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MACROSCOPIC MORPHOLOGICAL ANALYSIS OF DIFFERENT STAGES OF CYCLIC CORPUS LUTEUM IN INDIAN BUFFALO (*Bubalus bubalis*)

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ABSTRACT

The current study was undertaken with the aim to characterize the macroscopic morphological and morphometrical features of cyclic corpus luteum (CL; n=40) and CL of pregnancy (n=10) in buffalo. The four stages of cyclic CL were interpreted after ovarian analysis i.e., c olor, consistency, vasculature of CL, number and size of follicles into early (Stage I, 1 to 5 days, n=10), mid (Stage II, 6 to 11 days, n=10), late luteal phase (Stage III, 12 to 16 days, n=10) and follicular phase (Stage IV, 17 to 20 days, n=10). In Stage I, it was slightly protruded from the surface of ovary, bloody in appearance due to increased blood congestion, soft in consistency and termed as corpus haemorrhagicum. In Stage II, initially CL was bright red in color, later fleshy in color and soft in consistency. In Stage III, it was shrunken to great extent and pale yellow to creamish in color due to reduced vascularity. At Stage IV it was shrunken and rigid; texture became firmer, completely condensed into small whitish in color due to complete loss of vascularity. It varied in size and weight as well during the varying stages of estrus cycle depicting changes in its morphology. Therefore, by recording the macroscopic observations on cyclic CL and CL of pregnancy, it was further characterized into different stages.

Keywords: *Bubalus bubalis*, buffaloes, cyclic, corpus luteum, gross, morphology, pregnant

INTRODUCTION

Corpus luteum (CL) is a sole endocrine organ with a restricted or short existence that regulates numerous crucial functions in the reproductive processes explicitly estrous cycle, implantation of embryo (blastocyst), embryo persistence and maintenance of pregnancy (Niswender *et al*., 2000). CL is formed on the wall of Graafian follicle after ovulation and it progresses by extensive tissue remodelling, angiogenesis, cellular growth and differentiation during post-ovulation (Mishra *et al*., 2018). It synthesizes and secretes a varied range of hormones, the principle

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of which is progesterone (P4) in several domestic animals comprising buffalo (Zain and Omar, 2001; Kapoor *et al*., 2020). Progesterone regulates the duration of estrous cycle, relaxing of myometrium, stimulation of proliferation of endometrial cells and maintenance of conditions in uterus for development of the fetus (Miranda-Moura *et al*., 2016). The normal development of CL and its ability to synthesize progesterone, growth factors, angiogenic factors and vasoactive compounds depend on its vascularization and blood supply, although the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are the chief sources for the development and maintenance of dense network of neo-formed capillaries (Acosta and Miyamoto, 2004). Additionally, they also contribute in a paracrine and autocrine way in the production of progesterone, which should be sustained in appropriate levels to attain an efficacious reproduction in mammals (Needle *et al*., 2007).

The morphometry of reproductive organs is very crucial since the variations reflect the dynamic characteristics of the ovarian structure triggering the organ´s gametogenic and steroidogenic functions. Several reports have been published on changes occurring in the ovarian gross morphology during the estrous cycle. However, the description about macroscopic changes occurring in the CL during different stages of estrous cycle and pregnancy in Indian buffalo is still lacking. The morphological analysis of these changes during its evolution from formation to regression is essential. A detailed qualitative and quantitative investigation of the macroscopic changes in CL of buffalo was carried out in the present study with the aim to form a valuable basis for identifying the phases of development in cyclic CL which will serve as basic guide to facilitate further investigations in this arena.

MATERIALS AND METHODS

Collection of samples

The present study was conducted on buffalo corpus luteum (n=40) dissected from its ovaries. The ovaries at different stages of the estrus cycle were collected and dissected free from adherent tissue from reproductive system of buffaloes slaughtered at different abattoirs.

Macroscopic morphological observations

Immediately after collection, ovaries were washed in normal saline and categorized into four groups. The grouping was done according to macroscopic analysis of their appearance and morphological characteristics. The four stages of CL decoded after ovarian analysis i.e., color, consistency, vasculature of CL, number and size of follicles (Figure 1). They were categorized into early (Stage I, 1 to 5 days, n=10), mid (Stage II, 6 to 11 days, n=10), late luteal phase (Stage III, 12 to 16 days, n=10) and follicular phase (Stage IV, 17 to 20 days, n=10) (Ireland *et al*., 1980).

