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Studies on Ovarian Follicular Dynamics and Steroid Profiles in Sahiwal Cattle

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ABSTRACT

Background: Understanding of ovarian follicular dynamics and endocrine regulation is essential to design and use interventions to optimize reproductive efficiency. *Bos indicus* and *Bos taurus* cows have some differences in their follicular dynamics and ovarian steroidal hormones. The present study was planned to understand follicular dynamics vis-a-vis ovarian steroids profiles in Sahiwal cattle.

Methods: The study was conducted in normal cyclic, pluriparous, non-lactating Sahiwal cows (n=7). Trans-rectal ultrasonography was performed to record the location, size, number of follicles and size of corpus luteum (CL) from the beginning, till the end of experiment. The dataset was used to characterize the follicular wave emergence, growth, regression, time of selection and ovulation of the dominant follicle in each cow.

Result: Three Sahiwal cows showed two wave and four cows showed three wave estrous cycles with mean interovulatory length of 20.33±0.33 and 22.50±0.28 days, respectively. No significant difference was recorded in the maximum diameter of corpus luteum, P_4 and E_2 plasma levels between 2-wave and 3-wave estrous cycles. The peak progesterone values of 6.00±0.91 and 6.2±1.2 ng/ml and peak estradiol values of 15.83±0.60 and 14.31±0.44 ng/ml were recorded in 2-wave and 3-wave estrous cycle, respectively. The results showed that Sahiwal cows had 2-wave and 3-wave estrous cycle and the 3-wave estrous cycle had comparatively longer inter-ovulatory period and smaller maximum diameter of second wave dominant follicle than 2-wave estrous cycle.

Key words: Cattle, Estrous cycle, Follicular wave, Ovarian follicle, Sahiwal.

INTRODUCTION

There is urgent need to improve and propagate climate resilient breeds to alleviate the adverse effect of global warming on production and reproductive efficiency of dairy cattle. *Bos indicus* breeds of cattle have better heat tolerance and adaptability under harsh agro-climatic conditions prevailing in tropical regions compared to *Bos taurus* and their crosses (Khan *et al*., 2008). However, the *Bos indicus* cows had been found more sensitive to reproductive hormones like, luteinizing hormone (Griffin and Randel, 1978), progesterone and estradiol (Segerson *et al*., 1984). The differences were also reported in their follicular dynamics like higher incidence of four-wave interovulatory intervals in *Bos indicus* breeds like Brahman (Rhodes *et al*., 1995), Gir (Viana *et al*., 2000) and Rathi (Gaur and Purohit, 2007) had been reported compared to *Bos taurus.* The majority of estrous cycles in Holstein Friesian a well known *Bos taurus* breed were found to be having either two or three follicular waves (Adams *et al*., 2008). Smaller maximum diameter of the dominant follicle (10-12 mm vs. 14-20mm) and the corpus luteum (17-21 mm vs. 20-30 mm) had been recorded in *Bos indicus* cattle than *Bos taurus* cattle (Figueiredo *et al*., 1997, Bo *et al*., 1993). Sahiwal is considered as one of the best milch breeds of *Bos indicus* cattle; however, the studies on follicular dynamics and steroid profile in Sahiwal cows are scanty. The better understanding of follicular dynamics and their endocrine regulation is required for designing effective synchronization protocols which would help in conservation and the propagation of this breed. Therefore, the aim of the study was to understand follicular dynamics vis-a-vis ovarian steroids profiles in Sahiwal cattle.

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MATERIALS AND METHODS

The study was conducted at dairy farm of Directorate Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana, Punjab. Sahiwal cows (n=7), pluriparous, non lactating, weighing between 320 to 450 kg having apparently normal genitalia and normal estrous cycle were used for the study. The cows were maintained under loose housing system and were fed with chaffed green fodder, wheat straw, concentrates, common salt, mineral mixture and *ad libidum* drinking water. All cows were non pregnant and free from uterine abnormalities.

