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Ovarian follicular development, estradiol and progesterone profile during different days of early pregnancy in buffaloes *

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ABSTRACT

Improvement of reproductive performance of buffaloes requires a better understanding of the mechanisms controlling ovarian follicular growth and development. To study the follicular dynamics and ovarian steroid profile during early pregnancy, 6 buffaloes were selected on the basis of non return rate after insemination and subjected for ultrasonography to confirm the pregnancy as well as follicular development. The mean number of medium, large and total follicles was significantly lower on days 30, 40, 50 and 60 than day 20 of pregnancy indicating reduction in the follicular population with the advancement of pregnancy. There was no significant difference in the diameter of small and medium follicles on different days of pregnancy, however, large follicle significantly decreased with advancement of pregnancy. The progesterone levels fluctuated between 2.52±0.26 and 3.12±0.44 ng/ml until third month of pregnancy. The mean serum estradiol was significantly higher on day 0 and reduced into basal level on first and second month and remained low throughout the study period. In conclusion, on day 20 to 60 of pregnancy, the follicular development is continuous and the number and diameter of larger follicle is decreased as pregnancy advances in buffaloes.

Key words: Buffalo, Estrogen, Follicular development, Pregnancy, Progesterone

Buffaloes show waves of ovarian follicular development during estrous cycles similar to cattle (Awasthi *et al*. 2006). The fundamental features of follicle recruitment, selection, dominance and atresia during a follicular wave in buffaloes are similar to those described for cattle (Presicce *et al*. 2003). The number of follicular waves during an oestrous cycle can vary from 1 to 3 in buffaloes, with 2 waves being the most common (63%; Baruselli *et al*. 1997). The wave like phenomenon was also observed during early pregnancy in buffalo (Martin *et al*. 2008). Under various physiological conditions like pregnancy, postpartum, the pool of small antral follicles may be critical in the regulation of replenishment of follicles from the pre-antral pool and the ensuring depletion to large antral follicles. However, the information regarding follicular development during early pregnancy in buffalo is meager. Hence, the present work was carried out to study follicular growth and ovarian steroid profile during early pregnancy in buffalo.

MATERIALS AND METHODS

Experimental design: Adult female buffaloes (6), 5–8 year-old, with fairly good body condition were housed under intensive system of rearing confined in a large barn having asbestos roof and cemented floor. The animals were fed with green grass/fodder in addition to concentrate mixture and water *ad lib*. All the buffaloes were maintained under uniform feeding and management conditions. All buffaloes were observed for onset of estrus using a teaser bull and visual signs of estrus followed by rectal examination twice daily. The day of standing estrus was designated as day 0 of that cycle. Animals detected in estrus were inseminated using frozen semen of Murrah buffalo.

Pregnancy diagnosis and scanning of ovary for follicular development: The experimental animals, selected on the basis of non return rate, were subjected to ultrasonography. They were allowed to real time B-mode ultrasonographic examination at 10 days interval starting from day 20 to 60 of pregnancy. Monitoring of embryonic development was carried out by viewing embryonic vesicle through ultrasonography. The uterus was examined by guiding the

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probe slowly over the dorsal surface of the uterine horns. The location and dimensions of resolvable (>2mm), non - echogenic (black) areas of discrete structure within the uterus lumen were recorded. The structure was assumed to represent the fluid filled early conceptus i.e. embryonic vesicle. The presence of embryo was confirmed based on defined intrauterine echogenic (white) structure. Subsequent viability and development of embryo was assessed on follow up ultrasonic observation. After posterior part of uterus was viewed, the transducer was rotated 45° to 90° clockwise and counter clockwise to locate the ovaries. Follicles were counted, measured and classified according to Abdoon *et al*. (2001) as small (< 4.0 mm), medium (5–8 mm) and large (> 8mm).

Blood collection and hormonal assay by RIA: Blood samples were collected through jugular venipuncture from the experimental buffaloes on day 0, first and second month for estimation of progesterone and estradiol-17ß. Blood samples were kept in slanting position for 6–8 h and centrifuged at 2000×g for 15 min to harvest serum and stored at –20°C till assay. The progesterone and estradiol-17ß were estimated in serum using RIA kit. For estradiol analytical sensitivity of the kit was <0.6 pg/ml; the intra- and inter-assay coefficients of variation were 12.1% and 11.2%, respectively. For progesterone analytical sensitivity of the kit was 0.05 ng/ml; the intra- and inter-assay coefficients of variation were 5.8 and 9.0%, respectively.