Successively, the CL was dissected out completely from the ovaries and the gross observations and measurements were recorded i.e., color, consistency, diameter and weight. The data obtained was statistically analyzed to correlate the observations recorded.

RESULTS AND DISCUSSIONS

Colour

The early luteal phase CL (Stage I) i.e., the developing CL in its earliest phase of development

was somewhat protruded from the surface of the ovary which was bloody in appearance and soft in consistency. The bloody appearance observed at this stage was due to significantly increased blood congestion in the developing CL. Thus, this stage CL was termed as Corpus Haemorrhagicum (Figure 2a). The most congested part of the CL at this stage was its tip that protruded from the surface of ovary. This area had grossly apparent blood congestion and superficially visible micro capillaries as well (Figure 2a and 2c). However, when the CL was cut in longitudinal section, it was observed as pinkish red in color (Figure 2b). Moreover, its center had a cavity like a crater which was filled with blood and developing connective tissue filled clot like region (Figure 2c and 2d). These results were in accordance with the inferences of several other authors as well in buffaloes (El-Sheikh *et al*., 1967; Chandrahasan and Rajasekaran, 2004; Thangeval and Nayeem, 2004; Roy *et al*., 2006). However, Rakesh *et al*. (2013) reported that there was neither cavity at the apex nor crown formation at this stage in buffaloes.

At mid luteal phase (Stage II), initially the CL developed apex that protruded clearly from the surface of the ovary and was bright red in color (Figure 3a). Besides, when it was sectioned longitudinally, the parenchyma was fleshy in color and soft in consistency. Conversely, Kumar and Sharma (2015) reported that luteal phase CL was hard in consistency in Jaffrabadi buffaloes. The inside surface was slightly lobulated in appearance grossly because of connective tissue septa that divided the parenchymatous tissue in lobules established histologically as well (Figure 3b). A little later in this phase of CL, the protrusion increased in size and thus developed into crown like grossly appreciable structure. The protruded crown had a central cavity at its apex that was clearly discernible. Also, the area below the crown formed a neck like region and the part embedded inside the ovary constituted the body of CL (Figure 3c). In the longitudinally cut-section, embedded part was fleshy and yellowish to orange, brown in color at this stage of the estrus cycle (Figure 3d). Similar observations were made by Rakesh *et al*. (2013) in buffaloes.

The CL of late luteal phase (Stage III) was shrunken to great extent and was pale yellow to creamish in color due to reduced vascularity as the process of regression was already started at this stage (Figure 4a). The findings regarding the color and consistency were analogous to those made by Yadav *et al*. (2002); Chandrahasan and Rajasekaran (2004); Ghosh and Mondal (2006); Roy *et al*., 2006; Rakesh *et al*. (2013) in buffaloes. Consistency was observed to be firm at this stage. In cut-section, the CL at this stage still had slight lobulations grossly (Figure 4b).

At Stage IV i.e., the regressing stage, color of the CL was creamy to white whereas its texture became firmer, compact, shrunken and rigid (Figure 4c). It was completely condensed into a very small whitish area within the parenchyma of ovary following regression due to complete loss of vascularity (Figure 4d). Moreover, this pattern of CL regression associated with color was reported by many authors in different species also i.e., sheep (Nimunkar, 1999), cow (O'Shea *et al*., 1989) and buffalo (El-Sheikh *et al*., 1967; Roy *et al*., 2006; Jaglan, 2008; Rakesh *et al*., 2013) etc.

CL of pregnancy appeared to be larger than the luteal phase CL grossly and the color was bright red at the apex and fleshy to brown for rest of the parenchyma that constituted the body of CL of pregnancy (Figure 5a). Singh (1994) observed a similar gross appearance in the CL of early pregnancy. It was frequently observed as

completely embedded within the ovary. However, in some cases of early pregnancy CL, it was slightly protruded from the surface of ovary and rest of the proliferating parenchyma was embedded within the ovary (Figure 5a and 5b). As the CL progressed from early to mid-gestational CL and later especially, it characteristically occupied more or less the entire ovary and there was no protrusion on the surface (Figure 5c and 5d). This was a peculiarity observed in CL of pregnancy explicitly in buffaloes. Pathak and Bansal (2015) also observed that during the pregnancy, the ovary of buffalo was transformed as a whole into the CL with the yellowish luteal tissue occupying most of the ovarian stroma.