Ultrasonography and blood sampling

All the cows were scanned daily starting from random day of estrous cycle till ovulation to establish day 0. At least one complete estrous cycle starting from the day of ovulation till subsequent spontaneous ovulation was followed to characterize selection, growth and regression of follicles through ovarian ultrasonography using ultrasound scanner (Exago, ECM, France) equipped with B mode linear array transrectal transducer of 7.5 MHz frequency. Blood samples from all the cows were collected through jugular vein in heparinised vials from the day of ovulation (day 0) till subsequent ovulation. Following centrifugation of blood at 2000 rpm for 15 minutes, plasma was separated out and stored (at -20°C) until assay.

Observations recorded

Trans-rectal ultrasonography was performed to record the location, size and number of follicles and size of corpus luteum (CL) beginning from the start till the end of experiment. The dataset was used to characterize the follicular wave emergence, growth and regression patterns of the dominant and subordinate follicles, time of selection and ovulation of the dominant follicle in each cow. The synchronous emergence or growth of group of follicles (≥4 mm in diameter) was referred as wave emergence and it was followed by selection and continuous growth of dominant follicle. Ovulation was determined through scanning by the disappearance of the large follicle (Ginther *et al*., 1989).

Progesterone in plasma samples were measured using enzyme immunoassay PROG-EASIA (Catalogue number: KAP1451) and estradiol-17β was measured using E2-EASIA Kit, manufactured by DIA source Imunoassays S.A, Belgium.

Statistical Analysis

The data was analyzed for means and standard errors (SE) for all variables. The comparison of different parameters at different time point between 2 wave and 3 wave groups was done using t-test statistical procedure.

RESULTS AND DISCUSSION

The study was conducted to monitor follicular dynamics and steroid profiles in normal cyclic Sahiwal cows. Based on the diameter, the follicles were categorized into <4 mm, 4-6 mm, >6-8 mm and >8 mm groups. The follicles of <4 mm and 4-6 mm were observed during wave emergence and selection, respectively, whereas follicles of >6-8 mm and >8 mm were observed during dominance.

Follicular wave pattern

The characteristics of the follicular waves observed in Sahiwal cows are presented in Table 1. The ovarian follicles developed in a wave like pattern involving recruitment of small follicle (<4 mm), followed by selection, development and atresia or ovulation of the dominant follicle (DF). Similar wave like pattern of ovarian follicular development had been reported by various workers in cattle (Adams, 1998 and Fortune *et al*., 2001). In the present study, four cows revealed three wave follicular pattern (4/7, 57.1%) whereas, three showed two wave pattern. Similar predominance of three wave estrous cycles had been reported in *Bos indicus* breeds like in Brahman and Gir cows (Zeitoun *et al*., 1996 & Viana *et al*., 2000), Nelore heifers (Mollo *et al*., 2007) and European cattle breeds (Savio *et al*., 1988 and Sirois and Fortune, 1988). The studies had reported that bovine estrous cycles were composed of either two or three follicular waves (Adams, 1998) with predominance of two wave estrous cycles in *Bos taurus* cattle (Townson *et al*., 2002, Sartori *et al*., 2004). Noseir (2003) opined that the number of follicular waves in a single cycle is determined mainly by the size of follicle (>10 mm) and concentration of estradiol (>5.0 pg/ml), when both were achieved after emergence of the 2nd wave, the estrous cycles were of 2-wave but when not attained, the cycle continued to be 3-wave cycle.

Interovulatory length

The mean interovulatory length recorded in the present study was 20.33±0.33 and 22.50±0.28 days in 2-wave and 3-wave estrous cycle, respectively (Table 1). A similar correlation between number of follicular waves and estrous cycle length had been reported in Angus, Brahman, Senepol (Alvarez *et al*., 2000) and in Holstein dairy cows (Sartori *et al*., 2004) with average interovulatory interval of 19 and 23 days in 2-wave and 3-wave estrous cycle, respectively.

