



## RESEARCH ARTICLE

### Follicular Dynamics and Changes in Plasma Estradiol-17 $\beta$ and Progesterone Concentrations during Estrous Cycle in Beetal Goats

Ali Murtaza<sup>1</sup>, Muhammad Irfan-ur-Rehman Khan<sup>1,\*</sup>, Waqas Ahmad<sup>1</sup>, Tariq Sohail<sup>1</sup>, Imran Mohsin<sup>2</sup>, Muhammad Shahzad<sup>3</sup>, Muhammad Zahid Tahir<sup>1</sup> and Muhammad Ijaz<sup>4</sup>

<sup>1</sup>Department of Theriogenology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan

<sup>2</sup>Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan

<sup>3</sup>Nuclear Institute for Agriculture and Biology, Faisalabad-38000, Pakistan; <sup>4</sup>Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan

\*Corresponding author: irfan.khan@uvas.edu.pk

#### ARTICLE HISTORY (18-315)

Received: August 31, 2018

Revised: March 04, 2019

Accepted: March 06, 2019

Published online: April 06, 2019

#### Key words:

Beetal goats

Estradiol-17 $\beta$

Estrous cycle

Follicular dynamics

Progesterone

Ultrasonography

#### ABSTRACT

The study was designed to monitor ovarian follicular dynamics and plasma progesterone and estradiol-17 $\beta$  concentrations in Beetal goats (n=7; age: 3.7 $\pm$ 0.2 years). Following ovulation synchronization, two consecutive estrous cycles (n=14) were monitored via ultrasonography during the breeding season (Sep-Nov 2016). The interovulatory interval of Beetal goats averaged 21.2 $\pm$ 0.3 d and follicular and luteal phases were 3.9 $\pm$ 0.1 and 17.2 $\pm$ 0.3 days, respectively. The 4-wave follicular pattern was higher than 3-waves (71% vs. 29%; P>0.05). In 3-wave cycles, follicular waves emerged on Days -0.3 $\pm$ 0.3, 8.3 $\pm$ 1 and 14.5 $\pm$ 0.5 of estrous cycle (Day 0=ovulation), while in 4-wave cycles, waves emerged on Days 0.5 $\pm$ 0.2, 7.5 $\pm$ 0.5, 11.9 $\pm$ 0.4, and 16.1 $\pm$ 0.6. The maximum diameter of preovulatory follicle and corpus luteum was 7.2 $\pm$ 0.2 mm and 11.8 $\pm$ 0.3 mm, respectively. On an average 1.7 $\pm$ 0.2 follicles ovulated per cycle and luteolysis began on Day 17.2 $\pm$ 0.3 of the cycle. The largest follicles of first and ovulatory wave had greater diameters than those of 2<sup>nd</sup> and 3<sup>rd</sup> wave (P<0.05). The peak plasma estradiol-17 $\beta$  concentration was observed 33.6 $\pm$ 9.6 h before ovulation. The peak plasma progesterone concentration was attained by Day 12.2 $\pm$ 1 and reached <2 ng/mL within 1.6 $\pm$ 0.3 d following the onset of luteolysis. The plasma progesterone concentration and diameter of corpora lutea correlated throughout the estrous cycle (r=0.94; P<0.05). In conclusion, seventy-one percent of the cycles in Beetal goats were of 4-wave pattern and overall Beetal goats had tendency of twin ovulations.

©2019 PVJ. All rights reserved

**To Cite This Article:** Murtaza A, Khan MIR, Ahmad W, Sohail T, Mohsin I, Shahzad M, Tahir MZ and Ijaz M, 2019. Follicular dynamics and changes in plasma estradiol-17 $\beta$  and progesterone concentrations during estrous cycle in Beetal goats. Pak Vet J, 39(2): 193-198. <http://dx.doi.org/10.29261/pakvetj/2019.046>

#### INTRODUCTION

Sustained production of food is a challenge in Asia due to the huge human population. In this context, livestock and agriculture-based developing economies in subtropics require efficient production systems to ensure food security. China, India, and Pakistan constitute 46.9% of the world goat population (FAO, 2017) that provides livelihood and socio-economic benefits to the masses. Pakistan is ranked 3<sup>rd</sup> in the goat population with ~74.1 million heads (GoP, 2018). Beetal goats are ~10% of the total goat population of Pakistan and majority inhabits Punjab-Pakistan. Despite lack of genetic selection program, Beetal goats produce ~1.8-2.7 liters milk/day

and the average yearlings weigh ~27.8 kg (Ali and Khan, 2008). Beetal bucks are preferentially kept for meat and are slaughtered at Eid-ul-Adha festival. Contrary to the developed world, goats in Pakistan are raised on traditional systems such as sedentary, transhumant and household (Khan *et al.*, 2008). Consequently, the sustained goat production through genetic improvement remains obscure, and buildup of goat heads at the expense of human food may not suffice for boosting milk and meat production in the future.