Size

The CL varied in size along with the varying stages of estrus cycle. The mean diameter of CL at the earliest part of Stage I i.e., corpus haemorrhagicum was 0.73±0.2 cm (Table 1 and Figure 6). However, as the CL growth increased, it was shifted towards the luteal phase (Stage II) and the mean diameter was recorded to be 1.84±0.65 cm (Table 1 and Figure 6). Srikandakumar *et al.* (2001) reported the average diameter of CL on ovary of Arabian camel as 8.92±0.54 mm. At Stage III, i.e., the late luteal CL had started shrinking and there was a decrease in mean diameter which was recorded as 1.12±0.42 cm (Table 1 and Figure 6). The size of the regressed CL of Stage IV, the mean diameter of corpus albicans was greatly reduced and was 0.82±0.35 cm. Similarly, Jaglan (2008) observed the dimensions of CL in buffalo and stated that the size of CL increased significantly from Stage I (5.59 mm) to Stage II (10.98 mm), at Stage III (14.47 mm) and the size of CL finally decreased ($P<0.001$) markedly at Stage IV (7.87 mm) with the process of CL regression. According to him, the rate of increase in the size of CL was 2 times higher between Stages I to Stage II and 1.3 times between Stage II to Stage III. At Stage III, CL reached its maximum size being 2.6 times higher than Stage I and the decrease in size was 1.8 times lower (Stage IV) than its maximum size recorded at Stage III. The CL of early pregnancy had a mean diameter of 1.98±0.72 cm (Table 1 and Figure 6). The mean diameter, however, increased further to 2.4±0.85 cm, when CL progressed towards mid gestation (Table 1 and Figure 6) due to significant proliferation of luteal parenchyma at this stage. Okuda *et al.* (1988) measured the mean largest diameter of fully developed corpus luteum in cyclic cows as 2.5 cm, which increased to 2.6 cm in pregnant animals. Singh *et al.* (1990) reported the diameter of corpus luteum in pregnant buffaloes as 2.0 to 2.5 cm.

Weight

The mean weight of CL at Stage I was observed as 0.79±0.04 g (Table 6 and Figure 1). Subsequently, at mid luteal phase (Stage II), the mean weight of CL was recorded to be 1.81±0.16 g (Table 1 and Figure 6). Later, when the CL progressed towards the late luteal phase, the weight was found to decrease, and the mean weight was reduced to 0.98±0.08 g (Table 1 and Figure 6).

However, when the CL was regressed i.e., at Stage IV, the corpus albicans had mean weight of 0.72±0.13 g (Table 6 and Figure 1). O'Shea *et al.* (1989) measured the luteal weight of the CL in cows and documented it as 3.8±0.8 g. However, in sheep, Farin *et al.* (1986) reported that CL weighed 600 to 700 mg in just a few days. The mean weight recorded at early and mid-gestational period CL were 2.1±0.19 g and 2.6±0.21 g respectively (Table 1 and Figure 6). Similarly, Rakesh *et al.* (2013) recorded the mean weight of buffalo CL in Stage III

