RESEARCH ARTICLE

Pattern of Follicular Development, CL Size, Steroid Hormones and Biochemical Profile during Early Pregnancy in Heifers and Cows

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Abstract

The follicular development, CL size, plasma progesterone (P_4), estradiol-17β (E_2), total cholesterol and blood glucose profiles were studied in 6 post-pubertal cyclic heifers and 6 postpartum cyclic cows each of Gir and HF x Kankrej breeds at weekly interval from day 0 (estrus/AI) to day 21 post-AI and then on days 23, 28, 35 and 42 in non-return cases. The mean CL size in heifers (irrespective of breeds) as monitored by transrectal ultrasonography on days 0, 7, 14, 21, 23, 28, 35 and 42 post-AI was found to be 4.72 ± 0.33, 12.30 ± 0.55, 12.72 ± 0.62, 8.74 ± 1.39, 9.47 ± 1.17, 11.83 ± 0.87, 14.44 ± 0.61 and 14.50 ± 0.67 mm, respectively. Plasma P_4 concentrations on corresponding days were 0.49 ± 0.10, 3.16 ± 0.21, 5.18 ± 0.41, 3.01 ± 0.99, 3.50 ± 0.96, 5.66 ± 0.73, 8.54 ± 0.21 and 8.96 ± 0.15 ng/ml, respectively. The mean follicular size in heifers on days 0, 7, 14, 21, 23, 28, 35 and 42 post-AI was 12.70 ± 0.51, 5.86 ± 0.52, 5.23 ± 0.45, 9.21 ± 0.96, 7.41 ± 0.91, 6.14 ± 0.48, 4.80 ± 0.51 and 4.94 ± 0.30 mm, respectively. Plasma E_2 concentrations on corresponding days were 56.08 ± 2.98, 29.58 ± 2.52, 20.25 ± 2.43, 38.08 ± 5.70, 22.66 ± 4.97, 21.58 ± 2.84, 15.80 ± 2.53 and 22.40 ± 3.29 pg/mL, respectively. Nearly similar values and trend of observations were recorded for CL/follicular size and plasma P_4/E_2 profile in postpartum cows. The values of CL size and plasma P_4 were the lowest, and those of follicle size and plasma E_2 were the highest on the day of estrus/AI, changed highly significantly ($p < 0.01$) by day 7 to 14, and again around day 21–23 and fluctuated at or little lower levels around day 35 and 42 post-AI. These trends were associated with recurrence of estrus in few animals around day 21–23, and establishment of pregnancy in others. No significant variation was noted in any of the traits between breeds and between parity at any of the intervals studied. Further, the conceiving and non-conceiving cows (irrespective of breed or parity) revealed identical pattern and values of all 4 traits (CL, Follicle, P_4, E_2) till day 14 post-AI, and there was significant drop in CL size/P_4 and rise in follicular size/E_2 profile in non-pregnant animals on day 21 post-AI due to initiation of next cycle. The mean concentration of blood glucose and plasma cholesterol recorded varied from 60.96 ± 1.69 to 68.67 ± 2.10 and 169.79 ± 1.43 to 181.01 ± 1.85 mg/dL, respectively, which did not vary significantly between breeds or intervals post-AI. The values of glucose and cholesterol were non-significantly higher in non-pregnant Gir and pregnant crossbred cattle as compared to their counter parts.

Key words: CL Size, Crossbred, Gir, Follicular Dynamics, Normal cyclic cattle, Steroid Hormones and Biochemical Profile.