The insight of estrous cycle is essential to enhance reproductive efficiency for quick genetic gains (Bukar *et al.*, 2012). Various estrus synchronization protocols have been applied in Beetal goats with variable success rates. A

previous study in Beetal goats showed that estrus response and pregnancy rate did not differ between the synchronization protocols i.e. Ovsynch vs. PGF<sub>2α</sub> (Riaz *et al.*, 2012). Furthermore, administration of GnRH at the time of breeding did not improve pregnancy rate in Beetal goats. In another report, the diameter of preovulatory follicles and ovulation rate did not differ between Beetal and Teddy goats, while the number of small follicles (2-3 mm) was higher around estrus in Beetal than Teddy goats (Riaz *et al.*, 2013). The Beetal goats synchronized with double PGF<sub>2α</sub> showed a higher pregnancy rate than progesterone plus PGF<sub>2α</sub> treatment i.e., 78.9 vs. 55%, respectively (Andrabi *et al.*, 2015). However, the aforementioned studies made the interventions without any knowledge of follicular dynamics and hormonal profile of Beetal goats; therefore, a plausible explanation of the outcomes of a given protocol remains elusive.

To the best of our knowledge, follicular dynamics and estrous cycle physiology has not been explored in Beetal goats. Therefore, the present study characterizes ovarian follicular dynamics and its relationship with plasma concentrations of estradiol-17β and progesterone throughout the estrous cycle in Beetal goats.

## MATERIALS AND METHODS

**Geographical location, experimental animals, and estrous synchronization:** Seven cyclic multiparous Beetal goats (n=7; Age: 3.7±0.2 years; body condition score: 3.2±0.1 and body weight: 47.5±2.2 kg) kept at Small Ruminant Training and Research Centre, Pattoki, Kasur (31°03'29.0"N 73°52'42.9"E) were synchronized by two doses of d-cloprostenol (75 µg, i.m., Dalmazine®, Fatro, Italy) 11-days apart during the breeding season (September-November 2016). The goats were kept in free stalls, and each goat was provided seasonal green fodder (Sorghum: 4-5 kg) supplemented with concentrate (300 g; containing soybean meal, corn gluten, corn grain, canola meal, and wheat bran) on daily basis. All goats had access to clean water ad libitum. All procedures were approved by the Animal Care and Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore-Pakistan.

**Ultrasound examination and ovarian dynamics:** Ovarian changes in goats (n=7) were monitored for the two consecutive estrous cycles through B-mode ultrasound following 2<sup>nd</sup> PGF<sub>2α</sub> induced ovulation using a transrectal transducer (7.5 MHz, HS-1500®, Honda, Tokyo, Japan) (Khan *et al.*, 2015). The diameter of antral follicle (anechoic) and corpus luteum (hypoechoic) was measured daily by a single operator throughout the study. Ovarian changes were compared using identity method based on the examination of previous days (Ginther *et al.*, 2004).

The day at which the follicle achieved the largest diameter and did not increase subsequently was defined as the day of largest follicle diameter. The largest dominant follicle prior to ovulation was considered as preovulatory follicle and its sudden disappearance on the subsequent ovarian ultrasound scan was defined as ovulation (Day 0). The luteolysis was defined as the 1<sup>st</sup> day when a significant decrease in the diameter of CL was observed relative to its preceding diameter (De Castro *et al.*, 1999).

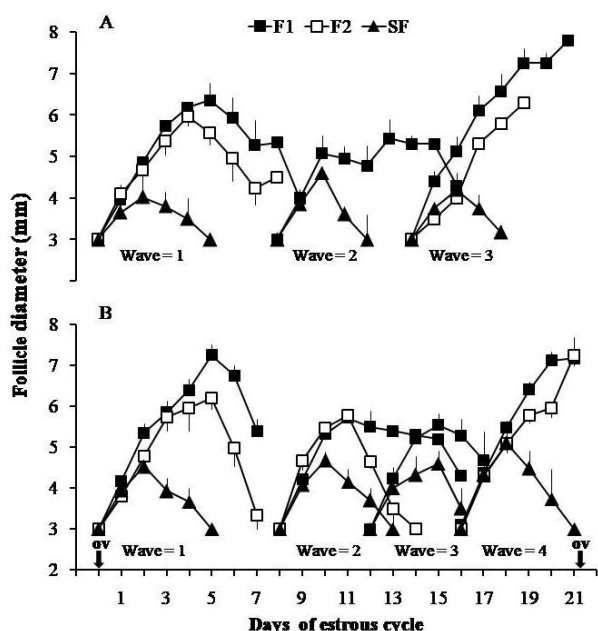
The follicular phase was the duration from luteolysis till ovulation. The wave emergence (WE) was characterized by sudden appearance of a cohort of follicles ≥3 mm of which one or two follicles reached a size ≥5 mm within next 48 h (Nogueira *et al.*, 2015). Inter-wave interval (IWI) was the time between the emergence of two successive waves (Simões *et al.*, 2006), whereas inter-ovulatory interval (IOI) was the time between two successive ovulations. For each estrous cycle, follicular waves, the day of wave emergence, IOI, IWI, ovulation rate, and luteal dynamics were estimated. For each wave, the number of follicles at wave emergence, diameters of 1<sup>st</sup> and 2<sup>nd</sup> largest follicles (F1 & F2), the day of largest follicle diameter, the growth rate of F1, the day of F1 selection, and growth and dominance phases of F1 were observed.

