Interwave interval (IWI)

Interval between the onset of a follicular wave and the subsequent follicular wave was recorded.

Diameter of largest follicles in each wave

In growing phase of follicular wave, when one or more dominant follicles attained a size greater than 5 mm the measurement was recorded.

Day of largest follicle in each wave

The day on which largest follicles of size ≥ 5mm in waves other than ovulatory wave were identified in three, four and five wave cycles.

Day of ovulation

Day of ovulation was identified by the disappearance of dominant follicles of size ≥ 5mm followed by the development of CL in ovaries.

Number and diameter of ovulatory follicles

Maximum diameter attained by ovulatory folliclesduring oestrous cycle and total number of follicles ovulated from both the ovaries were observed. The diameter of the follicle (mm) was estimated using the following equation.

D = (A+B)/ 2, where, A represents the maximum height and B, the maximum transverse diameter (Honnens *et al*., 2009).

Length of oestrous cycle and duration of oestrus

The interval between two successive behavioural oestrus was taken as oestrous cycle length. The duration of oestrus was assessed according to the onset and end of behavioural and physiological signs. A teaser buck was paraded to identify the onset and termination of oestrus. Onset of oestrus was taken as the time halfway between the last rejection and first acceptance of a teaser buck, while the end of oestrus was set halfway between the last acceptance and first rejection (Holtz *et al.*, 2008).

Result and discussion

Number of follicular waves per oestrous cycle

In the present study, cycles with two to five waves could be identified with predominant three and four wave patterns (Table 1). Two wave cycle was identified only during the first oestrous cycle and was associated with short cycles while five wave was observed in second cycle. This is in accordance with several studies in goats where the number of waves ranged between three to five with predominance of three and four wave pattern (Simoes *et al*., 2006; Rosales- Torres *et al*., 2012). The number of waves in a cycle is determined by the concentration of progesterone and the animals with four or five wave cycle had higher progesterone level than those with two or three waves in luteal phase (Menchaca and Rubianes, 2002). Another factor could be an early decrease in oestradiol level after ovulation that resulted in early rebound of FSH promoting the second wave to emerge earlier in four or five wave cycles (Rubianes and Menchaca, 2003).

Interwave interval

Mean interwave interval is depicted in Table 2. The mean duration of first interwave interval was 4.33± 0.28, 4.92± 0.29 and 3.25± 0.25 days for three, four and five wave cycles. The values were similar to the observations (4.0 ± 1.4 days) in Boer goats (Schwarz and Wierzchos, 2000) but lesser (5.6 ± 0.3 days) than in Serrana goat (Simoes *et al*., 2006). The interwave interval for ovulatory wave was longer than preceding waves in three and five wave cycle which might be due to the indirect progesterone action on follicular turnover and high level of oestradiol or inhibin from the largest follicles in ovulatory wave that prevented an immediate emergence of next cohort of growing follicles (Medan *et al*., 2005).

Diameter of largest follicles in each wave

The diameter of the largest follicles in the first wave of three, four and five wave cycles were larger than the follicles present in the subsequent waves (Table 3). The smaller diameter of second and third wave follicles could be due to the negative action of progesterone on follicular growth where the level was high during the mid-luteal phase than early or late of

Table 1. Number of follicular waves per oestrous cycle (per cent)

Oestrous cycle	Waves			
	2 wave	3 wave	4 wave	5 wave
1 (n=16)	25.00 (4)	43.80 (7)	31.30 (5)	0
2 (n=16)	0	31.30 ((5)	43.80 (7)	25.00 (4)
Overall	12.50 (4)	37.50 (12)	37.50 (12)	12.50 (4)

Figures in parenthesis are the number of animals

Table 2. Mean interwave interval (days) in three, four and five wave cycles

Waves	Interwave interval (days)				
	IWI 1	IWI 2	IWI 3	IWI 4	IWI 5
3 Wave (n=12)	4.33± 0.28	6.33 ± 0.51	6.92± 0.40	-	-
4 Wave (n= 12)	4.92± 0.29	3.75 ± 0.46	5.75 ± 0.39	5.25 ± 0.22	-
5 Wave (n= 4)	3.25± 0.25	3.50 ± 0.50	3.25± 0.63	4.0 ± 0.41	6.5 ± 0.65

oestrous cycle. Also, the growth phase of largest follicles was shorter for second and third waves when compared to first and last waves, which might be attributed to the negative feedback effect of progesterone (Ginther and Kot, 1994).

Day of largest follicle in each wave

The day of largest follicle in three and four wave cycles in Malabari goats (Table 3) were in agreement with the observations in Serrana goats by Simoes *et al.* (2006) who observed maximum follicular diameter on days 4.40 ± 2.10 and 9.80 ± 1.30 in three wave cycle and days 4.50 ± 1.20, 9.30 ± 1.80 and 13.90 ± 1.80 in four wave cycle. In five wave cycles, the mean day of appearance of largest follicles were lesser than that reported in Serrana goats *ie*., days 4.30± 1.10, 8.40 ± 2.10, 11.10± 1.90 and 14.60± 1.50. In the present study, the appearance of largest follicles could be observed from days 3 to 13 of oestrous cycle during peripubertal period of Malabari goats.

Day of ovulation

The day of ovulation occurred between days 13 and 23 of oestrous cycle in peripubertal Malabari goats among which short cycles (Days < 17) were more in first oestrous cycle. Majority of ovulations were observed on day 20 in both oestrous cycles (Table 4). In goats, short cycles have been reported to occur during peripubertal period, induced oestrus and at the onset of breeding season (Davila *et al.*, 2017; Fatet *et al*., 2011). Insufficient gonadotrophic support following ovulation, especially LH pulses could result in premature regression of CL and its functional failure (Alves *et al*., 2018).

