RESEARCH ARTICLE

Follicular Dynamics and Endocrine Profile during Normal Estrous Cycle and Early Pregnancy in Surti Buffaloes

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Abstract

The study was conducted on Surti buffaloes for follicular dynamics and endocrine profile during normal estrous cycle (n=6) and early pregnancy (n=6). In cyclic and early pregnant buffaloes, one-wave (33.33% vs. 50.00%) and two-waves (66.66% vs. 50.00%) follicular development was recorded, without three-wave pattern. In cyclic buffalo, the length of estrous cycle and duration of inter-ovulatory interval were two days shorter in one wave cycles than in two wave cycles (22.50±0.50 and 23.25±0.25 days). The number of follicles differed non-significantly within and between cyclic and early pregnant buffaloes. The mean maximum diameter of dominant follicle of second wave was significantly larger in cyclic than early pregnant animals for two wave pattern (1.09±0.02 vs 0.73±0.02 cm). The mean maximum CL size of early pregnant buffaloes was significantly larger than the cyclic buffaloes with one-wave cycle (1.50±0.04 vs. 1.21±0.02 cm), but it was identical in two-wave cycles (1.55±0.07 vs. 1.52±0.05 cm). In the normal cyclic animals, the CL size increased gradually up to day 8 (1.30±0.04 cm) and then remained stable up to day 18 (1.26±0.15 cm), followed by regression and display of next estrus between day 19 and 21, whereas in early pregnant animals the CL size gradually increased up to day 10 (1.48±0.07 cm) and then it was maintained till day 22 (1.46±0.04 cm) of monitoring. The mean diameter of largest follicle recorded was on the day of estrus (0.98±0.07 cm) with variable development of follicles during metestrus to diestrus phase with a new follicles developed during proestrus to estrus phase reaching maximum diameter of ovulatory size (0.99±0.09 cm) between days 19 and 21, whereas in early pregnant animals, the diameter of larger follicle (0.76±0.05 cm) was not recorded up to ovulatory size. The mean concentrations of serum progesterone were lowest during peri-estrus phase, increased through early-luteal phase to a maximum concentration during mid-luteal phase (4.50±0.88 to 4.67±0.95 ng/mL) and then gradually declined through late-luteal phase of diestrus in normal cyclic animals with inverse trend in estradiol profile. However, in early pregnant animals the progesterone levels gradually increased initially and then were maintained higher (6.17±0.32 to 8.13±0.55 ng/mL) with basal estradiol throughout the luteal phase. The serum progesterone/estradiol levels were in harmony with the ovarian dynamics in both cyclic and early pregnant buffaloes. The mean concentrations of serum FSH, LH and kisspeptin in normal cyclic and early pregnant animals did not vary statistically between periods within the status or between reproductive status of animals at any of the periods. The concentration of serum kisspeptin dropped significantly in advanced pregnancy as compared to first and second trimester.

Keywords: Buffalo, Early pregnancy, Endocrine profile ,Estrous cycle, Follicular dynamics.

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Introduction

The betterment of fertility in female buffalo is in close relationship with the knowledge of follicular development (Yilmaz *et al*., 2021). In order to optimize buffalo reproduction, the physiological controls of recruitment, selection, growth, dominance and atresia of ovarian follicles need to be better understood. Follicular dynamics is a continuous process of growth and regression of a group of antral follicles, one of which develops until it reaches the preovulatory follicular stage (Neglia *et al*., 2007). A good understanding of follicular dynamics can alleviate various reproductive problems and optimize the efficiency of buffalo reproduction (Das *et al*., 2013).

Ovarian follicular dynamics in buffaloes during unstimulated estrous cycle was similar to that observed in cattle and was characterized by waves of follicular recruitment, growth and regression. Baruselli *et al*. (1997) for the first time studied the buffalo follicular dynamics during estrous cycle and verified that 63.33, 33.73 and 3.33 % of Murrah buffaloes presented have two, three and one follicular waves, respectively. Later several studies also reported

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follicular dynamics in Murrah (Jerome *et al*., 2017), Swamp (Yindee *et al*., 2011), Nili-Ravi (Warriach and Ahmad, 2009), Mediterranean Italian (Presicce *et al*., 2005), and Egyptian (Barkawi *et al*., 2009) buffaloes. Almost all the reports have confirmed similar qualitative and quantitative follicular turn over. Follicular dynamics in Surti buffaloes during normal estrous cycle and early pregnancy is yet required to be investigated.