**Blood collection and hormones analyses:** Five goats were bled daily via jugular venipuncture (5 mL; BD Vacutainer®, USA) for a complete cycle. Plasma was obtained by centrifuging blood at 1200 × g for 13 min and stored at -20°C till analysis. The concentrations of progesterone and estradiol-17β were determined in duplicates by solid-phase RIA kits (Beckman Coulter®, Immunotech, France) (Khanum *et al.*, 2008). The sensitivity of progesterone and estradiol-17β assays was 0.2 ng/mL and 0.01 pg/mL, respectively. The inter-assay CV for progesterone and estradiol-17β were 8.66% and 14.5%, while the intra-assay CV for progesterone and estradiol-17β were 8.15% and 14.4%, respectively.

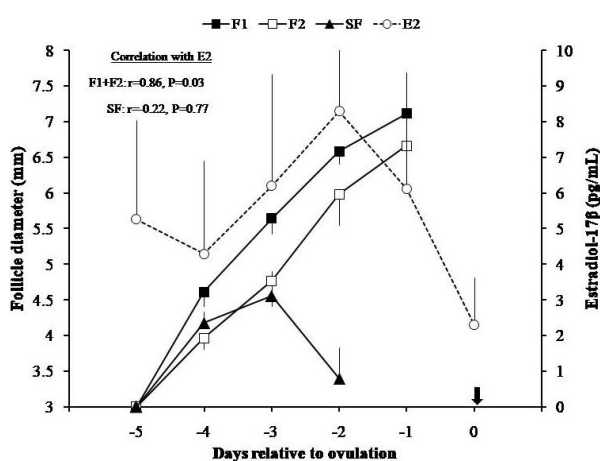
**Statistical analyses:** The mean ± SEM of follicular diameter, the growth rate of F1, the day of selection of F1, growth and dominance phase of F1, and IWI were compared within or between 3- and 4-wave cycles by one-way ANOVA (SPSS, version 20.0, NY). Tukey's HSD was used to compare means whenever P-value ≤0.05. Comparisons of IOI, the day of largest follicular diameter, the day of WE, duration of follicular and luteal phases and the number of ovulation between 3- and 4-wave cycles were done by Student's t-test. The skewed data of progesterone and CL were transformed to square root for comparison between 3- vs. 4-wave cycles by general linear model. Relationships between progesterone and CL diameter as well as between estradiol-17β and F1 of each wave were determined by Pearson's Correlation Coefficient.

## RESULTS

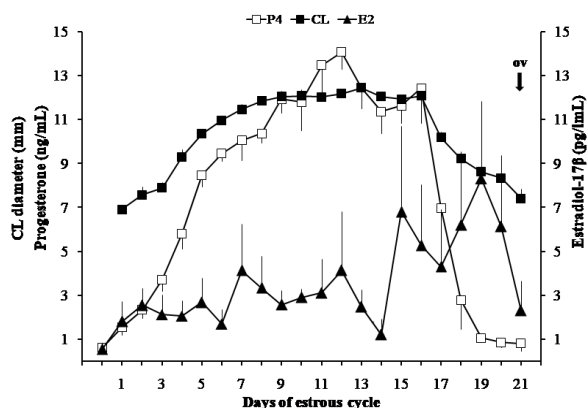
**Follicular wave pattern:** The average IOI of fourteen estrous cycles of seven Beetal goats was 21.2±0.3 d. The duration of follicular and luteal phase was 3.9±0.1 and 17.2±0.3 d, respectively. A cohort of six or seven antral follicles (≤3 mm diameter) emerged at regular intervals in both 3- or 4-wave cycles. In the 3-wave cycle, follicular waves emerged on Days -0.3±0.3 (Day 0=ovulation), 8.3±1 and 14.5±0.5 of the estrous cycle (Table 1). In 4-wave cycle, follicular waves emerged on Days 0.5±0.2, 7.5±0.5, 11.9±0.4, and 16.1±0.6 of the estrous cycle (Table 1). The frequency of 4-wave cycles was relatively higher than 3-wave cycles i.e., 71% vs. 29%, respectively; P>0.05. For 3- or 4-wave cycle, the characteristic changes in dominant (F1 & F2) and subordinate follicle's diameters



**Fig. 1:** Follicular pattern of 3- (n=4; A) and 4-wave (n=10; B) cycles in Beetal goats. Diameter (mean±SEM) of 1<sup>st</sup> (F1) and 2<sup>nd</sup> (F2) largest dominant follicles along with subordinate follicles (SF) were monitored daily for two consecutive ovulations (OV).



**Fig. 2:** Relationship between preovulatory follicles (1<sup>st</sup> largest; F1, 2<sup>nd</sup> largest; F2), subordinate follicles (SF) (diameter; mean±SEM) and estradiol-17 $\beta$  concentration (E2) during the preovulatory period in Beetal goats.



**Fig. 3:** Changes (mean±SEM) in diameter of corpus luteum (CL), plasma concentrations of progesterone (P4; n = 5) and estradiol-17 $\beta$  (E2; n = 4) during the cycle in Beetal goats. The data of one goat was excluded as it showed exceptionally high concentrations throughout estrous cycle in Beetal goats.

are shown in Fig. 1. The tendency to repeat a given wave pattern among goats was 66.7%. Moreover, there was no difference in growth rate of F1 within and between 3- and 4-wave cycles (Table 1). The IOI and duration of follicular and luteal phase did not vary between 3- and 4-wave cycles (Table 2).