Number and diameter of the ovulatory follicles

In the present study, only single and double ovulations could be identified, although multiple ovulations up to four ovulations are evident in Malabari breeds. Number of ovulatory follicles increased with parity of the animal and in peripubertal animals single and double ovulations are found. The incidence of single, twins, triplets and quadruplets in Malabari goat has been reported to be 56.45, 38.27, 3.59 and 1.69 per cent, respectively (Venkatachalapathy *et al*., 2016) and the overall values were comparable with regard to single and double ovulations of present study(Table 5).The mean size of ovulatory follicles in cycle 1, was 6.39 ± 0.13 mm (range 5.60- 7.50 mm) and in cycle 2 was 6.79 ± 0.13 mm (5.74 to 7.90 mm). The preovulatory follicle size in the present study was higher than reports by Filho *et al*. (2007) in Anglo Nubian goats (5.40 ± 0.20 mm) but lower than the size reported by Medan *et al*. (2005) and Simoes *et al*. (2006) in Shiba goats (8.20 mm) and Serrana goats (7.10 ±0.10 mm). In Malabari goats, an ovulatory size of 5.70± 0.20

Table 3. Mean diameter (mm) and day of largest follicles inthree, four and five wave cycles

Waves	Diameter (mm) and day of largest follicles							
	Wave 1		Wave 2		Wave 3		Wave 4	
	Diameter (mm)	Day	Diameter (mm)	Day	Diameter (mm)	Day	Diameter (mm)	Day
3 Wave (n=12)	5.85± 0.13	4.25 ± 0.22	5.36 ± 0.12	9.08 ± 0.54	-	-	-	-
4 Wave (n=12)	5.76 ± 0.10	4.75 ± 0.48	5.31 ± 0.10	8.58 ± 0.54	5.55 ± 0.12	13.00 ± 0.65	-	-
5 Wave (n=4)	6.15 ± 0.23	3.25 ± 0.25	5.56 ± 0.08	6.00 ± 0.41	5.24 ± 0.12	10.00 ± 0.41	5.50 ± 0.21	12.75 ± 0.48

Table 4. The day of ovulation in first and second oestrous cycles of Malabari doelings

Oestrous cycle	Day of oestrous cycle									
	D13	D15	D16	D17	D18	D19	D20	D21	D22	D23
1 (n=16)	18.8 (3)	6.3 (1)	0 (0)	18.8 (3)	0 (0)	12.5 (2)	25.0 (4)	12.5 (2)	0 (0)	6.3 (1)
2 (n=16)	0 (0)	6.3 (1)	0 (0)	0 (0)	0 (0)	12.5 (2)	37.5 (6)	31.3 (5)	6.3 (1)	6.3 (1)

Figures in parenthesis are the number of animals

Table 5. Number and mean diameter of ovulatory follicles in Malabari doelings

Oestrous cycle	Number of ovulatory follicles		Size of ovulatory follicle (mm)
	Single (per cent)	Double(per cent)	
1 (n=16)	75.00 (12)	25.00 (4)	6.39 ± 0.13
2 (n=16)	37.50 (6)	62.50 (10)	6.79 ± 0.13
Overall	56.30 (18)	43.80 (14)	6.59 ± 0.11

Figures in parenthesis are the number of animals

to 8.20 ± 0.07 mm was reported (Upasana, 2016a) which is in agreement with the present study.

Length of oestrous cycle and duration of oestrous

The mean length of first and second oestrous cycle was observed as 17.72± 0.79 and 20.19± 0.43 days, respectively. The first interoestrous interval was shorter than normal range reported for goats *ie*., 18 to 22 days (Jainudeen *et al*., 2000), which could be due to higher incidence of short cycles in peripubertal animals and incidence was reported as high as 45.58 per cent in native cross bred goats of Kerala (Krishnakumar, 1992). Length of oestrous cycle also varied depending upon latitude, breed, climate, feed availability, breeding system and presence of male. The mean duration of first and second oestrus was 36.88 ± 2.44 and 40.13 ± 1.84 h, respectively with a range of 24 to 48 h which was found to be within the normal range for goats. The average duration varies depending on breed, season and the presence of male (Jainudeen *et al*., 2000; Fatet *et al.*, 2011). Similar duration of oestrus (36h) was reported in Nubian goats (Romano and Fernandez Abella, 1997), Boer goats (37h) (Greyling, 2000) and Markhoz goats (39h) (Farshad *et al*., 2008). However, contrary to the present findings,Upasana *et al.* (2016b) has reported a lower mean duration of oestrus (22.67 ± 1.33 h) in postpartum Malabari crossbred does.

Conclusion

Ultrasonographic studies in peripubertal Malabari goats revealed that follicular development occurs in wave pattern as in several other goat breeds of tropical countries. Predominant wave pattern was found to be three and four wave cycles with the mean interoestrous interval of 17.72± 0.79 and20.19± 0.43 days in first and second cycle, respectively. Follicular dominance was present during the luteal phase of oestrous cycle with variable observations in size of follicles and day of appearance. Although short cycles were more in first oestrous cycle, the cycle length became regular in the subsequent cycle. Percentage of double ovulation and size of preovulatory follicles were higher in second oestrous cycle when compared to the first cycle.

Conflict of interest

The authors declare that they have no conflict of interest

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