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Introduction

It is established that normal cyclic cattle exhibit either 2-wave or 3-wave follicular growth during estrous cycle, but there are no clear studies to show how the follicular dynamics of early pregnant cows differ from non-conceived animals following breeding. Early researchers have demonstrated that after the first 20 days of pregnancy, follicular growth slows down; the size of largest follicle decreases and the CL has a negative effect on follicular growth on ipsilateral ovary (Guibault *et al*., 1986). The profiles of follicular dynamics were similar during the interovulatory interval and the first 21 days of pregnancy in heifers, whereas after approximately day 25, the conceptus or products of conceptus had unilateral negative influence (Pierson and Ginther 1987). It has also been documented that pregnant cows continue to have a regular wave-like pattern of follicular growth throughout first 60 days of pregnancy (Taylor and Rajamahendran, 1991). Information on follicular growth pattern and associated steroid hormones

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profile and biochemical changes is meagre in Gir and HFxK crossbred cattle from Indian subcontinent (Hadiya *et al.,* 2017). Hence, this study was aimed to know the follicular growth pattern, CL and follicular size as well as blood glucose, cholesterol and sex steroids profile of conceiving and non-conceiving Gir and HFxK crossbred cows and heifers during early post-breeding periods.

Materials and methods

Post-pubertal Gir heifers (n = 6) and normal cyclic Gir cows (n = 6) along with similar numbers of heifers and cyclic cows of HF x Kankrej crossbreds (total 12 heifers and 12 cows) of University farm at Anand, were used for present study that were inseminated between January and March 2014. The animals were thoroughly screened for their genital health through gynaeco-clinical examinations and only normal cyclic animals were included for present study. All the animals were loosely housed in the same shed with free access to pure drinking water and were managed under identical nutritional conditions. Heat detection was carried out in the herd by visual observations twice daily, each lasting for an hour in the morning and in the evening as well as by monitoring behaviour of animals through CCTV camera footage for the periods other than visual observation. The cows in estrus were identified by palpation per rectum as well as by transrectal ultrasound scanning, and were inseminated with good quality frozen-thawed semen.

Transrectal ultrasound examination of all the inseminated experimental animals was performed at weekly interval from day 0 (estrus/AI) to day 21 post-AI, and then on days 23, 28, 35 and 42 using a real time B-mode ultrasound scanner (M-5 Vet, Mindray, China) equipped with a 5.0–8.5 MHz linear array transducer. The animals returning to estrus for non-conception or EED were monitored up to day 28 post-AI. The scanning of both the ovaries and uterine horns was accomplished in several planes through rectal wall to identify all the follicles greater than 4 mm in diameter and the corpus luteum as well as uterine contents (Ginther, 1995; Hadiya *et al.,* 2017). The cows that were found pregnant on transrectal ultrasonography between days 28 and 42 were later confirmed by examination per rectum on day 60. Pattern of follicular development, and follicular and luteal size were recorded for first 42 days post-AI together with blood sampling on corresponding days to that of ultrasound scanning to estimate the plasma levels of sex steroids, blood glucose and total cholesterol.

Plasma progesterone (P_4) and estradiol-17β (E_2) concentrations were determined by employing standard RIA techniques of Kubasic *et al.* (1984) and Robertson and King (1979), respectively. Labelled antigen (I^{125}), antibody coated tubes and standards were procured from Immunotech-SAS, Marseille Cedex, France. The sensitivity of the progesterone and estradiol assays was 0.1 ng/mL and 9.58 pg/mL, respectively. The intra- and inter-assay coefficients of variation were 5.4 and 9.1% for progesterone and 14.4 and 14.5% for estradiol 17β, respectively. Blood glucose was measured in fresh whole blood by using Glucometer (GlucoOne, Model No: BG-03, Morepen Lab. Ltd., Himachal Pradesh). Plasma cholesterol was estimated by using Assay kits and procedure of Crest Biosystem, Goa on biochemistry analyser. The data on plasma profile were analysed using ANOVA, DMRT and t test through SPSS software version 20.00 to know the variations within and between the groups for each trait (Snedecor and Cochran, 1994).

Results and discussion

The findings on follicular growth pattern, CL size, blood glucose and plasma progesterone (P_4), estradiol-17β (E_2) and total cholesterol profiles studied in all experimental animals at different time interval from estrus/AI are presented in Tables 1 to 3.