The selection of dominant follicle (F1) after wave emergence did not vary between 3 vs. 4-wave cycle (Table 2) and occurred on  $1.7 \pm 0.1$  d following the emergence. The day of the maximum diameter of F1 in each wave following wave emergence was greater for 3- as compared to 4-wave cycles ( $P < 0.05$ ; Table 2). Among first, second and ovulatory waves, the day of F1 maximum diameter was similar for the 3- or 4-wave cycle (Table 1). In contrast, the diameter of the F1 differed within waves in the 3-wave cycle ( $P < 0.05$ ). In the 4-wave cycle, F1 of the 2<sup>nd</sup> and 3<sup>rd</sup> wave was smaller ( $P < 0.05$ ) in diameter than 1<sup>st</sup> and ovulatory waves (Table 1). In 4-wave cycles, the first IWI was longer ( $P < 0.05$ ) than 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> IWI; however, no difference was found among IWI of 3-wave cycles. The growth and dominance phases of F1 in ovulatory waves were longer in 3-wave than 4-wave cycles ( $P < 0.05$ ; Table 1).

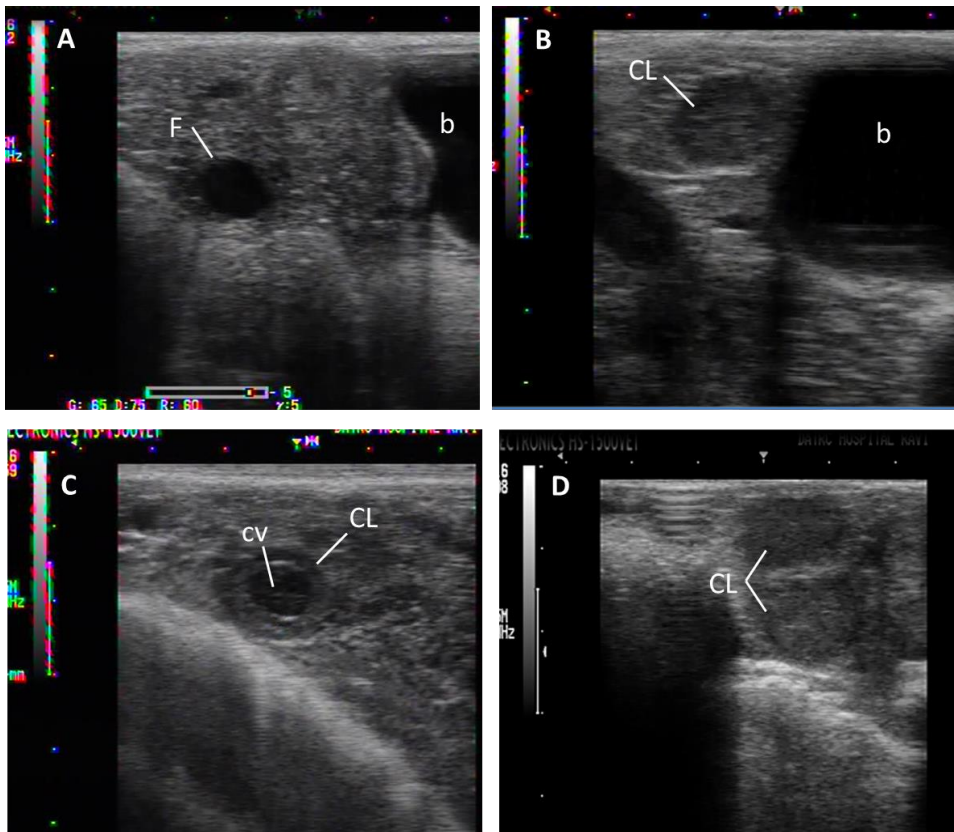
#### Preovulatory follicle and plasma estradiol-17 $\beta$ concentration:

The mean diameter of preovulatory follicle was  $7.2 \pm 0.2$  mm. In 3- or 4-wave cycle, the number of ovulatory follicles averaged  $1.7 \pm 0.2$  and achieved their maximum diameter 30.3±2.5 h prior to ovulation. The ovulatory follicles emerged  $5.5 \pm 0.3$  d prior to ovulation (Fig. 2). Of the observed estrous cycles, the frequency of double ovulations was 57.1% while single and triple ovulations were 35.7 and 7.1%, respectively. Most double ovulations involved single ovary than both ovaries i.e., 75 vs. 25%. The ovulation rate did not differ between 3- vs. 4-wave cycle (Table 2). However, single follicle ovulated in 75% of 3-wave cycles while the most 4-wave cycles (80%) had two to three ovulatory follicles. Furthermore, both the preovulatory follicles (F1 and F2) had similar growth pattern.

