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Characterization of ovarian follicular development and steroid profile during estrous cycle and seasonal anestrous in buffalo

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ABSTRACT

The experiment was conducted to study ovarian follicular population, its diameter, steroid profile (estradiol 17ß and progesterone) and corpus luteum development during estrous cycle and seasonal anestrous in Murrah buffaloes. They were categorized into 2 groups based on the estrus signs i.e. cyclic (6) and anestrous (6) and subjected to ultrasound scanning of ovaries on day 0 (day of estrus in cyclic and day of first scanning in anestrous buffaloes), 6, 10 and 16 using B-mode scanner equipped with 6.0 MHz linear array transducer. Blood samples were collected on the above days for the estimation of the progesterone and estradiol-17ß. The mean number of small follicles on day 0 and 10 and medium follicles on day 10 were significantly higher within as well as between the cyclic and anestrous buffaloes, while mean number of large follicles on day 0, 10 and 16 were significantly higher in the cyclic as compared to anestrous buffaloes. However, mean number of total follicles were significantly higher in cyclic as compared to anestrous buffaloes on all the days of scanning. The maximum diameter of large follicle was observed in cyclic buffaloes on day 0 (1.40±0.49 cm). The diameter of CL increased with its development and reached to its peak on day 10 (1.39±0.04 cm), thereafter, it reduced on day 16 (0.72±0.30 cm) of the cycle. The mean serum progesterone concentration was significantly higher on day 6, 10 and 16 than day 0 of the estrous cycle indicating development of functional corpus luteum, however, mean serum estradiol-17ß concentration was significantly higher on day 0 than the other days of cycle signifying follicular growth and development secreting higher amount of estrogen.

Key words: Anestrous, Buffalo, Estrus, Follicle, Steroid

Pattern of follicular development has been studied using various techniques, however, a major breakthrough in understanding of ovarian physiology is possible with the advent of ultrasound technology in farm animals (Pierson and Ginther 1987) as well as other animal species which provided base for improving fertility, synchronizing estrus, and superovulatory responses (Lucy *et al*. 1992). Ultrasonography is a reliable method for identifying and measuring follicles for the assessment of ovarian functions (Pierson and Ginther 1987) and especially in buffaloes where manual palpation per rectum is always not accurate. Lower number of primordial and antral follicles, a slower shift from small to large follicles and a higher incidence of atresia were reported as major reasons for lower response of superovulatory protocol in buffalo as compared to cattle (Manik *et al*. 2002).

In riverine buffalo, during summer, occurrence of estrous cycles either reduced or absent affect the reproductive efficiency adversely. Information on follicular dynamics during estrous and anestrous conditions in buffalo is meager. An in-depth understanding of follicular development will be of immense use to improve reproductive efficiency in buffaloes. The present experiment, therefore, was designed to study ovarian follicular population, its diameter, steroid profile (estradiol 17ß and progesterone) and corpus luteum development during estrous cycle and seasonal anestrous in buffalo.

MATERIALS AND METHODS

Experimental animals: The study was conducted on adult female Murrah buffaloes of 5–7 years of age maintained under uniform feeding and managmental conditions at experimental animal sheds of the institute. The animals were kept in intensive system and fed with green fodder in addition to concentrate mixture and water *ad lib*. The cyclic buffaloes (6) were selected on the basis of expression of

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estrus signs and teaser bull parading. The same animals were used during hot season i.e. non-breeding season as anestrous buffalo with absence of estrus signs even after 2 cycle period has been passed.

Ultrasonography: Experimental animals were subjected to ultrasound scanning of ovaries for follicular development on day 0, 6, 10 and 16 using B-mode scanner equipped with 6.0 MHz linear array transducer. The day of standing estrus was designated as day 0 of that cycle. Anestrous buffaloes were also scanned in the similar way randomly on day 0 (day of first ultrasonography scanning), 6, 10 and 16. Animals were kept off-feed for 12h prior to ultrasonographic scanning. The transducer was inserted and manipulated in the rectum to view urinary bladder, vagina and cervix in the longitudinal plane. Follicles of both the ovaries were counted, measured and classified as small (<0.40cm), medium (0.50–0.90 cm) and large (>1cm) follicles after locating the ovaries by the transducer on clockwise and anti- clockwise rotation.