The length of estrous cycles was shorter by 2.17 days in 2-wave compared to 3-wave estrous cycle in our study which was in agreement with Ginther *et al*., (1989) who reported that the interovulatory interval was shorter by 2.4 days in 2-wave compared to 3-wave estrous cycles in Holstein Friesian heifers. Noseir (2003) also reported shorter mean length of estrous cycle in 2-wave compared to 3-wave estrous cycle in cattle (19.8±0.6 vs. 22.5±0.8 days P<0.05). Baruselli *et al*., (2003) observed that the wave patterns were affected by parity in Nelore cattle. It was observed that low plane of nutrition and heat stress could also lead to increased proportion of 3-wave estrous cycles in *Bos taurus* cattle (Adams, 1998 and Baruselli *et al*., 2003). The nutritional supplementation also influence post partum fertility in crossbred cows (Dhami *et al*., 2017).

Day of wave emergence

The results of the present study showed that the first follicular wave emerged on the day of ovulation (day 0) in 2-wave as

Table 1: Interovulatory interval and day of wave emergence in Sahiwal cows showing 2-wave and 3-wave estrous cycle.

Characteristics	2-wave n=3	3-wave n=4
Interovulatory interval (Days)	20.33±0.33	22.50±0.28
First wave	0	0
Day of wave emergence	(Day of ovulation)	(Day of ovulation)
Second wave		
Day of wave emergence	7.50±0.57	7.00±0.28
Third wave		
Day of wave emergence	N.A	14.50±0.81

Table 2: Day of selection and differences in diameter of dominant (DF) and largest subordinate (SOF) follicle of first wave in Sahiwal cows.

Days from wave emergence	Difference in diameter of DF and SOF (mm)Cows (n=7)						
	#1	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**
0	0	0	0	0	0	1	0
1	0.5	1	1.4	1	1	0.1	0
2	1.7	**2.8**	1.2	**2**	0.5	**2.1**	1
3	**2**		**3**		1.4		**3.8**
4							**3.5**
Mean day of selection of dominant follicle							2.71±0.28
Mean diameter of DF at the time of selection (mm)							7.14±0.24

Shaded blocks indicates day of selection (when the difference in diameter of DF and SOF was ≥2mm) in respective cows.

well as 3-wave estrous cycles (Table 1). Similar results with respect to emergence of first follicular wave had been reported in dairy cows by Noseir (2003). However, Viana *et al*., (2000) observed emergence of first follicular wave on day 0.78±0.44 in Gir cows and Muraya *et al*., (2015) on day 0.61±0.06 and 0.42±0.16 in the Kenyan Boran cows in 2-wave and 3-wave estrous cycle, respectively. In contrast, Gaur and Purohit (2007) reported emergence of first follicular wave on day 2.10±0.36 and 0.7±0.5 after ovulation in the Rathi cows in 2-wave and 3-wave estrous cycle, respectively. In the present study, the emergence of the second wave in Sahiwal cows was earlier showing 3-wave compared to 2-wave estrous cycle (on day 7±0.28 vs. 7.5±0.57, respectively). Noseir (2003) and Gaur and Purohit (2007) reported emergence of second follicular wave on day 7.2±1.0 in 3-wave cycle which was comparable to emergence of second follicular wave on day 7.0±0.28 in 3-wave cycle in the present study.

The emergence of second wave observed in the present study was earlier in 2-wave as well as in 3-wave estrous cycle compared to Gir cows and Kenyan Boran cows reported by Gaur and Purohit (2007) and Muraya *et al*., (2015), respectively. In the present study, the third wave emerged on day 14.5±0.81 in Sahiwal cows showing 3-wave estrous cycle, whereas, Muraya *et al*., (2015) reported emergence of third wave on day 15.00±0.98 in Kenyan Boran cow having 3-wave estrous cycle. However, Gaur and Purohit (2007) observed emergence of third wave on day 13.2±3.4 in Rathi (*Bos indicus*) cows which was earlier compared to the emergence of third wave observed in Sahiwal cows in the present study.

Mean number of antral follicles

The mean±SEM number of antral follicles at first wave emergence was 25.0±5.93 in Sahiwal cows. Similar mean number of antral follicles at wave emergence had been reported in *Bos taurus* cattle by Ginther *et al*., (1996) while this number was less in Kenyan Boran cow (17.5±2.67; Muraya *et al*., 2015) and in Angus (21±4; Alvarez *et al*., 2000). In Nelore heifers, the number of antral follicles observed at the time of onset of wave emergence was double (approximately 50; Buratini Jr. *et al*., 2000) than the present study. Alvarez *et al*., (2000) also reported higher number of follicles at wave emergence in Brahman (39±4) and Senepol (33±4) cows.