The different phases of reproductive cycle are regulated by intricate sequential events and interaction between hypothalamic releasing factors, gonadotropic hormones from the pituitary gland and sex steroids. Lack of integration and endocrine imbalances at any phase of the sequence may result in reproductive failure (Raval *et al.,* 2021). Knowledge of follicular patterns and folliculogenesis are necessary to successfully implement both estrus synchronization and superovulation protocols. Therefore, the present investigation was undertaken to study ovarian dynamics and endocrine profile during estrous cycle and early pregnancy and to compare follicular and luteal dimensions with endocrine profile in Surti buffaloes.

Materials and Methods

Experimental Animals and Design

The investigation was undertaken during July, 2019 to March, 2021 on normal cyclic buffaloes (n=12) of Reproductive Biology Research Unit of the College in Anand (Gujarat). The study evaluated follicular dynamics and endocrine profile during estrous cycle (n=6) as well as during early pregnancy (n=6) in buffaloes. The animals were followed for USG (A real time, B mode, with 6.5 MHz linear array transducer (DC-6 Vet, Mindray, China) as well as blood sampling on alternate days at least up to first 22 days. The animals (n=6) that did not repeat and found pregnant at 6 to 7 weeks post-AI were taken as early pregnant, and were further monitored for kisspeptin profile during early (<10 weeks), mid (>10 to <20 weeks) and late (>20 to 36 weeks) stages of pregnancy.

The animals were subjected to jugular vein-puncture for blood collection in serum clot activator tubes on alternate days soon after USG for estimation of the endocrine profile. The blood serum was separated immediately by centrifugation at 750 x g and stored at -80˚C with a drop of 0.01 % merthiolate until assayed for various hormones.

Monitoring of Ovarian Follicular Dynamics

Upon trans-rectal ultrasonography from the day of estrus/AI, the animals were assessed for the follicular/luteal growth and regression pattern as well as number of follicles and their diameters. During each examination, the diameters of identified follicles with antral diameter ≥3/4 mm were recorded and monitored for their growth pattern as growing, static and regressing follicles (Baruselli *et al.,* 1997). During USG examinations, the desired images were frozen on the screen and the measurements were taken using a built in calliper system, and the parameters like total number of follicles (≥ 3 mm), maximum size of dominant follicle (cm), growth rate of dominant follicle (cm/d), ovulatory follicle size (cm), corpus luteum size (cm), length of estrous cycle (days) and inter-estrus interval (days) etc. were recorded/calculated for further evaluation.

Endocrine Assay

The blood serum samples preserved were analyzed for Kisspeptin (ELISA, Cloud-Clone Corp., USA Cat. No. CEC559Bo), FSH and LH (IRMA- immuno-radiometric assay, Beckman Coulter, Immunotech, France Cat. Nos. IM2125 and IM1381), and Estradiol and Progesterone (RIA, Beckman Coulter, Immunotech, France Cat. Nos. A21854 and IM1188) by using kits and procedures described in the manufacturers' instructions. The analytical and functional sensitivity for FSH was 0.17 and 0.5 IU/L, respectively; and the intra- and inter-assay coefficients of variation were 4.05 and 8.20%, respectively. For LH assay, the corresponding values were 0.16 and 0.48 IU/L, and 7.33 and 8.42%. The sensitivity of the assay for progesterone and estradiol was 0.1 ng/mL and 9.58 pg/mL, respectively. The intra- and inter- assay coefficients of variation for progesterone were 5.4 and 9.1 % and for estradiol 14.4 and 14.5%, respectively.

Statistical Analysis

The data generated on ovarian dynamics and hormone profile were analyzed statistically for ANOVA using completely randomized design and critical difference test, and 't' test to compare between cyclic and early pregnant animals.