Blood collection for steroid hormone assay: Blood samples were collected on day 0, 6, 10 and 16 of the cycle in estrous and anoestrous group of buffaloes and the serum was separated and stored at –20°C until analysis. The concentration of estradiol and progesterone were estimated using the diagnostic I^{125} kits. For estrodiol hormone, analytical sensitivity of the kit was < 11pg/ml; the intra-assay and inter-assay coefficient of variation were 12.1 and 11.2%, respectively. For progesterone hormone, analytical sensitivity was 0.05ng/ml; the intra-assay and inter-assay coefficient of variation was 5.8 and 9.0%, respectively.

Statistical analysis of data: The statistical analysis was carried out using SPSS version 13.0 for windows. The data for follicular dynamics, corpus luteum and hormonal profiles were analyzed by paired 't' test to compare the differences within groups and one-way ANOVA to compare the differences between the groups.

RESULTS AND DISCUSSION

Follicular population: The mean number of small follicles were significantly higher (P<0.05) on day 0 and 10 within as well as between the groups, however, it did not differ significantly during different days of scanning in the anestrous buffaloes (Table 1). The mean number of large follicles were significantly (P<0.05) higher in estrous buffaloes on day 0, 10 and 16. The mean number of total follicles was significantly higher on day 0 and 10 within as well as between the groups. Higher number of small follicles was observed on day 0 and 10 as compared to other days may be due to existence of 2 wave follicular patterns, commonly observed in buffaloes (Baruselli *et al*. 1997). Presence of significantly higher (P<0.05) number of medium size follicles on day 10 within as well as between the groups corroborates to the finding of Manik *et al*. (2002) who observed higher number of medium size follicles during mid luteal stage as compared to other stages of estrous cycle in cattle and buffalo. Baruselli *et al*. (1997) reported higher number (2.5–3.0) of medium size follicles during day 5–6 and 14 of estrous cycle. The significantly higher number of medium-size follicles during mid luteal phase of the cycle might have resulted from growth of the large pool of small follicles present on day 0 or 1 due to systemic or local (intraovarian) action of estrogens.

The mean number of large follicle(s) in the estrous buffaloes were significantly (P<0.05) higher on day 0, 10 and 16 within as well as between the groups which might be due to emergence of follicular waves recruiting higher number of follicles that develop together and 1 or 2 of these follicles become dominant/large follicle. Baruselli *et al*. (1997) also observed higher number of larger follicles on day 0, 10–11 and 17 of estrous cycle in buffalo supporting the findings of present study. The mean number of total follicles in estrous buffaloes was significantly higher as compared to anestrous buffaloes indicating ovarian function. The total number of surface follicles on the buffalo ovary collected from local abattoir ranged from 5.14 to 6.06 (Madan *et al*. 1996) which is more or less similar as observed in anestrous buffaloes. The mean number of total follicles observed in this study was much lower than cattle (Manik *et al*. 2002) which primarily might be due to species difference. Irrespective of the wave patterns (1- or 2- or 3-wave) the number of antral follicles that appear as cohort at the time of each wave emergence (recruitment) is lesser in buffalo than that in cattle. The variation among the

Table 1. Follicular population during different days of estrous cycle and anestrous period in buffaloes