Selection of first wave dominant follicle

The day of dominant follicle selection was defined as the day on which the difference in diameter of dominant follicle and SOF reached ≥2 mm. The mean day of selection of dominant follicle was 2.71±0.28 days and the average diameter of dominant follicle at the time of selection was 7.14±0.24 mm in seven Sahiwal cows under the study (Table 2).

The selection of dominant follicles was earlier and the diameter of selected follicle was higher in Sahiwal cows under the study compared to Kenyan Boran cows (Muraya *et al*., 2015). However, in *Bos taurus* breeds, follicular selection occurred when the largest developing follicle reached 8.5 to 9.0 mm in diameter (Ginther *et al*., 1996, Sartori *et al*., 2001) whereas, in case of ovulatory wave the diameter at the time of selection was between 8.3 to 9.8 mm in Holstein cattle (Bastos *et al*., 2010).

Various studies had shown that the diameter of follicle at selection differed in *Bos indicus* and *Bos taurus* breeds. However, Sartori *et al*., (2016) observed that the time for follicle deviation after ovulation or after wave emergence was similar between *Bos taurus* and *Bos indicus* (Nelore) which could be due to a slower growth rate of follicle in Nelore (0.8 to 1.2 mm per day; Sartori and Barros, 2011) compared to Holstein cattle (1.2 to 1.6 mm per day; Sartori *et al*., 2001).

The inhibin produced by follicles along with increased circulating estradiol (0.2 to 1 pg/ml) near the time of follicular deviation cause final depression in FSH and was associated with selection of the dominant follicle (Adams *et al*., 1992). The inhibition of FSH by estradiol was synergized with inhibin (Webb *et al*., 2004).

Growth pattern of Dominant Follicles

The growth pattern of dominant follicle of first wave in Sahiwal cows and having 2-wave and 3-wave estrous cycle was analyzed. The development of dominant follicle was categorized into growth, static and regression phases (Fig 1 and 2).

Growth phase of DF

In the present study, the first wave DF diameter observed at selection was 6.58±0.30 mm and 7.56±0.15 mm and showed a growth rate of 1.04±0.29 and 0.69±0.13 mm in 2-wave and 3-wave estrous cycle, respectively (Table 3, Fig 1 and 2). Whereas, the duration of growth phase were 3.33±0.33 and 3.5±0.50 days in 2-wave and 3-wave estrous cycle, respectively.

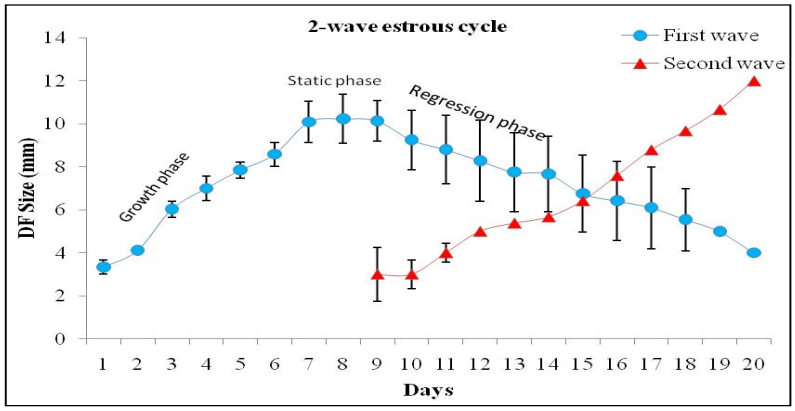

Fig 1: Characteristics of dominant follicles (DFs) development in 2-wave estrous cycle.