Results and Discussion

Follicular Dynamics

The characteristics of follicular dynamics observed in cyclic Surti buffaloes indicated that 33.33% (n=2) and 66.66% (n=4) of buffaloes had one- and two-waves of follicular development, respectively, while in early pregnant buffaloes one- and two-waves of follicular patterns were in equal proportion (50.00% n=3 each, Table 1, 2). Similar predominant two-wave pattern of follicular development in buffaloes ranging from 53.0 to 83.3% was reported by Baruselli *et al*. (1997), Presicce *et al*. (2005), Noseir *et al*. (2014) and Gaur and Purohit (2019). However, 62.5% of one-wave pattern in water buffaloes (Awasthi *et al*., 2006) and 53.6% and 83.3% of three wave pattern in Egyptian buffaloes (Barkawi *et al*., 2009) and in Graded Murrah buffaloes (Satheshkumar *et al*., 2011) were also reported. It was suggested that the breed, physiological and nutritional status, lactation, environmental conditions and the time/season of study play an important role in influencing wave pattern (Ginther *et al.,* 1989; Jerome *et al*., 2017). In addition, genetic predisposition or uncontrolled environmental conditions also have role in regulation of development of the one, two or three follicular waves within the estrous cycle (Baruselli *et al.,* 2013). The time of luteal regression is responsible for having either two or three waves estrous cycle (Ginther *et al*., 1989). The fundamental features of follicle recruitment, selection, dominance and atresia observed during a follicular wave were in association with endocrine events (Baruselli *et al*., 1997; Singh *et al.,* 2000).

Table 1: Characteristics (mean ± SE) of estrous cycle in cyclic Surti buffaloes having one- and two-waves follicular development

Characteristics	Number of waves	
	One	Two
No. of buffaloes (n=6)	2 (33.33%)	4 (66.66%)
Duration of estrous cycle (days)	20.50±1.50	22.50±0.50
Duration of inter-ovulatory interval (days)	21.50±1.50	23.25±0.25
Total no. of follicles at emergence of first wave	4.00±1.00	3.50±0.50
Total no. of follicles at emergence of second wave	--	5.00±0.41
Maximum diameter of anovulatory dominant follicle (cm)	--	0.94±0.03
Growth rate of anovulatory dominant follicle (cm/day)	--	0.05±0.00
Maximum diameter of ovulatory follicle (cm)	1.00±0.12	1.09±0.02
Growth rate of ovulatory follicle (cm/day)	0.03±0.00	0.07±0.00*
Maximum size of corpus luteum (cm)	1.21±0.02	1.52±0.05**

* p<0.05, ** p<0.01 between one- and two-wave cycles.

Table 2: Characteristics (mean ± SE) of estrous cycle in early pregnant Surti buffaloes having one- and two-waves follicular development

Characteristics	Number of waves	
	One	Two
No. of buffaloes (n=6)	3 (50%)	3 (50%)
Total no. of follicles at emergence of first wave	4.00±0.58	4.33±0.33
Total no. of follicles at emergence of second wave	--	3.67±0.33
Maximum diameter of anovulatory dominant follicle of first wave (cm)	0.85±0.01	0.78±0.06
Growth rate of anovulatory dominant follicle of first wave (cm/day)	0.04±0.01	0.05±0.01
Maximum diameter of anovulatory dominant follicle of second wave (cm)	--	0.73±0.02
Growth rate of anovulatory dominant follicle of second wave (cm/day)	--	0.04±0.00
Maximum size of corpus luteum (cm)	1.50±0.04	1.55±0.07
Diameter of ovulatory follicle on the day of breeding (cm)	1.08±0.12	0.97±0.02

In cyclic Surti buffalo, the length of estrous cycle and duration of inter-ovulatory interval were insignificantly shorter in one wave cycle than in two wave cycles (Table 1). These findings are akin to the observations of Baruselli *et al*. (1997), Presicce *et al*. (2005), Awasthi *et al*. (2006), Satheshkumar *et al*. (2011) and Ojeda *et al*. (2014). The variation in length of estrous cycle between one and two wave buffaloes might be attributed to time of luteal regression and progesterone concentration.

During the present study, successive anovulatory follicular wave-like pattern was identified and each wave showed a dominant follicle and a variable number of other follicles, being smaller in size (subordinate follicles). Statistically, there were no significant differences in mean total numbers of follicles at emergence of first or second wave within and between cyclic and early pregnant buffaloes (Table 1, 2). These observations were in agreement with the previous findings of Melvin *et al*. (1999) and Singh *et al*. (2017), who reported mean number of total follicles as 2.4 to 4.3 and 2.83±0.31 to 5.67±0.80 in buffaloes. However, a higher mean number of total follicles (7.50±0.50 to 9.33±1.69) were noticed by Kumar *et al.* (2012) in non-cyclic buffaloes. Insignificant differences observed in terms of number of follicles for the different waves for both cyclic as well as early pregnant buffaloes under study were in agreement with those reported in different breeds of buffalo (Baruselli *et al*., 1997; Neglia *et al*., 2007), supporting the hypothesis that the number of follicles recruited depends on the individual animals. Barkawi *et al*. (2009) however found significant difference in number of follicles (5.3±0.4 vs. 7.0±0.4) recruited during second wave in two and three waves in Egyptian buffaloes. The differences in number of total follicles might be attributed to cyclic condition of animal (Kumar *et al.,* 2012), breed and managemental variations (Singh *et al*., 2017).