Category of follicle	Groups	Days			
		0	6	10	16/17
Small	Estrous	5.67±0.56aA	4.83±0.31b	5.0±0.37aA	4.67±0.56aA
	Anestrous	4.17±0.40B	3.67±0.42	4.17±0.40B	3.83±0.31B
Medium	Estrous	2.33±0.33a	2.83±0.17a	3.00±0.26Ab	2.5±0.22a
	Anestrous	1.50±0.34	1.67±0.36	1.67±0.33B	1.33±0.21
Large	Estrous	1.33±0.21aA	0.50±0.22b	1.67±0.17a	1.17±0.37aA
	Anestrus	0.33±0.20B	0.33±0.21	1.17±1.10	0.17±0.17B
Total	Estrous	9.33±0.33aA	8.16±0.45Ab	9.67±0.45Aa	8.33±0.56Ab
	Anestrous	6.0±0.37B	5.67±0.22B	7.01±0.31B	5.33±0.33B

a,b, Mean±SE with different superscript in a row differ significantly (P<0.05); A,B, mean±SE with different superscript in a column differ significantly (P<0.05).

number of recruited follicles between the different waves is small in buffaloes. Further, the number of follicles recruited in a follicular wave is relatively constant and lower in buffaloes as compared to cattle (Das *et al*. 2013)

There was no difference in small, medium, large and total follicular population during different days of observation in anestrous buffalo indicating emergence of relatively constant number of follicles, however, significantly lower number of total follicles were observed in anestrous than the estrous buffaloes. The LH output is suppressed during the entire period of anestrous, however, rhythmic FSH secretion may initiate the emergence of follicular development up to pre-ovulatory size (Rubianis and Menchaca 2003). Apparently, the absence of an LH drive prevents ovulation of larger follicle in anestrous buffalo, however, ovarian antral follicular turnover is not impaired during anestrous, which closely resembles to that of cycling buffaloes. Reports indicated that most of the buffalo ovaries remain smooth and inactive without any maturing Graafian follicle during summer. Further, preliminary observations made from slaughter house ovaries revealed that the buffalo ovaries have lower number of visible surface follicles during summer (2.4 per ovary) than winter (Das *et al.* 2013). In the present study, all categories of follicles were observed during different days of scanning in anestrous buffaloes indicated follicular growth except number of large follicles and ovulation.

Follicular diameter: The diameter of small, medium and large follicle during different days of estrous cycle in buffaloes has been shown in Fig 1. The mean diameter of large follicle was significantly higher on day 0 and 10 than

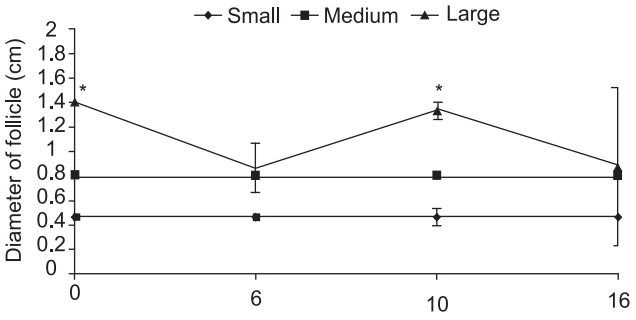

Fig. 1. Mean diamter of small, medium and large follicles during different days of estrous cycle in buffaloes.

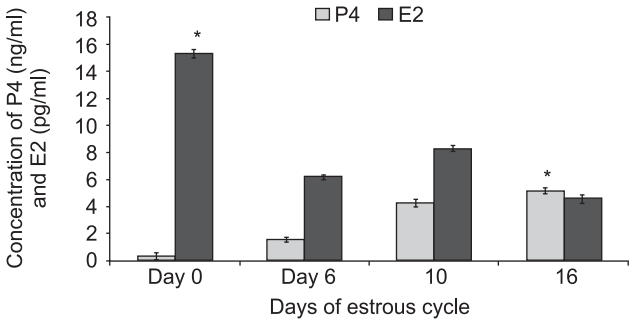

Fig . 2. Serum mean concentration of P_4 and E_2 during different days of estrous cycle in buffaloes.