CL Size and Plasma Progesterone Profile

The mean size of regressing CL and plasma P_4 concentrations on the day of estrus/AI were the lowest or at basal level, and increased highly significantly ($p < 0.01$) by day 7 and 14 in

Table 1: Mean (± SE) corpus luteum size and plasma progesterone concentration in conceiving and non-conceiving heifers and cows (irrespective of breed Gir and crossbred) at different intervals post-estrus/AI

	Cyclic heifers				*Cyclic cows*			
	CL size (mm)		*Plasma progesterone (ng/mL)*		*CL size (mm)*		*Plasma progesterone (ng/mL)*	
Days post-AI	*Conceiving (n = 5)*	*Non-conceiving (n = 7)*	*Conceiving (n = 5)*	*Non-conceiving (n = 7)*	*Conceiving (n = 7)*	*Non-conceiving (n = 5)*	*Conceiving (n = 7)*	*Non-conceiving (n = 5)*
0	$4.42^{a} \pm 0.49$	$4.88^{a} \pm 0.34$	$0.26^{a} \pm 0.10$	$0.43^{a} \pm 0.15$	$4.35^{a} \pm 0.39$	$4.82^{a} \pm 0.72$	$0.33^{a} \pm 0.08$	$0.46^{a} \pm 0.17$
7	$12.72^{b} \pm 0.44$	$11.41^{c} \pm 0.39$	$4.56^{b} \pm 0.84$	$3.58^{c} \pm 0.57$	$11.88^{b} \pm 0.93$	$12.16^{b} \pm 0.81$	$4.19^{b} \pm 0.46$	$4.42^{b} \pm 0.52$
14	$12.46^{b} \pm 0.40$	$11.54^{c} \pm 0.41$	$6.94^{c} \pm 0.76$	$5.67^{d} \pm 0.48$	$12.37^{b} \pm 0.95$	$12.68^{b} \pm 1.08$	$5.35^{b} \pm 0.48$	$4.36^{b} \pm 0.56$
21	$13.12^{bc} \pm 0.59$	$7.40^{b} \pm 1.12$**	$7.68^{cd} \pm 0.34$	$1.87^{ab} \pm 0.85$**	$12.94^{b} \pm 0.95$	$5.78^{a} \pm 1.40$**	$6.62^{bc} \pm 0.40$	$1.19^{a} \pm 0.88$**
23	$13.10^{bc} \pm 0.46$	$6.81^{b} \pm 0.71$**	$8.30^{cd} \pm 0.41$	$1.70^{ab} \pm 0.50$**	$13.24^{b} \pm 0.89$	$7.69^{ab} \pm 1.44$**	$6.58^{bc} \pm 0.38$	$2.15^{a} \pm 1.18$**
28	$13.96^{bcd} \pm 0.49$	$8.58^{b} \pm 1.33$**	$8.96^{d} \pm 0.19$	$2.27^{bc} \pm 0.52$**	$13.80^{b} \pm 0.83$	$11.00^{b} \pm 0.59$	$7.28^{bc} \pm 0.38$	$4.96^{b} \pm 0.63$*
35	$14.48^{cd} \pm 0.53$	-	$8.46^{d} \pm 0.18$	--	$14.21^{c} \pm 0.83$	--	$7.65^{bc} \pm 0.44$	--
42	$14.72^{d} \pm 0.46$	-	$9.00^{d} \pm 0.11$	--	$14.04^{bc} \pm 0.85$	--	$8.11^{c} \pm 0.40$	--

Figures in parentheses indicate number of animals/observations. ** $p < 0.01$ between C-NC subgroups.
Means bearing uncommon superscript within the column differ significantly between days post-AI ($p < 0.05$).