The diameter of preovulatory follicles and plasma concentration of estradiol-17 $\beta$  were positively correlated ( $r = 0.86$ ;  $P < 0.05$ ; Fig. 2). Estradiol-17 $\beta$  concentration increased markedly from Day 14 onward, and attained peak concentration ( $11.1 \pm 2.9$  pg/mL) before ovulation i.e.,  $33.6 \pm 9.6$  h (range: 24-72 h). The basal concentration of estradiol-17 $\beta$  during luteal phase ranged between 0.53 to 6.8 pg/mL.

#### Corpus luteum and plasma progesterone concentration:

Corpus luteum (CL) was first detectable ( $7.4 \pm 0.2$  mm) by Day  $2.7 \pm 0.2$  and 19% corpora lutea had a central cavity, which disappeared by Day 11. The twin corpora lutea had similar diameters. Morphologically, CL increased gradually and reached a plateau by Day  $7.9 \pm 0.2$  (Fig. 3). The CL attained maximum diameter ( $12.5 \pm 0.2$  mm) on Day  $11.6 \pm 0.5$  and the luteolysis began by Day  $17.2 \pm 0.3$ . The CL diameter was directly associated ( $r = 0.94$ ;  $P < 0.05$ ) with the plasma progesterone concentration during the cycle (Fig. 3). The CL achieved maximum physiological competence in terms of progesterone ( $14.1 \pm 0.2$  ng/mL) by Day  $12.2 \pm 1$ . The CL became



**Fig. 4:** Ultrasound images showing the preovulatory follicle (A; F=follicle; b=urinary bladder), single CL (B; CL=corpus luteum), a CL with central cavity (C; CV=cavity) and double CL (D).

**Table 1:** Comparison between follicular characteristics among different waves of 3- and 4-wave estrous cycles in Beetal goats

	3-wave cycle (n = 4)			4-wave cycle (n=10)			
	W1	W2	W3	W1	W2	W3	W4
Day of wave emergence	-0.3±0.3	8.3±1	14.5±0.5	0.5±0.2	7.5±0.5	11.9±0.4	16.1±0.6
Diameter of 1 <sup>st</sup> largest follicle (F1)	6.7±0.2 <sup>a</sup>	5.8±0.2 <sup>Ab</sup>	7.9±0.3 <sup>a</sup>	7.1±0.2 <sup>a</sup>	6.2±0.1 <sup>Bb</sup>	6.2±0.2 <sup>b</sup>	7.3±0.2 <sup>a</sup>
Diameter of 2 <sup>nd</sup> largest follicle (F2)	6±0.2	4.6±0.2 <sup>A</sup>	6.3±0	6.6±0.2	5.8±0.1 <sup>B</sup>	-	6.8±0.3
Day of maximum F1 diameter	5.5±0.3	11.3±0.6	20.3±0.5	5.2±0.3	10.3±0.3	15.2±0.6	20.1±0.6
Growth rate of F1 (mm/d)	0.9±0.1	0.9±0.3	0.9±0.1	1±0	1±0.1	1±0.1	1±0.1
Day of selection F1 after WE	1.3±0.3	2±0	1.8±0.5	1.9±0.3	1.8±0.3	1.6±0.2	1.6±0.2
Growth phase of F1 (d)	4.3±0.3	3.8±0.8	5.8±0.5 <sup>A</sup>	4.1±0.3 <sup>ab</sup>	3.1±0.3 <sup>b</sup>	3±0.4 <sup>b</sup>	4.4±0.3 <sup>Ba</sup>
Dominance phase of F1 (d)	5.8±0.6	4.8±1.3	5.8±0.5 <sup>A</sup>	4.8±0.4	4±0.3	3.3±0.4	4.3±0.3 <sup>B</sup>
No. of follicles at WE	6.5±0.5	6±1	6.3±1	7.3±0.5 <sup>ab</sup>	5.6±0.4 <sup>b</sup>	6.4±0.9 <sup>ab</sup>	7.9±0.7 <sup>a</sup>
Inter-wave interval (IWI; d)	W1-W2	W2-W3	W3-W1	W1-W2	W2-W3	W3-W4	W4-W1
	7.3±1	5.3±0.9 <sup>A</sup>	6.3±0.7 <sup>A</sup>	6±0.3 <sup>a</sup>	3.5±0.4 <sup>Bb</sup>	3.6±0.4 <sup>b</sup>	3.9±0.4 <sup>Bb</sup>

<sup>AB</sup> denotes the differences between corresponding waves in 3- vs. 4-wave cycles. Ovulatory wave of 3-wave cycle (W3) is compared with the ovulatory wave of 4-wave cycle (W4). <sup>abc</sup> denotes differences within 3- or 4-wave cycles. Values with similar superscripts in a row have statistically insignificant difference ( $P>0.05$ ). Days=d.