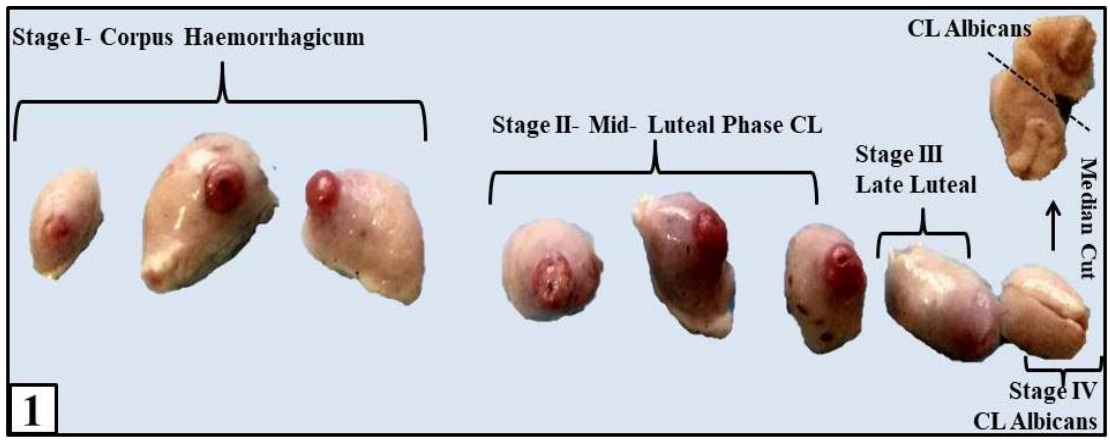

Figure 1. Showing stages of corpus luteum (CL) i.e., early luteal, mid-luteal, late luteal and regressing phase (CL AB) based on macroscopic analysis of buffalo CL.

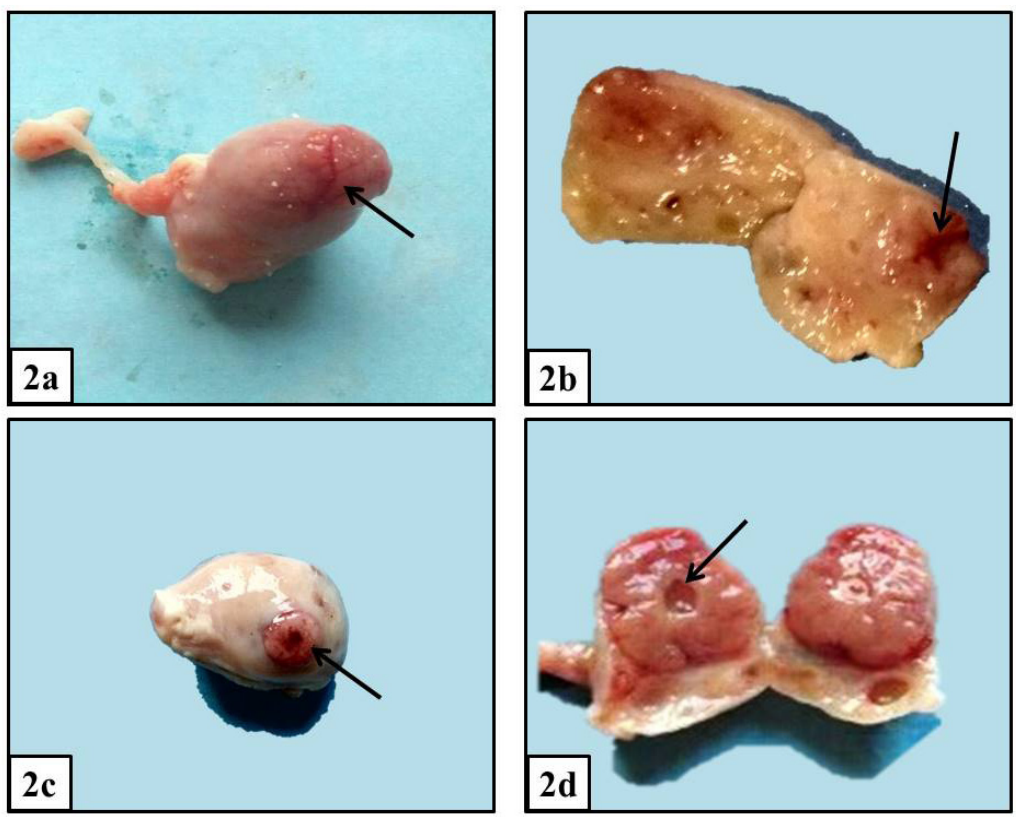

Figure 2. Showing Corpus haemorrhagicum (a) with its congested part protruding from the surface of ovary and superficially visible micro capillaries (arrow). (b) with grossly apparent blood congestion (arrow) that filled the central cavity present (Longitudinal section). (c) central cavity (arrow) or crater with blood congestion (d) with central cavity that shrinked (arrow) and gradually getting filled by developing luteal parenchyma (Longitudinal section).