Table 2: Mean (± SE) follicle size and plasma estradiol-17β concentration in conceiving and non-conceiving heifers and cows (irrespective of breed Gir and crossbred) at different intervals post-estrus/AI

	Cyclic heifers				*Cyclic cows*			
	Follicle size (mm)		*Plasma estradiol-17β (pg/mL)*		*Follicle size (mm)*		*Plasma estradiol-17β (pg/mL)*	
Days post-AI	*Conceiving (n = 5)*	*Non-conceiving (n = 7)*	*Conceiving (n = 5)*	*Non-conceiving (n = 7)*	*Conceiving (n = 7)*	*Non-conceiving (n = 5)*	*Conceiving (n = 7)*	*Non-conceiving (n = 5)*
0	$14.24^{c} \pm 0.69$	$12.94^{d} \pm 1.20$	$51.00^{b} \pm 5.61$	$50.57^{b} \pm 5.68$	$15.61^{b} \pm 0.81$	$14.84^{c} \pm 1.85$	$65.57^{c} \pm 3.14$	$62.40^{b} \pm 4.94$
7	$5.60^{a} \pm 0.19$	$5.67^{ab} \pm 0.64$	$25.60^{a} \pm 2.20$	$22.00^{a} \pm 3.84$	$6.72^{a} \pm 0.83$	$7.54^{a} \pm 1.96$	$31.85^{bc} \pm 3.57$	$28.00^{a} \pm 1.81$
14	$5.88^{a} \pm 0.43$	$4.53^{a} \pm 0.28$	$23.40^{a} \pm 5.47$	$26.86^{a} \pm 2.85$	$6.08^{a} \pm 0.97$	$5.90^{a} \pm 0.79$	$19.03^{a} \pm 2.77$	$21.00^{a} \pm 4.31$
21	$7.82^{b} \pm 0.63$	$8.88^{c} \pm 1.33$	$30.80^{a} \pm 3.06$	$37.86^{ab} \pm 7.55$	$5.20^{a} \pm 0.54$	$11.16^{bc} \pm 1.95$**	$16.42^{a} \pm 2.25$	47.20 ± 9.63**
23	$6.28^{ab} \pm 0.95$	$7.71^{bc} \pm 0.88$	$27.00^{a} \pm 5.62$	$20.57^{a} \pm 4.92$	$6.20^{a} \pm 0.67$	$8.02^{ab} \pm 1.65$	$22.00^{a} \pm 3.52$	$30.80^{a} \pm 9.78$
28	$6.02^{a} \pm 0.55$	$8.02^{b} \pm 1.14$	$25.20^{a} \pm 3.62$	$34.43^{ab} \pm 7.21$	$6.30^{a} \pm 0.60$	$6.54^{a} \pm 0.71$	$19.14^{a} \pm 4.03$	$19.20^{a} \pm 2.98$
35	$5.84^{a} \pm 0.67$	-	$22.00^{a} \pm 5.65$	-	$6.05^{a} \pm 0.94$	--	$19.57^{a} \pm 2.59$	--
42	$4.78^{a} \pm 0.18$	-	$19.00^{a} \pm 2.24$	-	$5.55^{a} \pm 0.54$	--	$24.00^{ab} \pm 1.97$	--

Figures in parentheses indicate number of animals/observations. ** P<0.01 between C-NC subgroups.
Means bearing uncommon superscript within the column differ significantly between days post-AI ($p < 0.05$).

Table 3: Mean (± SE) blood glucose and plasma total cholesterol concentration in conceiving and non-conceiving heifers and cows (irrespective of breed Gir and crossbred) at different intervals post-estrus/AI