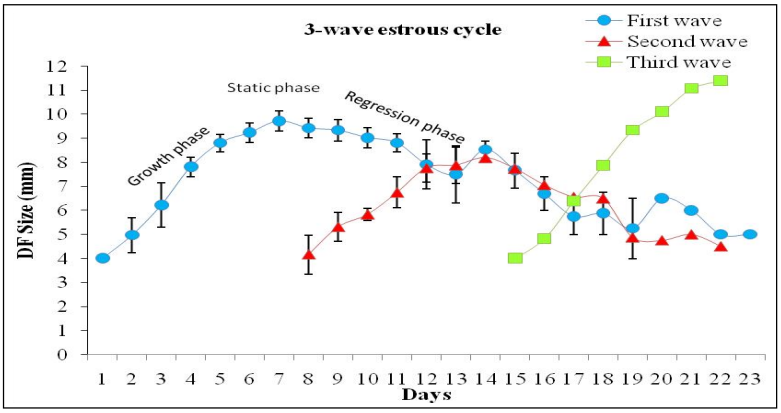

Fig 2: Characteristics of dominant follicles (DFs) development in 3-wave estrous cycle.

the present study, respectively. Whereas, the duration of growth phase of 3.10±0.50 days observed in Rathi cows by Gaur and Purohit (2007) was comparable to the duration of growth phase recorded in the present study. In contrast to present study, Viana *et al*., (2000) reported longer duration of growth phase of 5.67±0.71 days in 3-wave estrous cycle in Gir cows.

Static phase of DF

The duration of static phase was 3±0.57 and 1.75±0.47 in 2-wave and 3-wave estrous cycle, respectively (Table 3, fig 1 and 2). The static phase of first wave DF was non-significantly longer in 2-wave than 3-wave estrous cycle. This could also be one of the reasons for longer duration of first follicular wave in 2-wave compared to 3-wave estrous cycle in cattle.

Regression phase of DF

The duration of regression phase and regression rate of dominant follicle was 7.66±0.66 and 0.66±0.19 in 2-wave; 7.5±2.21 and 0.95±0.44 in 3-wave estrous cycle, respectively (Table 3, Fig 1 and 2). The difference in regression rate of first wave dominant follicle in 2-wave and 3-wave estrous cycle was non-significant.

Gaur and Purohit (2007) reported that regression rate of first wave DF in 2-wave estrous cycle was 0.55±0.20 mm/day in Rathi cows which was comparable to our results.

Table 3: Characteristics (Mean ± SEM) of first wave dominant follicle in 2-wave and 3-wave estrous cycle in Sahiwal cows.

Characteristics	2-wave (n=3)	3-wave (n=4)
First wave dominant follicle		
Growth phase		
Duration (days)	3.33±0.33	3.50±0.5
Beginning day diameter (mm)	6.58±0.30	7.56±0.15
End day diameter (mm)	10.25±1.12	9.81±0.27
Growth rate (mm/day)	1.04±0.29	0.69±0.13
Static phase		
Duration (days)	3.00±0.57	1.75±0.47
Beginning day	4.33±0.33	4.50±0.5
Beginning day diameter (mm)	10.23±1.13	9.81±0.27
End day diameter (mm)	10.13±0.93	9.87±0.30
Regression phase		
Start day	8.33±0.88	7.25±0.75
Diameter (mm)	9.00±1.14	8.93±0.29
Duration(days)	7.66±0.66	7.50±2.21
Regression Rate (mm/day)	0.66±0.19	0.95±0.44

n= total number of cows in each group.

Gaur and Purohit (2007) reported higher growth rate of 1.81±0.32 and 1.03±0.24 mm/day for first wave DF in 2-wave and 3-wave estrous cycle in Rathi cows compared to

However, Muraya *et al*., (2015) reported higher regression rate of 1.01±0.29 and 0.91±0.10 mm/day compared to the regression rate recorded in the present study in 2-wave and 3-wave estrous cycle, respectively.

Size variations of dominant follicles during 2-wave and 3-wave estrous cycle

The maximum diameter of first wave and the second wave DF was 10.3±1.09 and 11.66±0.60 mm in 2-wave estrous cycle, respectively. The difference between the first and second wave dominant follicle diameter was non-significant (Table 4). The diameters of first, second and third (ovulatory) wave DFs recorded in the present study were 9.90±0.31, 8.82±0.52 and 11.42±0.20 mm in 3-wave estrous cycle, respectively. The diameters of first wave dominant follicle recorded in 2-wave and 3-wave estrous cycle did not differ significantly (10.3±1.09 vs. 9.90±0.31; P>0.05).

