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Morphological and functional characterization of corpus luteum during different stages of estrous cycle in buffalo

H B RAKESH[1], S K SINGH[2], G CHETHAN SHARMA [3], N JESSIEHUN[4] and S K AGARWAL[5]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

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ABSTRACT

The objective of the present investigation was to study morphological changes in corpus luteum and luteal progesterone content during different stages of estrous cycle in buffalo (*Bubalus bubalis*). Uterus along with the ovaries were procured from the local slaughter house and classified into 4 stages; early (stage I, 1 to 5 days), mid (stage II, 6 to 11 days), late luteal phase (stage III, 12 to 16 days) and follicular phase (stage IV, 17 to 20 days) of estrous cycle based on morphological characteristics of corpus luteum and presence of surface follicles on the ovary. Dimensions of the corpus luteum gradually increased with its growth and significant peak values were observed at stage III followed by a decrease at stage IV of estrous cycle, suggesting regression of corpus luteum. The mean progesterone concentration per gram and content per corpus luteum significantly increased from stage I to III of estrous cycle with a peak at stage III and thereafter, it decreased at stage IV. The present study suggested gradual increase in dimension of CL and luteal progesterone contents with the growth of corpus luteum.

Key words: Buffalo, Corpus luteum, Dimension, Morphology, Progesterone

The corpus luteum (CL) plays a central role in the regulation of estrous cycle and maintenance of pregnancy (Niswender *et al*. 2000). These functions are carried out largely by progesterone, which is the principal steroid hormone synthesized by this transient endocrine gland (Stocco *et al*. 2007). Granulosa and theca cells of the dominant follicle following ovulation undergo morphological changes to form CL. In mammals, CL is composed mainly of steroidogenic and non-steroidogenic cells (O'Shea *et al*. 1989, Fields and Fields 1996) responsible for production of progesterone. Size of CL and secretion of progesterone increase until the mid-luteal phase of the estrous cycle, where both CL size and progesterone reach plateau (McCracken *et al*. 1999). During the luteal phase, progesterone down regulates estrogen receptors and restricts luteinizing hormone and follicle stimulating hormone secretion to a low frequency pulses through a negative feedback on gonadotropin releasing hormone in the hypothalamus (Niswender *et al*. 2000).

Luteolysis is initiated by endometrial $PGF_{2\alpha}$ released between day 15 and 17 of the cycle (Arosh *et al*. 2002). Oxytocin from the CL and posterior pituitary and $PGF_{2\alpha}$ from the uterus are involved in a positive feedback loop to cause luteolysis (McCracken *et al*. 1999). Buffalo CL is not well projected over the surface of ovary as observed in cattle thus many times it is difficult to palpate CL accurately for the diagnostic purposes. Factors that regulate CL function during estrous cycle are not clearly understood particularly in buffalo. Therefore, relationship between dimension of corpus luteum and its progesterone content would be valuable in understanding corpus luteum function and its maintenance. The objective of the present study was to study morphological characteristics of corpus luteum in association with the luteal progesterone content during different stages of estrous cycle in buffalo (*Bubalus bubalis*).

MATERIALS AND METHODS

Apparently healthy female buffalo uterus (48) along with ovaries collected from a local slaughter house, transported to the laboratory on ice within 30 min and were categorized into early (stage I, 1 to 5 days, n=12), mid (stage II, 6 to 11 days, n=12), late luteal phase (stage III, 12 to 16 days, n=12) and follicular phase (stage IV, 17 to 20 days, n=12) as per Ireland *et al.* (1980).

Morphology and progesterone content of corpus luteum: The morphological characteristics of CL such as colour, consistency, vasculature, length, width and weight were recorded. Luteal tissues of different stages were stored in

Present address: [1, 3, 4]Research scholar (drrakeshhb@gmail.com, chetu_vet@yahoo.com, jessie.vet@gmail.com), [2]Senior Scientist, (singhsanjayk69@gmail.com). [5]Director, CIRG, Makhdoom (skadr@rediffmail.com).

PBS at –20°C for progesterone assay. Before going for assay, tissue was thawed and triturated using pestle and mortar. The CL triturated fluid was diluted with PBS in a ratio of 1: 10 and 50µl of this fluid was taken for estimation of progesterone by radio-immuno assay kit (Baithalu *et al.* 2011).

Statistical analysis: Numerical data are presented as mean±SEM. The statistical analysis was carried out using SPSS version 16.0 software and data was analyzed by one-way ANOVA to compare the differences between the stages.

RESULTS AND DISCUSSION

Morphological appearances of CL at different stages of estrous cycle are depicted in the Fig. 1. The colour of CL at stage I was observed as bright red, very soft in consistency, slightly protruded from the surface of the ovary and there was neither cavity at the apex nor crown formation. At stage II, colour of the apex part of CL was reddish with flesh coloured body and soft in consistency, however, at stage III the colour of CL (both apex and body) was flesh coloured and compact in consistency along with well formed crown and cavity at the apex. At stage IV, colour of CL was yellowish-white with firm consistency, started sinking into ovary and vascularity reduced to almost nil indicating regression of CL. The dimension of CL gradually increased with the advancement of stage of estrous cycle and peak values were observed at stage III of estrous cycle (Table 1). Length, width and weight of CL were significantly higher (P<0.01) at stage III than the other stages. The mean length, width and weight of CL at stage III and IV of luteal phase were 1.58±0.09 cm, 1.45±0.06 cm, 1.62±0.11 g vs. 1.45±0.08 cm, 0.92±0.06 cm, 0.97±0.05 g, respectively, demonstrating a declining trend from stage III to IV, which is indicative of the process of CL regression. Overall dimensions and weight of the CL as described in the present study coincides with the observations made by Luktuke and Rao (1962) and Baithalu *et al.* (2011), but lower than that of El-Wishy *et al.* (1988) in buffalo which might be due to difference in breed. The dimensions and weight of cyclic CL observed in the present study are lower than reported in cattle demonstrating species specific differences (Edwards 1962, Ireland *et al.* 1980).