The observations on mean maximum diameter and growth rate of dominant and anovulatory follicles in cyclic and early pregnant Surti buffaloes studied in different wave patterns revealed that only the mean maximum diameters of dominant follicle of second wave was significantly higher in cyclic animals than early pregnant animals having two wave pattern (1.09±0.02 vs 0.73±0.02 cm, Table 1, 2). These observations were in line with Yindee *et al*. (2011), Kachiwal *et al*. (2012) and Singh *et al*. (2017). The comparable findings were also recorded by Barkawi *et al*. (2009) (1.3±0.06 cm), Noseir *et al*. (2014) (1.05±0.04 and 1.12±0.04 cm) and Jerome *et al*. (2017) (10.00±1.20 and 11.00±0.95 mm) in Murrah buffaloes. However, the mean maximum diameters of dominant follicle reported by Baruselli *et al*. (1997) (1.51±0.24 cm) and Heleil and Deeb (2010) (1.45±0.34 cm) were higher than present results. It was speculated that during non-ovulatory wave, one follicle is subsequently selected from the cohort for continued growth and becomes dominant (Adams, 1999). Similar follicular sequential changes (recruitment, selection and dominance) were observed during the present study. The life span of dominant follicle is controlled by LH secretion.

Awasthi *et al*. (2006) attributed the minimized growth of dominant follicle to lower availability of LH to developing follicle, which affected the bioavailability or synthesis of various growth factors and/or their binding proteins needed for terminal growth of the follicle. Martin *et al*. (2008) also added that dominant follicles with smaller diameters might be due to an inadequate supply of LH receptors.

In cyclic Surti buffaloes, the mean maximum CL size recorded during the cycle was 1.21±0.02 and 1.52±0.05 cm in one- and two-wave cycles, respectively, having significant difference between waves, whereas, in early pregnant Surti buffaloes, difference (1.50±0.04 and 1.55±0.07 cm) was insignificant. The mean maximum CL size of early pregnant buffaloes was significantly larger than the cyclic buffaloes (1.50±0.04 vs. 1.21±0.02 cm) with one-wave cycle (Table 1, 2). If the ovum has been fertilized, the CL persists for several months, otherwise the CL degenerates and shrinks. So the CL has a direct effect on pregnancy of animal. Corpus luteum continuously produces progesterone, which is responsible for maintaining pregnancy. The size of CL is believed to have a relationship with progesterone production and thereby influences success of pregnancy. The present findings on CL size are comparatively lower than those of Ojeda *et al*. (2014) in multiparous buffaloes (1.95±0.42 cm) and of Raval *et al*. (2021) in Jaffarabadi buffaloes (1.37 to 2.39 cm). The variation in results can be attributed to breed, season, and nutritional and managemental differences.

Follicular/Luteal Biometry and Endocrine Profile

The CL developed at the site of ovulated follicle gradually increased up to day seven/eight and then remained stable up to day 17/18 of the estrous cycle, which then regressed to non-measurable size, and the normal cyclic animals displayed next estrus between day 19 and 21. Whereas in early pregnant animals, the CL size gradually increased initially up to day eight/nine and then it was maintained throughout the period *i.e.,* day 22 of ultrasonography examination, and these animals did not return to estrus again indicating establishment of early pregnancy.

The mean serum progesterone concentration was the lowest (0.17±0.04 ng/mL) during the estrus and increased through the early-luteal phase to a maximum (4.67±0.95 ng/mL) concentration during the mid-luteal phase and then gradually declined through the late-luteal phase of diestrus in normal cyclic animals. However, in early pregnant animals the mean serum progesterone levels gradually increased initially and then were maintained throughout the length of luteal phase, and all these animals were found to be early pregnant by day 26-27 by USG and were confirmed by day 60-65 through per rectal palpation. The mean serum progesterone levels on different days of monitoring were in harmony with the CL sizes observed through real time ovarian ultrasound examination in both cyclic and early pregnant buffaloes (Table 3, Fig. 1).