However, the diameter of the ovulatory (second dominant) follicle of 2-wave estrous cycle was significantly larger than the diameter of second wave DF of 3-wave estrous cycle (11.66±0.60 vs. 8.82±0.52 mm; P<0.05). In 3-wave estrous cycle the diameter of third DF i.e. ovulatory follicle was also significantly larger compared to first and second wave dominant follicles (11.42±0.20 vs. 9.90±0.31 and 8.82±0.52 mm, respectively; P<0.05) shown in Table 4. The results of the present study were in agreement with Gaur and Purohit (2007), who also reported significantly larger second wave dominant follicle in 2-wave cycle compared to the second DF in 3-wave estrous cycle in Rathi cows (14.65±1.24 vs. 10.44±2.13mm, respectively; P<0.05). The maximum diameter of second DF recorded in 3-wave was smaller (P<0.05) than the diameter of first and third DFs in 3-wave estrous cycle in the present study.

Similar results had been reported by Viana *et al*., (2000), who reported that the maximum diameter of second DF was smaller than the diameter of first and third DFs in 3-wave estrous cycle in Gir cows (10.44±2.13 vs. 11.78±1.20 and 12.44±1.59 mm, respectively; P<0.05). This difference could be due to the fact that the second wave emerged during the period of higher progesterone production by the corpus luteum, whereas the first and third waves emerged during the luteogenic and luteolytic periods, respectively (Viana *et al*., 2000). The maximum diameters of both the ovulatory and non-ovulatory follicles recorded in Sahiwal cow in the present study seemed to be smaller compared to *taurine* cows (Ginther *et al*., 1989) and Zebu cows (Figueiredo *et al*., 1997 and Sartorelli *et al*., 2005).

Luteal dynamics

The luteal development was divided into three phases; a growth phase characterized by regular increase in the CL diameter, than a lag phase characterized by slight or no increase in the diameter of CL and finally the regression phase where there was decrease in the diameter of CL. In the present study, the maximum diameter of corpus luteum recorded was 19.16±1.30 mm and 17.93±0.85 mm in 2-wave and 3-wave estrous cycle, respectively. The difference was non-significant (Table 5). Similar diameter of corpus luteum ranging from 17 to 21 mm had been reported in *Bos indicus* cattle by Figueiredo *et al*., (1997).

However, diameter of corpus luteum measuring 20 to 30 mm had been reported in *Bos taurus* by Ginther *et al*., (1989) and Sartori *et al*., (2004) which was higher compared to the present study. It was observed during the study that the onset of CL regression occurred early in 2-wave than 3-wave estrous cycle (12.66±0.88 day vs. 13.75±1.03 day, respectively). Similar findings were observed in Holstein heifers by Ginther *et al*., (1989). Jaiswal (2007) observed that the decrease in CL size corresponded with decreasing progesterone and increasing estradiol concentrations resulting in pre-ovulatory LH surge and ovulation.

Mean progesterone concentration in 2-wave and 3-wave estrous cycle

The mean P„ concentration (ng/ml) varied according to the size of CL in Sahiwal cows under study. The peak progesterone values of 6.00±0.91 and 6.2±1.2 ng/ml were observed in 2-wave and 3-wave estrous cycle, respectively (Fig 3 and 4). It was reported that despite smaller diameter of CL in zebu compared to *taurine* breeds of cattle, higher circulating concentrations of estradiol (E,) and progesterone (P„) were recorded in *B. indicus* than *B. taurus by* Carvalho *et al*., (2008) and Bastos *et al*., (2010). These physiological differences may due to greater circulating cholesterol, insulin and IGF-1 in *Bos indicus* compared to *Bos taurus*.

Similar pattern of progesterone concentration had been reported by Randel (1980) in Brahman cattle and Hansel (1981) in Holstein heifers. Liewelyn *et al*., (1987) observed that plasma progesterone concentration on 2 days before estrus to 3 days after estrus was less than 1.0 ng/ml and reached a maximum 8.0 to 10.0 ng/ml at 11 to 15 days after estrus in Ethiopian highland zebu cattle. The results of the present study were in

Table 4: Maximum diameter (Mean±SEM) of DFs in 2-wave and 3-wave estrous cycle.