Statistical analysis: Data for follicular population and diameter and hormonal profile between groups and days within groups were evaluated by using paired t-test and Student's t-test, respectively (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

The detection of embryonic vesicle (the amnion containing conceptus and amniotic fluid) were recorded on day 20 onwards in the present study. The embryonic vesicle identified as non-echogenic structure (black) through ultrasound scanning. In the present study, the embryonic vesicle was accessible only during the first 60 days of gestation, as by the progress of pregnancy, it became too long to be presented efficiently on the screen. The mean diameter of embryonic vesicle diameter (EVD) was 0.52±0.12, 1.56±0.16, 2.94±0.07, 3.80±0.14 and 6.37±0.15 on day 20, 30, 40, 50 and 60 of pregnancy, respectively. The mean diameter of EVD was significantly (P<0.01) greater on days 30, 40, 50 and 60 of pregnancy. Our result is in agreement with others on buffaloes (Pawshe *et al*. 1994, Ali and Fahmy 2008) and cattle (Rosiles *et al.* 2005). The findings of EVD in the present study on day 40 was higher as compared to cattle (2.94±0.07 vs 2.50±0.36; Rosiles *et al.* 2005) which might be due to rapid development of buffalo embryo than the cattle (Karaivanov *et al.* 1987).

The small, medium, large and total mean number of follicles during early pregnancy in buffalo are shown in Table 1. There was no significant difference in small follicle on different days of early pregnancy. However, the mean number of medium, large and total follicles were significantly (P<0.05) lower on days 30, 40, 50 and 60 than day 20 of pregnancy indicating reduction in the follicular population with the advancement of pregnancy. This might be due to increased rate of follicular atresia during pregnancy. Furthermore, continuous progesterone exposure during pregnancy has negative effect on follicular development and suppressing the diameter of dominant follicle (Martin *et al*. 2008). Our result is in accordance with the findings of Taylor and Rajamahendran (1990) who also reported the higher number of total follicles at an early pregnancy (day 19 to 24), thereafter, it reduced with advancement of pregnancy.

The mean diameters of small, medium and large follicle (cm) during early pregnancy in buffaloes are shown in Table 2. There was no significant difference in the diameter of small and medium follicles on different days of pregnancy. The diameter of large follicle, however, significantly

Table 1. Mean number of small, medium, large and total follicles during different days of early pregnancy in buffaloes (n=6)

Category of follicle	Days				
	20	30	40	50	60
Small	4.16±0.16	4.33±0.42	4.80±0.59	5.00±0.93	4.16±0.94
Medium	1.80±0.16^{a}	1.33±0.49^{a}	0.67±0.33^{b}	2.00±0.44^{a}	1.50±0.34^{a}
Large	1.00±0.25^{a}	0.51±0.22^{b}	0.50±0.22^{b}	0.66±0.21^{b}	0.16±0.16^{b}
Total	7.00±0.25^{a}	6.16±0.47^{b}	6.00±1.32^{b}	6.00±0.77^{a}	5.83±0.90^{b}

a, b, Mean±SE with different superscript in a row differ significantly (P<0.05).

Table 2. Mean diameter of small, medium and large follicles during different days of early of pregnancy in buffaloes (n=6)

Category of follicle	Days				
	20	30	40	50	60
Small	0.40±0.01	0.43±0.01	0.44±0.01	0.42±0.02	0.44±0.09
Medium	0.72±0.02	0.69±0.02	0.72±0.02	0.69±0.02	0.69±0.03
Large	1.39±0.13^{a}	1.01±0.15	0.92±0.09	0.87±0.06^{b}	0.89±0.07^{b}

a, b, Mean±SE with different superscript in a row differ significantly (P<0.05).

($P<0.05$) decreased with advancement of the pregnancy. The findings are in accordance with Baruselli *et al*. (1997) who also observed that the dominant follicles during pregnancy were smaller than those described for the first dominant follicle and the ovulatory follicle, respectively, in 2 and 3 waves estrous cycles in buffaloes. Probably, the high levels of progesterone reduced the maximum size of the dominant follicle during buffalo pregnancy (Martin *et al*. 2008).

In pregnant cows, the wave like pattern of follicular growth continued through the first 60 days of pregnancy. Each wave resulted in a large dominant follicle corresponding to the mid-cycle and ovulatory follicles of a normal estrous cycle. Guilbault *et al*. (1986) showed that after the first 20 days of pregnancy follicular growth slowed, the size of largest follicle decreased and CL has a negative effect on follicular growth on ipsilateral ovary. Martin *et al*. (2008) suggested that growth of dominant follicle does not slow, but that dominant follicle after 20 days of pregnancy are smaller than those observed during estrus. The presence of a conceptus promotes a rapid turnover of small to medium follicles and then limits further growth by increasing atresia. In addition, it was suggested that one of the major differences between pregnant and non pregnant cows with regard to ovarian function, is the absence of a large ovulatory follicle in pregnant animals (Thatcher *et al*. 1988).

The progesterone levels fluctuated between 2.52±0.26 and 3.12±0.44 ng/ml until third month of pregnancy. The mean progesterone concentration in buffalo during early pregnancy continued to increase on day 22 onwards (Terzano *et al*. 2012). The mean serum estradiol-17ß was significantly higher on day 0 (12.57±0.85) and reduced into basal level (6.45±0.34- first month, 8.65±0.33- second month) and remained low throughout the study period. The present findings are in accordance with various authors reported in cattle (Patel *et al*. 2009) and buffalo (Batra *et al*. 1982). In conclusion, the follicular growth is a continuous process in early pregnancy (day 20 to 60) and subsequent development and atresia of dominant follicle without ovulation is characteristic due to progesterone dominance during pregnancy in buffaloes.

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