Neglia *et al*. (2007) opined that progesterone during the luteal phase affects the growth of the anovulatory dominant follicles, decreasing their diameter. In addition, maximum progesterone levels from the luteal concentrations also suppress follicular development and results in lower dominant follicles size and lower growth rates (Baruselli *et al*., 1997). From the above endocrinological findings observed by several authors, it was hypothecated that the lower LH pulses and higher progesterone levels during mid-luteal phase might prevent further growth and ovulation of dominant follicle.

Table 3: Mean (±SE) CL size and serum progesterone profile on different days in cyclic and early pregnant Surti buffaloes

Days of Estrous Cycle	CL Size (cm)		Serum Progesterone (ng/mL)	
	Cyclic (n=6)	Early Pregnant (n=6)	Cyclic (n=6)	Early Pregnant (n=6)
0 (Estrus)	$0.00±0.00^{a}$	$0.00±0.00^{a}$	$0.17±0.04^{a}$	$0.16±0.04^{a}$
2	$0.00±0.00^{a}$	$0.63±0.20^{b*}$	$0.40±0.10^{ab}$	$0.67±0.06^{a*}$
4	$0.99±0.08^{bc}$	$1.10±0.03^{c}$	$1.16±0.20^{abc}$	$2.75±0.42^{b*}$
6	$1.13±0.06^{c}$	$1.19±0.06^{cd}$	$2.20±0.33^{bcd}$	$3.67±0.45^{b*}$
8	$1.30±0.04^{c}$	$1.30±0.06^{cde}$	$3.55±0.72^{def}$	$4.97±0.32^{c*}$
10	$1.30±0.06^{c}$	$1.48±0.07^{e}$	$4.05±0.79^{def}$	$5.77±0.40^{cd}$
12	$1.33±0.08^{c}$	$1.43±0.04^{e}$	$4.50±0.88^{ef}$	$6.17±0.32^{cde}$
14	$1.26±0.14^{c}$	$1.41±0.06^{de}$	$4.67±0.95^{f}$	$6.60±0.26^{de}$
16	$1.26±0.15^{c}$	$1.43±0.06^{e}$	$3.52±0.82^{def}$	$7.37±0.49^{ef*}$
18	$1.04±0.26^{bc}$	$1.45±0.04^{e}$	$2.62±0.65^{cde}$	$8.10±0.72^{f**}$
20	$0.67±0.23^{b}$	$1.46±0.04^{e*}$	$1.44±0.57^{abc}$	$8.13±0.55^{f**}$
22	$0.23±0.14^{a}$	$1.46±0.04^{e**}$	$0.36±0.05^{ab}$	$8.13±0.50^{f**}$

Means bearing different superscripts within column (a,b,c,d,e,f) differ significantly ($p<0.05$).*$p<0.05$, **$p<0.01$ for cyclic vs. early pregnant animals on same day for particular parameter.

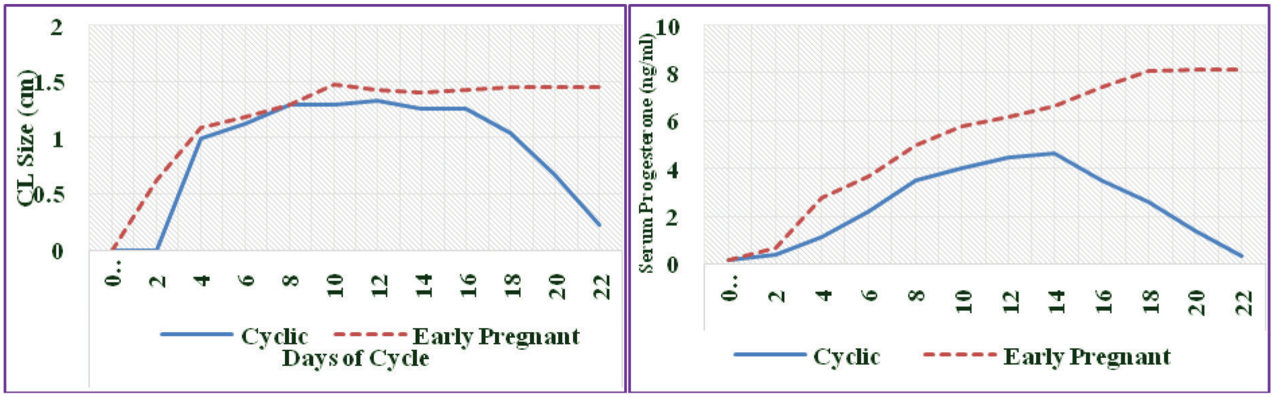

Fig. 1: Mean CL size (cm) and serum progesterone (ng/mL) profile on different days of estrous cycle in cyclic and early pregnant Surti buffaloes