Characteristics	2-wave (n=3)(mm)	3-wave (n=4)(mm)
First wave		
Maximum diameter of DF	10.31±1.09	9.90±0.31[a]
Second wave		
Maximum diameter of DF	11.66±0.60*	8.82±0.52 [a]*
Third wave		
Maximum diameter of DF	N.A	11.42±0.20[b]

Values bearing different superscripts within the column differ significantly (P<0.05).

Values bearing (*) sign within the row differ significantly (p<0.05).

Table 5: Characteristics of luteal development in Sahiwal Cows.

Characteristics	2-wave (n=3)	3-wave (n=4)
Interovulatory interval	20.33±0.33	22.50±0.28
Maximum diameter of CL (mm)	19.16±1.30	17.93 ±0.85
Day of maximum diameter of CL	11.00±1.0	12.00±1.08
Day of onset of CL regression	12.66±0.88	13.75±1.03

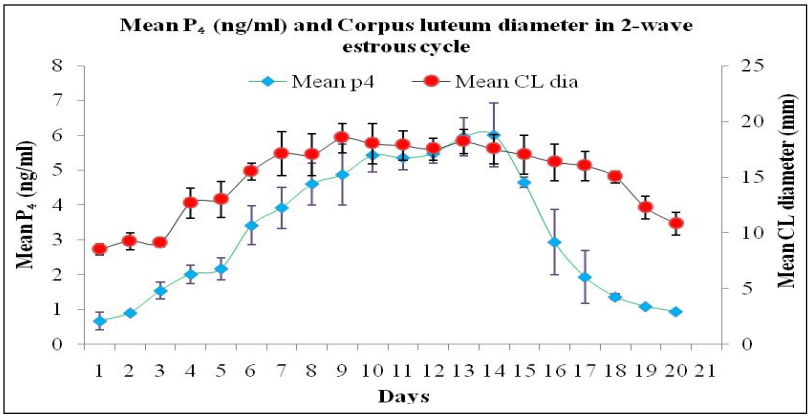

Fig 3: Mean plasma P_4 level and corpus luteum diameter in 2-wave estrous cycle.

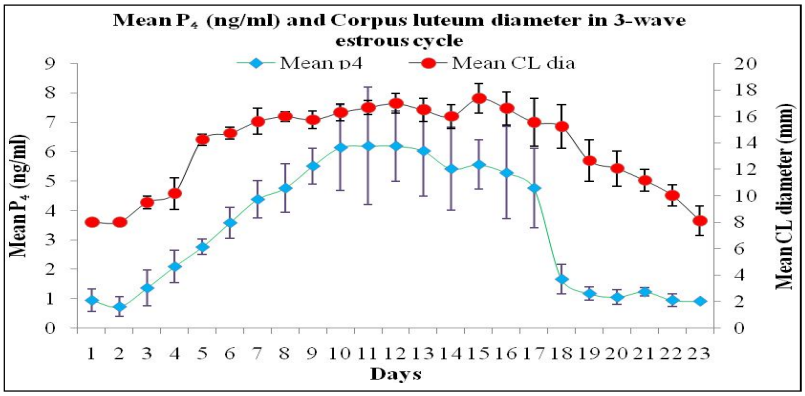

Fig 4: Mean plasma P_4 level and corpus luteum diameter in 3-wave estrous cycle.

agreement with Schams *et al*., (1977) and Coetzer *et al*., (1978) who observed a drop in the concentration of progesterone in the blood of zebu cows about 13 days after estrus. Mondal *et al*., (2004) stated that the mean plasma progesterone concentrations declined from 0.85 ± 0.06 ng/ml (on day – 4) to 0.40 ± 0.02 ng/ml on the day of estrus, thereafter, increased to reach maximum concentration of 3.03 ± 0.91 on day 10.