the other days of estrous cycle. The maximum diameter of large follicle in estrous buffalo on day 0 was larger than day 10. The diameter of large follicle on day 0 is comparable with other studies (Baruselli *et al*. 1997a). The size of large follicle on the day of estrus was reported between 1.10 and 1.80 cm in buffaloes against 2.0 –2.2 cm in cows (Agarwal and Tomar 2003). The maximum diameter of dominant follicle as observed on day 0 was larger than that of the dominant follicle on day 10 (1.40±0.49 vs. 1.33±0.070) but lower than reported in cattle (Manik *et al*. 2002) which may be due to difference in species. The lower diameter of dominant follicle on day 10 might be due to suppressive effect of higher progesterone level (5.19±0.20 ng/ml) during this period. Our result is in accordance to Fortune (1993) in cattle and Baruselli *et al*. (1997) in buffalo. Bruke *et al*. (1994) also supported the suppressive role of progesterone in follicular development. Progesterone influences the size attainable by dominant follicles by regulating LH secretion (Savio *et al*. 1993) which is important for steroidogenesis in dominant follicles and reduction in LH secretion depletes androgen substrate for aromatization that initiates atresia (Lucy *et al*. 1992). The mean diameter of different follicles in anestrous buffaloes were not much variable and were not significantly different during different days of examination.

Corpus luteum diameter: The mean diameter of CL was 0.97±0.13, 1.39±0.042 and 0.72±0.30 cm on day 6, 10 and 16, respectively, which was significantly ($P<0.05$) higher on day 10 than the other days of estrous cycle. The diameter of CL increased with its development and reached to its peak on day 10 thereafter it reduced on day 17 of estrous cycle indicating luteolysis. The present findings are in concurrence with Patel *et al*. (2009) who also reported diameter of CL as 14.50±3.28 mm on day 10 of estrous cycle in buffaloes. The diameter of CL was lower than that reported in cattle. Regression of the CL in the absence of conception, and the associated discontinuation of progesterone secretion, is obligatory for the initiation of a new ovarian follicular wave and commencement of the next estrous cycle.

Steroid hormone profile: The mean serum progesterone concentration was significantly ($P<0.01$) higher on days 6, 10 and 16 than that on day 0 of estrous cycle (Fig. 2). The mean serum estradiol-17ß concentration was significantly ($P<0.01$) higher on day 0 than the days 6, 10 and 16 of estrous. Higher progesterone concentration on day 6, 10 and 16 of the cycle indicating presence of functional corpus luteum, is in accordance with Takkar *et al*. (1983) who have also reported lower concentration up to day 6 and peak concentration at around day 15 after estrus in buffaloes. In the present study, mean serum estradiol-17ß concentration was significantly ($P<0.01$) higher on day 0 than the other days of cycle. Higher estrogen concentration on day 0 may be due to presence of dominant follicle secreting higher amount of estrogen which is in agreement with the findings of Batra and Pandey (1982) who have recorded higher estrogen concentrations during estrus compared to luteal

phase (30–32 vs. 10–20 pg/ml) in buffaloes. A smaller peak of estrogen during mid luteal phase (day 10) observed in the present study indicated the presence of wave like follicular development and dominant follicle secreting higher estrogen. The serum concentration of estrogen and progesterone during anoestrous was on basal level and not much variable during different days of anoestrous. The present study concluded that follicular population during different days of estrous cycle in buffaloes was lower than that of cattle but showed similar trend of development and the follicular development in anestrous buffaloes were not impaired, however, it showed close resemblance to estrous buffaloes. Although, several studies are available on follicular dynamics in buffalo, there is lack of information on functional dominance of follicle during mid luteal phase affecting embryonic survivability by inducing $PGF_{2\alpha}$ secretion. Therefore, further studies are warranted to determine the influence of functional dominance of follicle during critical period, environmental factors and nutrition regulating follicular development in buffaloes.

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