**Table 2:** Comparison of various parameters between 3- and 4-wave cycles in Beetal goats

Parameter	3-wave	4-wave
Interovulatory interval (IOI; d)	21.3±0.5	21.2±0.4
Follicular phase (d)	4.5±0.3	3.9±0.1
Luteal phase (d)	16.8±0.3	17.3±0.4
Ovulation rate	1.3±0.3	1.9±0.2
Mean diameter of preovulatory follicle (mm)		
Single ovulation	7.7±0.4	6.7±0.3
Double ovulations	6.7±0.3	7.2±0.3
Day of max. preovulatory follicle diameter after WE (d)	5.5±0.5	4.2±0.4
Selection of F1 of each wave after WE (d)	1.6±0.2	1.7±0.1
Mean day of max. F1 diameter after WE (d)	4.6±0.4 <sup>a</sup>	3.7±0.2 <sup>b</sup>
Mean max. progesterone concentration (ng/mL)	14.2±0.1	16.0±1.5
Day of max. progesterone concentration (d)	11.0±1.0	13.0±1.5
CL diameter (mm)		
Early luteal phase (Day 0-7)	9.8±0.4	10.0±0.2
Mid-late luteal phase (Day 8-16)	11.6±0.2	12.3±0.1
Follicular phase (Day 17-21)	8.5±0.3	9.2±0.2
Progesterone concentration (ng/mL):		
Early luteal phase (Day 0-7)	5.7±0.7	5±0.5
Mid-late luteal phase (Day 8-16)	11.5±1.0	12.6±1.4
Follicular phase (Day 17-21)	1.6±0.3	3.2±1.4

<sup>ab</sup> denotes the differences between 3- vs. 4-wave cycles. Days = d.

physiologically inactive when progesterone concentration declined  $<2$  ng/mL within  $1.6±0.3$  d after the onset of luteolysis and reached nadir ( $0.68±0.1$  ng/mL) at ovulation (Fig. 3). However, regressing CL was detectable until one day before ovulation. The diameter of CL and plasma progesterone concentration was not different during various phases of 3- and 4-wave cycles (Table 2). Moreover, the mean maximum plasma progesterone concentration and the day of maximum progesterone concentration did not differ in 3- and 4-wave cycles (Table 2). Also, there were no differences in progesterone secretions of cavitated and non-cavitated CL(s) (Fig. 4).

## DISCUSSION

Analogous to cows (Adams, 1994) and ewes (Driancourt, 1991), goats also exhibit a wave-like pattern of follicular growth in breed specific manner (Medan *et al.*, 2003). In goats, follicular waves vary from two to five or six-waves per cycle (Nogueira *et al.*, 2015). In this

study, most Beetal goats exhibited 4-wave follicular pattern comparable to Serrena (Simões *et al.*, 2006) and Saanen goats (Ginther and Kot, 1994). In cattle, IOI varies due to the follicular wave pattern, and the cycles with more waves show late luteal regression (Ginther *et al.*, 1989). In contrast, such a relationship between number of waves and length of the cycle has not been reported in sheep (Seekallu *et al.*, 2010) and goats (Menchaca and Rubianes, 2002). Likewise, the cycle lengths in Beetal goats were similar between 3- vs. 4-wave pattern and IOI ( $21.2 \pm 0.3$  d) was comparable to other goat breeds (Orita *et al.*, 2000; Simões *et al.*, 2006). Moreover, the days of WE and F1 maximum diameter for 3 or 4-wave cycle in Beetal goats were similar to Saanen goats (De Castro *et al.*, 1999). It was found that 33% Beetal goats alternated their wave patterns similar to cattle (Jaiswal *et al.*, 2009) and ewes (Ginther *et al.*, 1995), and likely to be associated with the time in the breeding season.

In contrast to mono-ovulatory species (Ginther *et al.*, 1989), follicular dominance in goats is inconspicuous and more than one large follicles co-dominate; in particular during first and ovulatory waves (Ginther and Kot, 1994). In the current study, most Beetal goats exhibited co-dominance and growth rates of the two largest follicles (F1 & F2) were similar within first and ovulatory waves. It has been suggested that small ruminants exhibit follicular co-dominance as the hypothalamic-pituitary axis is either less sensitive to inhibin (Martin *et al.*, 1988) or the small follicles have sustained FSH support due to the inadequate inhibitory mechanism by the largest follicle (Driancourt, 1991). Moreover, whether the selection of co-dominant follicles occurs concurrently or separately within a few hours, as reported in cattle (Ginther, 2016), remains unknown to-date in goats.

In Beetal goats, the first IWI between 3- and 4-wave cycles (Table 1) was similar to those reported in Saanen ( $7.3 \pm 0.9$  d) (De Castro *et al.*, 1999) and Serrena goats ( $5.6 \pm 0.3$  d) (Simões *et al.*, 2006). In the current study, unlike constant IWI in the 3-wave cycles, the first IWI in the 4-wave cycles was longer than subsequent waves. Although the length of first IWI has been related to the subsequent number of waves in a cycle (Menchaca and Rubianes, 2002), such a relationship was not found in Beetal goats. During the early luteal phase, the delayed rise in FSH under increasing concentration of progesterone has been associated with longer first IWI in goats (Simões *et al.*, 2006). Furthermore, first and ovulatory wave's dominant follicles were larger than those of other waves in Beetal goats, and corresponds well with studies in sheep (Seekallu *et al.*, 2010) and goats (Nogueira *et al.*, 2015). The discrepancy in size of the largest follicle of different waves in goat may be due to decreased LH pulses caused by elevated progesterone level during mid-luteal phase (Bartlewski *et al.*, 1999).