Mean estradiol levels in 2-wave and 3-wave estrous cycle

The blood plasma levels of estradiol measured during the estrous cycle are represented in Fig 5 and Fig 6. The peak estradiol values of 15.83±0.60 and 14.31±0.44 pg/ml were observed in 2-wave and 3-wave estrous cycle, respectively. The peak values of estradiol in the present study were higher than that of previously reported in Brahman, Angus and Senepol cows (Alvarez *et al*., 2000). Rantala and Taponen (2015) reported that estradiol reached to peak value (8.2±1.8pg/ml) on Day 20 in Dairy cows.

Glencross and Pope (1981) cited that plasma estradiol-17β concentration was low at the start of luteal regression (2.2 ±0.5 pg/ml), increased to 3.8 ±0.6 pg/ml on next day and reached 6.6 ±0.9 pg/ml when the concentration of progesterone in the blood had fallen to a minimum in the Holstein heifers. The highest concentration of estradiol-17β (10.1 pg/ml) was recorded one or two days after complete luteolysis. Probably the drop in progesterone concentration following luteal regression allowed the pre-ovulatory follicle to increase its secretion of oestradiol-17β (Karsh *et al*., 1978).

Summary

Sahiwal cows showed 2-wave and 3-wave estrous cycle with inter-ovulatory period of 20.33±0.33 and 22.50±0.28 days, respectively. The first and second wave emerged on day 0 and 7.5±0.57 in 2-wave estrous cycle whereas first, second and third wave emerged on day 0, 7±0.28 and 14.5±0.81 in 3-wave estrous cycle, respectively. Not much difference was observed for maximum diameter of dominant follicle in 2 wave and 3 wave cycle. The interovulatory period was longer in 3-wave as compared to 2 wave cycle (22.50±0.28 vs 20.33±0.33 days).

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Compliance with ethical standards

The research was conducted in accordance with the ethical standards of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.

Conflict of interest

The authors declare that there is no conflict of interest.

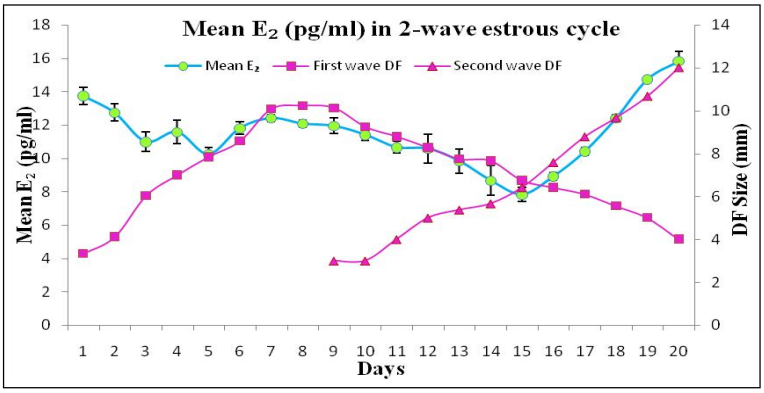

Fig 5: Mean plasma E, level in 2-wave estrous cycle.

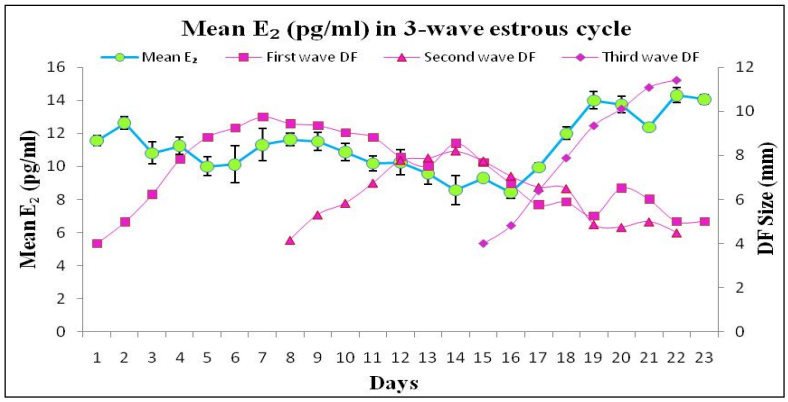

Fig 6: Mean plasma E, level 3-wave estrous cycle.

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