The mean progesterone concentration per gram of CL and per CL at different stages of estrous cycle is given in Table 2. The results indicated significant increase (P<0.01) in progesterone concentration (µg/g of CL) from stage I to III of estrous cycle. Peak values of progesterone were observed at stage III and thereafter, it decreased with regression of CL i.e., at stage IV. Total progesterone contents (µg/CL) also increased up to stage III and thereafter declined (stage IV). Our results demonstrated maximum progesterone secretory ability of corpus luteum at stage III (days 12 to 16). The luteal progesterone concentration coincided with the increase or decrease in weight of CL at different stages of estrous cycle. Progesterone content of CL at different stages of estrous cycle observed in the present study are in accordance with Mittal (1991), Shah and Mehta (1992), Baithalu *et al.* (2011) in buffalo, but lower than values reported by El-Sheikh *et al.* (1967) in Egyptian buffaloes. The present findings of progesterone content per g of CL at different stages of estrous cycle were comparable with cattle (Mares *et al.* 1962), however, the total progesterone content per CL seems to be much lower than the cattle which may be due to species difference regarding the morphology and physiology of corpus luteum.

It may, therefore, be concluded that with the advancement of stage of estrous cycle the dimension of CL increased, peak values were obtained at stage III of the cycle and thereafter decreased with the regression of CL. The luteal progesterone

Table 1. Dimension of corpora lutea at different stages of estrous cycle in buffalo

Parameters	Stages of estrous cycle				Overall mean
	Stage In=12	Stage IIn=12	Stage IIIn=12	Stage IVn=12	
Length (cm)	1.02±0.08 [a]	1.50±0.20 [b*]	1.58±0.09 [b]	1.45±0.08 [b*]	1.39±0.07
Width (cm)	0.85±014 [a]	1.28±0.09 [b*]	1.45±0.06 [b]	0.92±0.06 [a]	1.13±0.07
Weight (g)	0.69±0.18 [a]	1.32±0.19 [bc]	1.62±0.11 [c]	0.97±0.05 [ab]	1.15±0.10

Mean±SEM bearing different superscript (a, b, c) in a row differ significantly (P<0.01) and *(P<0.05).

Table 2. Progesterone concentration (µg/g) and content (µg/CL) of corpus luteum at different stages of estrous cycle in buffalo

Progesterone (µg)	Stages of estrous cycle			
	Stage I; n=12	Stage II; n=12	Stage III; n=12	Stage IV; n=12
P_4/g of CL	19.07±0.78 [b]	28.62±1.41 [c]	39.83±1.26 [d]	12.33±1.77 [a]
P_4/CL	12.81±1.54 [a]	31.53±3.01 [b]	52.85±3.12 [c]	9.37±2.59 [a]

Mean±SEM bearing different superscript (a, b, c, d) in a row differ significantly at 1% level (P<0.01).

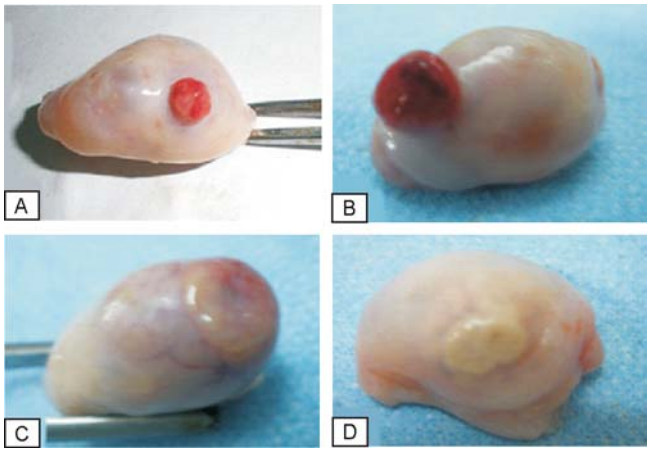

Fig. 1. (A-D). Morphology of corpora lutea at different stages of estrous cycle in buffalo. A. Stage I: Bright red, slightly protruded, highly vascular, no cavity and crown at apex.B. Stage II: Reddish apex, flesh color body, cavity and crown formed at apex. C.Stage III: Flesh colored apex and body, well formed crown and cavity at apex. D.Stage IV: Yellowish-white color, almost nil vascularity.

concentration (µg/g of CL) and its total content (µg/CL) also significantly increased (P<0.01) from stage I to III of estrous cycle and thereafter decreased indicating maximum progesterone secretory ability at stage III (days 12 to 16). The increase and decrease in luteal progesterone concentration coincided with the weight of CL at different stages of estrous cycle.

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