The ovulatory follicle's diameter and ovulation rate in Beetal goats were similar to Boer and Serrena goats (Simões *et al.*, 2006; Nogueira *et al.*, 2015). However, in Shiba goats the ovulatory follicle's diameter and ovulation rate were  $5.5 \pm 1$  mm and  $2.8 \pm 1$ , respectively (Orita *et al.*, 2000). Therefore, ovulation rate and size of ovulatory follicle may be breed specific in goats. As good as other goat breeds (De Castro *et al.*, 1999; Medan *et al.*, 2003), Beetal goats ovulated within 24 to 72 h following a

preovulatory peak of estradiol-17 $\beta$  i.e.,  $33.6 \pm 9.6$  h. Previously, it has been shown that the estradiol-17 $\beta$  concentration increased twice in goats; first during the early luteal phase and second during the preovulatory period (De Castro *et al.*, 1999). The present study found maximum estradiol-17 $\beta$  concentration during the follicular phase and multiple low peaks during the luteal phase in Beetal goats (Fig. 3). The rise in estradiol-17 $\beta$  concentration appeared to be associated with the dominant follicle(s) of each wave. As a corroboration, the overall plasma concentrations of estradiol-17 $\beta$  increased with the size of largest follicle(s) ( $r=0.43$ ;  $P<0.05$ ) during the cycle in Beetal goats. Similarly, the positive correlation of estradiol-17 $\beta$  with the preovulatory follicles ( $r=0.86$ ;  $P<0.05$ ) was in agreement to a previous report in Beetal goats (Murtaza *et al.*, 2018). Moreover, multiple peaks of estradiol-17 $\beta$  under the luteal phase have been suggested as a marker of high prolificacy of goat breed (Pang *et al.*, 2010).

In this study, the maximum plasma progesterone concentration on Day 12 post-ovulation was comparable to that in Dwarf (Khanum *et al.*, 2008) and Shiba goats (Orita *et al.*, 2000) under subtropical conditions. However, on the given day, Beetal goats had higher progesterone concentration than Shiba goats (Orita *et al.*, 2000) i.e., 14.1 vs. 8.2 ng/mL; probably due to the greater CL diameter ( $12.5 \pm 0.2$  mm) in Beetal goats. On the other hand, first ultrasonographical detection of CL (Day  $2.7 \pm 0.2$ ) and days to maximum CL diameter (Day  $11.6 \pm 0.5$ ) were comparable to other goat breeds (De Castro *et al.*, 1999). The correlation between CL diameter and progesterone concentration in Beetal goats was indicative of progesterone plasma index as has been reported in sheep (Amiridis *et al.*, 2002). In the current study, the physiological and morphological demise of CL began concurrently from Day 17 onwards. However, CL remained detectable via ultrasounds even though progesterone reached  $<2$  ng/mL by Day 18.6 of the cycle. A similar demise of CL has been reported in Shiba goats where CL remained detectable albeit progesterone reached basal levels (De Castro *et al.*, 1999). On contrary, another study in Shiba goats suggests that CL becomes physiologically inactive 48 h prior to morphological regression (Orita *et al.*, 2000). However, the early progesterone decline in the former study may be associated with higher variation in progesterone assay.

In conclusion, the Beetal goats exhibited a wave-like pattern of follicular development and seventy-one percent of the cycles had 4-wave pattern. The follicular co-dominance was demonstrated during first and ovulatory waves of the cycle. No obvious differences existed in follicular characteristics and endocrine profiles between 3- vs. 4-wave cycles. In future, the mechanism for the selection of dominant follicle and the factors affecting the number of waves during estrous cycles may require further understanding in goats.

**Acknowledgments:** Authors would like to thank Higher Education Commission (HEC), Pakistan for providing funding to the Ali Murtaza (PIN NO. 213-60800-2Av2-117) for completion of his studies under "Indigenous Ph.D. Fellowship Program for 5,000 Scholars". In addition, this work was partially supported through an HEC grant # 554.

**Authors contribution:** MIRK, AM conceived, designed, analyzed and wrote the manuscript. AM, WA, TS, and IM executed the study and statistically analyzed the data. MS performed the hormonal analysis. MZT, MI, and all other authors critically reviewed the manuscript.