	Cyclic heifers				*Cyclic cows*			
	Blood glucose (mg/dL)		*Plasma total cholesterol (mg/dL)*		*Blood glucose (mg/dL)*		*Plasma total cholesterol (mg/dL)*	
Days post-AI	*Conceiving (n = 5)*	*Non-conceiving (n = 7)*	*Conceiving (n = 5)*	*Non-conceiving (n = 7)*	*Conceiving (n = 7)*	*Non-conceiving (n = 5)*	*Conceiving (n = 7)*	*Non-conceiving (n = 5)*
0	64.84 ± 3.04	75.46 ± 4.08	165.04 ± 6.36	181.17 ± 5.06	68.50 ± 3.69	55.48 ± 2.23*	178.05 ± 4.27	163.05 ± 8.23
7	67.37 ± 1.90	75.20 ± 4.67	167.85 ± 4.19	184.20 ± 2.93	68.91 ± 2.65	60.66 ± 2.88	180.03 ± 5.06	161.02 ± 6.30
14	67.80 ± 1.96	74.46 ± 4.13	177.94 ± 1.79	180.07 ± 3.90	66.60 ± 3.11	57.92 ± 3.44	171.65 ± 4.43	175.20 ± 5.72
21	69.88 ± 1.32	77.00 ± 2.88	169.53 ± 5.75	179.62 ± 2.89	64.53 ± 2.61	63.60 ± 2.98	176.01 ± 3.92	167.51 ± 5.62
23	71.40 ± 2.11	71.81 ± 1.67	171.72 ± 3.42	179.02 ± 4.41	68.41 ± 4.10	61.62 ± 2.16	180.42 ± 3.21	171.12 ± 1.59
28	64.42 ± 3.92	75.43 ± 2.71	172.95 ± 6.42	182.64 ± 3.48	67.78 ± 3.36	59.45 ± 2.99	172.71 ± 3.63	176.24 ± 1.67
35	63.88 ± 2.50	-	176.00 ± 4.69	-	67.06 ± 2.33	--	173.60 ± 3.26	--
42	67.30 ± 3.00	-	175.69 ± 2.92	-	65.20 ± 3.91	--	176.16 ± 3.25	--

Figures in parentheses indicate number of animals/observations.
None of the means within the column differ significantly between periods or days post-AI.

both heifers and cows due to ovulatory estrus and new CL formation. The CL size and plasma P_4 levels then remained more or less static at higher level till day 42 in conceived cows and heifers, irrespective of breed. However, in non-conceived groups, both these traits dropped down significantly ($p < 0.01$) around day 21–23 and again rose slightly at day 28 post-AI in both the heifers and cows (Table 1). These trends were associated with establishment of pregnancy in 5 heifers and 8 cows, irrespective of breed, and return of rest of the animals to next cycle around day 21–23, and also the cause of embryonic mortality in 1 heifer and 2 cows between day 23 and 28 post-breeding. As far as Gir and crossbreds are concerned no significant variation was noted in CL size or plasma P_4 profile at any of the intervals studied and the trend was the same in both the Gir and crossbred heifers as well as cows. Further, the CL size and plasma P_4 concentrations in conceiving and non-conceiving animals (irrespective of breed and parity) revealed identical pattern and values till day 14 post-AI, and there was significant ($p < 0.01$) drop in both these traits in non-pregnant animals due to re-establishment of next cycle around day 21–23 and persistence of CL and continuance of P_4 production in conceived animals (Table 1).

Follicular Size and Plasma Estradiol-17β Profile

The mean values of follicular size and plasma estradiol-17β concentration on the day of estrus/AI were the highest and decreased highly significantly ($p < 0.01$) by day 7 and 14, and then fluctuated non-significantly till monitored up to day 42 post-AI in conceived groups of heifers and cows, while in non-conceived groups these traits rose significantly around day 21–23 and fluctuated at or little lower levels till day 28 post-AI in both heifers and cows, irrespective of Gir and crossbred breeds. These trends were associated with recurrence of estrus in non-conceived animals around day 21–23, and establishment of pregnancy in conceived animals, and also due to embryonic mortality in three animals between day 23 and 28 post-breeding in non-conceived group. No significant variation was noted in follicular size or plasma E_2 profile between day 7 to 42 post-AI in conceived, and day 7-14 and again on day 23–28 in non-conceived groups (Table 2). Further, the distribution of animals (irrespective of breed or parity) according to conceiving and non-conceiving status revealed identical pattern and values of follicular size and plasma E_2 concentrations till day 14 post-AI, and there was significant rise ($p < 0.05$) in follicular size as well as E_2 profile in non-pregnant animals on day 21–23 due to initiation of next cycle.