Table 4: Mean (±SE) size of largest follicle (cm) and serum estradiol (pg/mL) profile on different days in cyclic and early pregnant Surti buffaloes

Days of Estrous Cycle	Largest follicle Size (cm)		Serum estradiol (pg/mL)	
	Cyclic (n=6)	Early Pregnant (n=6)	Cyclic (n=6)	Early Pregnant (n=6)
0 (Estrus)	0.98±0.07[ef]	1.02±0.06[d]	170.83±27.94[e]	185.00±28.84[a]
2	0.45±0.04[a]	0.46±0.04[a]	38.00±9.55[ab]	64.67±10.57[b]
4	0.57±0.03[ab]	0.57±0.03[ab]	54.67±6.19[abc]	57.17±11.20[b]
6	0.69±0.04[bc]	0.66±0.03[bc]	39.33±17.68[ab]	30.33±9.46[b]
8	0.79±0.03[cd]	0.70±0.04[bc]	60.00±27.57[abc]	37.83±9.74[b]
10	0.83±0.03[cde]	0.76±0.05[c]	35.67±18.33[ab]	37.67±7.29[b]
12	0.84±0.03[def]	0.70±0.03[bc*]	47.67±15.56[abc]	33.83±10.23[b]
14	0.86±0.05[def]	0.67±0.03[bc*]	22.83±5.83[a]	48.00±11.85[b]
16	0.85±0.04[def]	0.71±0.04[bc*]	22.67±8.01[a]	64.33±18.30[b]
18	0.85±0.02[def]	0.67±0.07[bc*]	108.00±27.57[cd]	59.00±12.48[b]
20	0.86±0.07[def]	0.64±0.03[bc*]	97.67±18.87[bcd]	52.17±8.58[b]
22	0.99±0.09[f]	0.64±0.04[bc*]	133.33±28.51[de]	66.50±17.37[b]

Means bearing different superscripts within column (a,b,c,d,e,f) differ significantly ($p<0.05$). *$p<0.05$ for cyclic vs. early pregnant animals on same day for particular parameter.

In the present study, the mean serum progesterone concentrations were the lowest during the peri-estrus phase, which increased through early luteal phase to a maximum concentration during mid luteal phase in cyclic Surti buffaloes. These results were in agreement with earlier observations (Mondal *et al.,* 2010; De Tarso *et al.,* 2017; Gaur and Purohit, 2019). The cyclic pattern of circulatory progesterone concentrations follows the known changes in corpus luteum function in buffalo during estrous cycle. The decline in progesterone levels towards the end of the cycle and a sharp rise during luteal development suggest that functioning of CL can be monitored by progesterone determination. Similarly, in cattle and buffalo that exhibited overt estrus and silent estrus, progesterone level was the lowest during peri-estrus phase and increased to maximum concentration during mid-luteal phase (Mondal *et al.,* 2010). In pregnant buffaloes covered under present study, the levels of serum progesterone concentrations were maintained high, which corroborated with earlier observations (De Tarso *et al.,* 2017; Gaur and Purohit, 2019).

The mean diameter of the largest follicle was measured to be maximum on the day of estrus. After estrus phase, variable development of follicles took place during metestrus to diestrus phase and finally during proestrus to estrus phase a new follicle again developed to maximum diameter of ovulatory size, and the cyclic animals displayed estrus signs again. Whereas, in early pregnant animals, the diameter of larger follicle was not recorded up to ovulatory size during the period of ultrasonography monitoring, and the animals also did not show estrus signs again (Table 4, Fig. 2). The serum estradiol concentration was estimated to be the maximum on the day of estrus. The levels remained low during metestrus to diestrus phase and finally during proestrus to estrus phase the levels were found to increase in cyclic animals and again