## REFERENCES

- Adams G, 1994. Control of ovarian follicular wave dynamics in cattle: implications for synchronization & superstimulation. *Theriogenology* 41:19-24.
- Ali A and Khan M, 2008. Environmental factors affecting growth and reproductive traits of Beetal goats in Pakistan. *Ind J Anim Genet Breed* 24:271-2.
- Amiridis G, Rekkas C, Fthenakis G, *et al.*, 2002. Progesterone concentration as an indicator of ovarian response to superovulation in Chios ewes. *Theriogenology* 57:1143-50.
- Andrabi S, Anwar M and Mahmood A, 2015. Efficacy of short-term estrus synchronization protocols and timed artificial insemination in subtropical goats. *J Anim Plant Sci* 25:298-300.
- Bartlewski P, Beard A, Cook S, *et al.*, 1999. Ovarian antral follicular dynamics and their relationships with endocrine variables throughout the oestrous cycle in breeds of sheep differing in prolificacy. *J Reprod Fert* 115:111-24.
- Bukar M, Rosnina Y, Ariff O, *et al.*, 2012. Corpora lutea diameter, plasma progesterone concentration and follicular development in PGF2 $\alpha$  and CIDR estrus synchronized goats. *Pak Vet J* 32:216-20.
- De Castro T, Rubianes E, Menchaca A, *et al.*, 1999. Ovarian dynamics, serum estradiol and progesterone concentrations during the interovulatory interval in goats. *Theriogenology* 52:399-411.
- Driancourt M, 1991. Follicular dynamics in sheep and cattle. *Theriogenology* 35:55-79.
- FAO. FAOSTAT [Internet]. Food and Agriculture Organization of the United Nations; 2017 [20-02-2019]. Available from: <http://www.fao.org/faostat/en/#data/QA>.
- Ginther O, 2016. The theory of follicle selection in cattle. *Domest Anim Endocrinol* 57:85-99.
- Ginther O, Gastal E, Gastal M, *et al.*, 2004. Comparative study of the dynamics of follicular waves in mares and women. *Biol Reprod* 71:1195-201.
- Ginther O, Knopf L and Kastelic J, 1989. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *J Reprod Fert* 87:223-30.
- Ginther O and Kot K, 1994. Follicular dynamics during the ovulatory season in goats. *Theriogenology* 42: 987-1001.
- Ginther O, Kot K and Wiltbank M, 1995. Associations between emergence of follicular waves and fluctuations in FSH concentrations during the estrous cycle in ewes. *Theriogenology* 43:689-703.
- GoP. Economic Survey of Pakistan 2017-18 [Internet]. Finance Division, Government of Pakistan (GoP); 2018 [28-05-2018]. Available from: [http://www.finance.gov.pk/survey/chapters\\_18/Economic\\_Survey\\_2017\\_18.pdf](http://www.finance.gov.pk/survey/chapters_18/Economic_Survey_2017_18.pdf).
- Jaiswal R, Singh J, Marshall L, *et al.*, 2009. Repeatability of 2-wave and 3-wave patterns of ovarian follicular development during the bovine estrous cycle. *Theriogenology* 72:81-90.
- Khan MIR, Channa AA and Sattar A, 2015. Diagnosis of pseudopregnancy in a Beetal goat using real-time B-mode ultrasonography. *Pak Vet J* 35:537-9.
- Khan MS, Khan M and Mahmood S, 2008. Genetic resources and diversity in Pakistani goats. *Int J Agric Biol* 10:227-31.
- Khanum S, Hussain M and Kausar R, 2008. Progesterone and estradiol profiles during estrous cycle and gestation in dwarf goats (*Capra hircus*). *Pak Vet J* 28:1-4.
- Martin G, Price C, Thiery J, *et al.*, 1988. Interactions between inhibin, oestradiol and progesterone in the control of gonadotrophin secretion in the ewe. *J Reprod Fert* 82:319-28.
- Medan MS, Watanabe G, Sasaki K, *et al.*, 2003. Ovarian dynamics and their associations with peripheral concentrations of gonadotropins, ovarian steroids, and inhibin during the estrous cycle in goats. *Biol Reprod* 69:57-63.
- Menchaca A and Rubianes E, 2002. Relation between progesterone concentrations during the early luteal phase and follicular dynamics in goats. *Theriogenology* 57:1411-9.
- Murtaza A, Ahmad W, Sohail T, *et al.*, 2018. Follicular dynamics and changes in oestradiol-17 $\beta$ , progesterone and LH profiles following PGF2 $\alpha$  induced oestrus in early and late ovulating Beetal goats. *Reprod Dom Anim*. doi: <https://doi.org/10.1111/rda.13392>
- Nogueira DM, Cavalieri J, Gummow B, *et al.*, 2015. Comparison of follicular dynamics and hormone profiles in Boer goats examined during the breeding and non-breeding seasons in the tropics of Queensland, Australia. *Small Rumin Res* 125:93-100.
- Orita J, Tanaka T, Kamomae H, *et al.*, 2000. Ultrasonographic observation of follicular and luteal dynamics during the estrous cycle in Shiba goats. *J Reprod Devel* 46:31-7.
- Pang X, Wang Z, Zhu T, *et al.*, 2010. Concentrations of progesterone and estradiol in peripheral plasma during the estrous cycle and after ovariectomy in Huanghuai goats of high or poor prolificacy. *Asian-Aust J Anim Sci* 23:188-96.
- Riaz H, Ahmad N and Yousuf MR, 2013. Ovarian follicular dynamics around estrus in Beetal and Teddy goats. *Pak Vet J* 33:120-2.
- Riaz H, Sattar A, Arshad M, *et al.*, 2012. Effect of synchronization protocols and GnRH treatment on the reproductive performance in goats. *Small Rumin Res* 104:151-5.
- Seekallu S, Toosi B, Duggavathi R, *et al.*, 2010. Ovarian antral follicular dynamics in sheep revisited: Comparison among estrous cycles with three or four follicular waves. *Theriogenology* 73:670-80.
- Simões J, Almeida JC, Valentim R, *et al.*, 2006. Follicular dynamics in Serrana goats. *Anim Reprod Sci* 95:16-26.