Blood Glucose and Plasma Cholesterol Profile

The mean values of blood glucose and plasma total cholesterol profile did not vary significantly between different intervals from day 0 till day 42 post-AI in any of the groups. The values of both blood glucose and cholesterol were slightly on higher side in non-conceived heifers than cows throughout the study period indicated better nutritional status of heifers. In conceived animals the trend of cholesterol profile was however reverse, being higher in cows than heifers (Table 3). As far as breed is concerned no significant variation was noted in blood glucose or plasma cholesterol profile at any of the intervals between breed or parity. The results in general did not reveal any specific role of glucose or cholesterol in folliculogenesis, CL function or pregnancy establishment in animals under study (Table 3).

The literature on the aspects we studied in normal cyclic animals from the day of insemination till day 28 in non-pregnant or up to day 42 post-AI in pregnant ones, particularly with reference to CL or follicle size and steroid hormones or blood biochemical profile was scarce (Hadiya *et al.,* 2017), except scattered work on progesterone profile during early pregnancy. Energy, fertility and progesterone are directly related to each other. Higher levels of progesterone favour prompt induction of estrus and early conception with minimum services per conception. The fate of dominant follicle depends on function of corpus luteum, and under elevated plasma P_4 concentrations, the dominant follicle becomes atretic due to negative influence of the P_4 on pulsatility of LH secretion (Ireland *et al.,* 2000).

Follicular size and estradiol concentration have been postulated to have an important role in controlling follicular development and in determining whether an estrous cycle will have 2 or 3-waves in dairy animals (Noseir, 2003). A significant positive correlation ($p < 0.05$) between serum glucose and E_2 and a significant negative relationship between total cholesterol and E_2 ($p < 0.05$) in postpartum cows have been documented by Saleh *et al*. (2011). The plasma progesterone concentration on day six after AI was reported to be higher in pregnant than in non-pregnant cows, but the difference was significant only after 10 days of AI (Komoto *et al.,* 1991). Dhabale *et al*. (2000) reported mean serum progesterone concentrations of 1.98 ± 0.47, 2.43 ± 0.75, 1.97 ± 0.59 and 1.85 ± 0.78 ng/mL on days 0, 10, 20 and 30 post-estrum in eight crossbred cows. Rectal palpation for ovarian changes in these cows revealed that two and three cows had ovulatory and anovulatory heats with normal cycle length and three cows had ovulatory heat with prolonged cycle length of 32–37 days.

Similarly, the progesterone level was reported to be significantly higher in fertile than infertile cycle on day 21 post-breeding, but not at estrus, day 7 or day 14 (Patel *et al*., 2006), indicative of luteal dysfunction in infertile cows. The pooled plasma P_4 profile of conceived HF cows was significantly higher than in non-conceived ones (3.23 ± 0.21 vs 2.42 ± 0.12 ng/mL) (Patel *et al.,* 2006). In Surti buffaloes, Sharma *et al*. (2010) observed significantly higher ($p < 0.01$) weekly P_4 levels in fertile (n = 10) than the infertile (n = 12) cycles on day 14 (3.89 ± 0.36 vs. 2.39 ± 0.24 ng/mL) and 21 (3.70 ± 0.28 vs. 0.36 ± 0.08 ng/mL), but not at estrus or day 7. The P_4 levels in fertile cycles showed a significant rise from the day of estrus to day 7 till day 14 and then maintained almost same level till day 21st post-breeding, whereas in fertile cycles the levels recorded on day 0 and 21 were at par and significantly lower/basal ($p < 0.05$) than those found on day 7 and 14 post-breeding.

In our study, the glucose and cholesterol profiles were similar in conceiving and non-conceiving cows throughout the 42 days of post-AI period. However, Srivastava and Sahni (2000) observed higher levels of cholesterol at estrus/AI in cows and buffaloes turned out to be pregnant than non-pregnant. Kavani *et al*. (2005) studied plasma glucose and cholesterol levels on days 0, 7, 14 and 21 of fertile and infertile cycles post-breeding in Surti buffaloes, and found that the levels were apparently higher at most intervals in fertile than the infertile cycles.

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