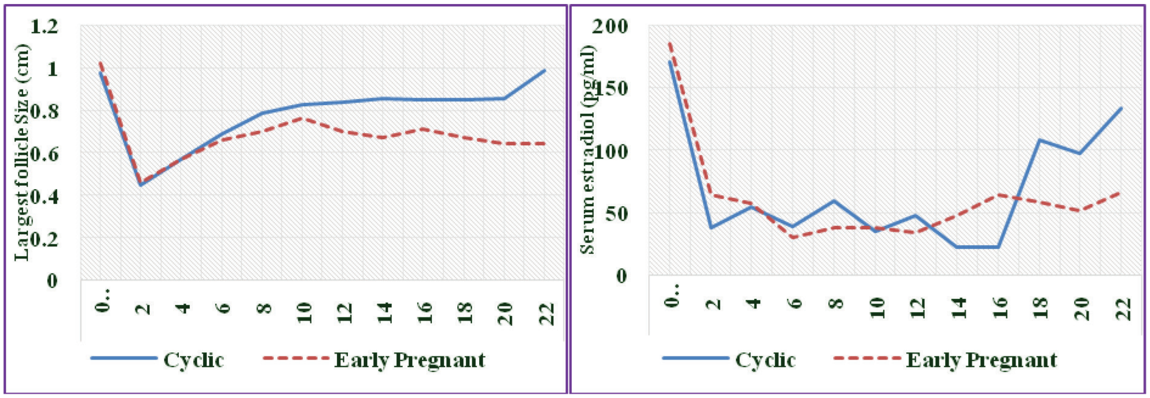

Fig. 2: Mean largest follicle size (cm) and serum estradiol (pg/mL) profile on different days of estrus cycle in cyclic and early pregnant Surti buffaloes

Table 5. Mean (±SE) serum Kisspeptin (KP), FSH and LH profiles on different days in cyclic and early pregnant Surti buffaloes

Days of Estrous Cycle	Serum Kisspeptin (pg/mL)		Serum FSH (IU/L)		Serum LH (IU/L)	
	Cyclic (n=6)	Early Pregnant (n=6)	Cyclic (n=6)	Early Pregnant (n=6)	Cyclic (n=6)	Early Preg-nant (n=6)
0 (Estrus)	403.42±101.75	250.61±94.39	0.53±0.13[ab]	0.83±0.18	1.13±0.54	1.00±0.32[ab]
2	348.98±86.40	311.93±120.08	0.55±0.35[ab]	0.40±0.26	1.58±0.42	1.05±0.56[ab]
4	374.90±81.10	386.59±148.21	0.60±0.18[ab]	0.56±0.30	1.05±0.56	0.75±0.38[a]
6	361.63±81.39	336.42±135.88	0.98±0.22[b]	1.01±0.36	1.05±0.56	0.75±0.17[a]
8	236.11±56.78	356.86±124.45	0.70±0.09[ab]	0.65±0.28	2.92±0.89	1.50±0.58[ab]
10	418.56±133.17	332.10±119.35	0.44±0.16[ab]	0.43±0.20	2.25±0.94	2.22±0.51[b]
12	405.47±158.46	282.10±91.95	0.69±0.14[ab]	0.98±0.42	1.88±0.51	1.00±0.32[ab]
14	378.81±107.68	246.56±76.97	0.81±0.19[ab]	0.68±0.35	1.58±0.77	1.08±0.38[ab]
16	330.58±124.89	223.06±88.69	0.63±0.08[ab]	0.93±0.08*	1.30±0.63	1.27±0.52[ab]
18	372.23±126.38	295.57±114.66	0.28±0.12[a]	0.63±0.36	1.08±0.47	0.80±0.39[a]
20	429.59±120.17	332.16±121.66	0.71±0.21[ab]	0.84±0.14	1.25±0.40	0.67±0.11[a]
22	374.99±95.45	348.91±124.53	0.67±0.21[ab]	0.88±0.11	1.97±0.35	1.22±0.53[ab]

Means bearing different superscripts within column (a,b) differs significantly ($p<0.05$). *$p<0.05$ for cyclic vs. early pregnant animals on the same day.

reached to highest level upon next estrus along with estrus signs. In early pregnant animals, however, the mean levels of estrogen remained low subsequent to estrus throughout the study period in the buffaloes found to be early pregnant by day 26-27 through USG. These observations agreed well with earlier reports in buffalo (Mondal *et al.,* 2010). The proestrus rise of estradiol may be associated with triggering of LH release by positive feedback on hypothalamo-hypophyseal axis (Kim, 2018). Proestrus rise in estradiol secretion after progesterone withdrawal is considered to be a prerequisite event for the initiation of both behavioural estrus and preovulatory LH surge in most livestock.

The mean serum kisspeptin (Kiss-1) levels at 3-4, 6-7 and 9-10 month of pregnancy in Surti buffaloes were 620.48±52.79, 650.76±47.53 and 433.57±44.67 pg/mL, respectively, with a significant drop in advanced pregnancy. However no such study could be seen in the literature. The serum kisspeptin, FSH and LH levels had showed inconclusive trend of change during the period of estimation in both cyclic as well as pregnant animals (Table 5, Fig. 3). Mondal *et al.* (2015) observed three peaks of kisspeptin, one on a day before appearance of preovulatory LH surge, second at day 6 and third one at day 18 of the estrous cycle in non-lactating cyclic cows. Rizzo *et al.* (2018) recorded relatively low plasma kisspeptin levels (124.69±42.76 pg/mL) in early postpartum dairy cows. They stated that kisspeptin enhanced hypothalamic GnRH release as well as pituitary gonadotropin secretion, thus promoting follicular growth and the increase in estradiol levels, which might have further enhanced kisspeptin release through a positive feedback loop.

The previous reports suggested that the peripheral plasma FSH concentrations have been highest on the day of estrus, which decline thereafter gradually and fluctuate at basal levels before rising again to peak levels at the next estrus

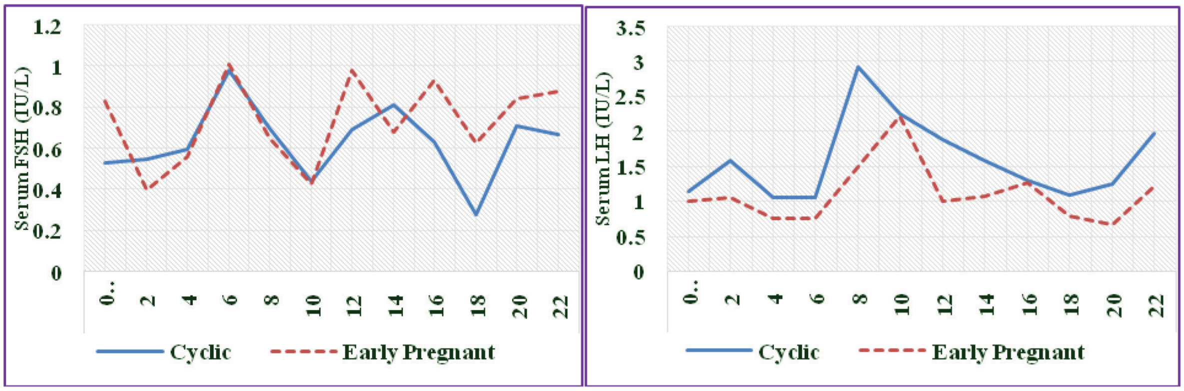

Fig. 3: Mean serum FSH and LH (IU/L) profile on different days of estrus cycle in cyclic and early pregnant Surti buffaloes

(Mondal *et al.,* 2010). After the occurrence of simultaneous pre-ovulatory surges of FSH and LH, LH levels decline sharply to the basal levels, whereas FSH concentrations show a gradual decline (Mondal *et al.,* 2010). The peripheral concentration of LH remain at basal levels throughout the estrous cycle till the day of estrus when a pre-ovulatory LH surge occurs (Kim*,* 2018). However in present investigation, the serum FSH and LH levels had shown inconclusive trend of change during the period of estimation and did not reveal clear match with ovarian follicular dynamics recorded in both cyclic as well as pregnant animals. Hence further investigations are required.

Conclusions

The present study on ultrasound scanning of ovaries revealed two patterns of follicular development, *viz.,* one- and two-waves during normal estrous cycle and early pregnancy in Surti buffaloes without evidence of three-wave cycle. During early pregnancy, the corpus luteum has negative influence on growth of dominant follicle, but does not affect the growth of small and medium sized follicles. The CL size and serum progesterone levels showed positive association. The transrectal ultrasound scanning of genital organ and serum progesterone profile over a period of time gives a clear picture of the reproductive status of the animal.

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