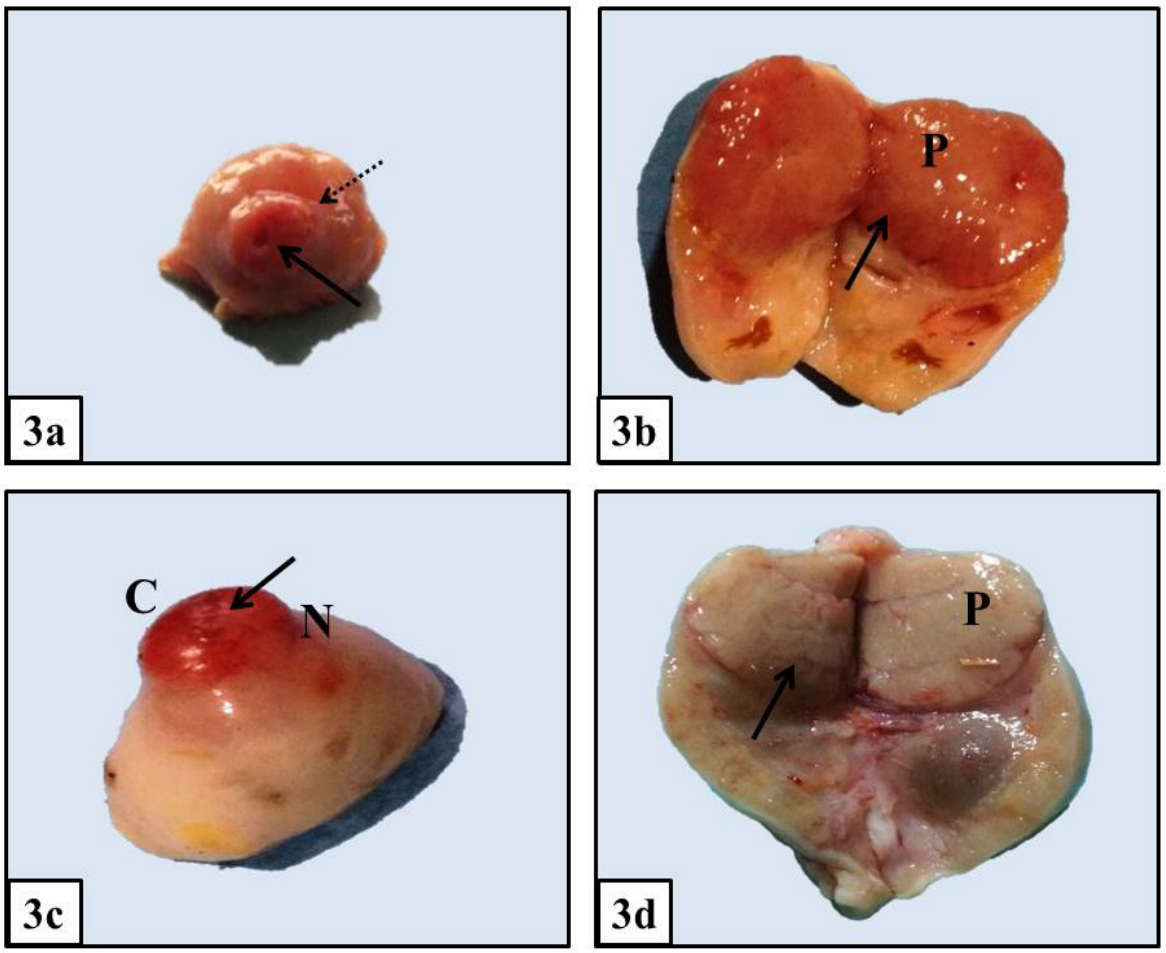

Figure 3. Mid luteal phase corpus luteum showing (a) bright red apex (arrow) that protruded from the surface of the ovary and neck region (dotted arrow) below (b) soft, flesh colored luteal parenchyma (P) with slight lobulations visible in cut section grossly (c) protruded crown (C) with central cavity (arrow) at its apex, neck like region (N) below it and body embedded within the ovary (d) further luteal development into yellowish to orange brown luteal parenchyma (P) with distinct lobulations (arrow) in cut section.

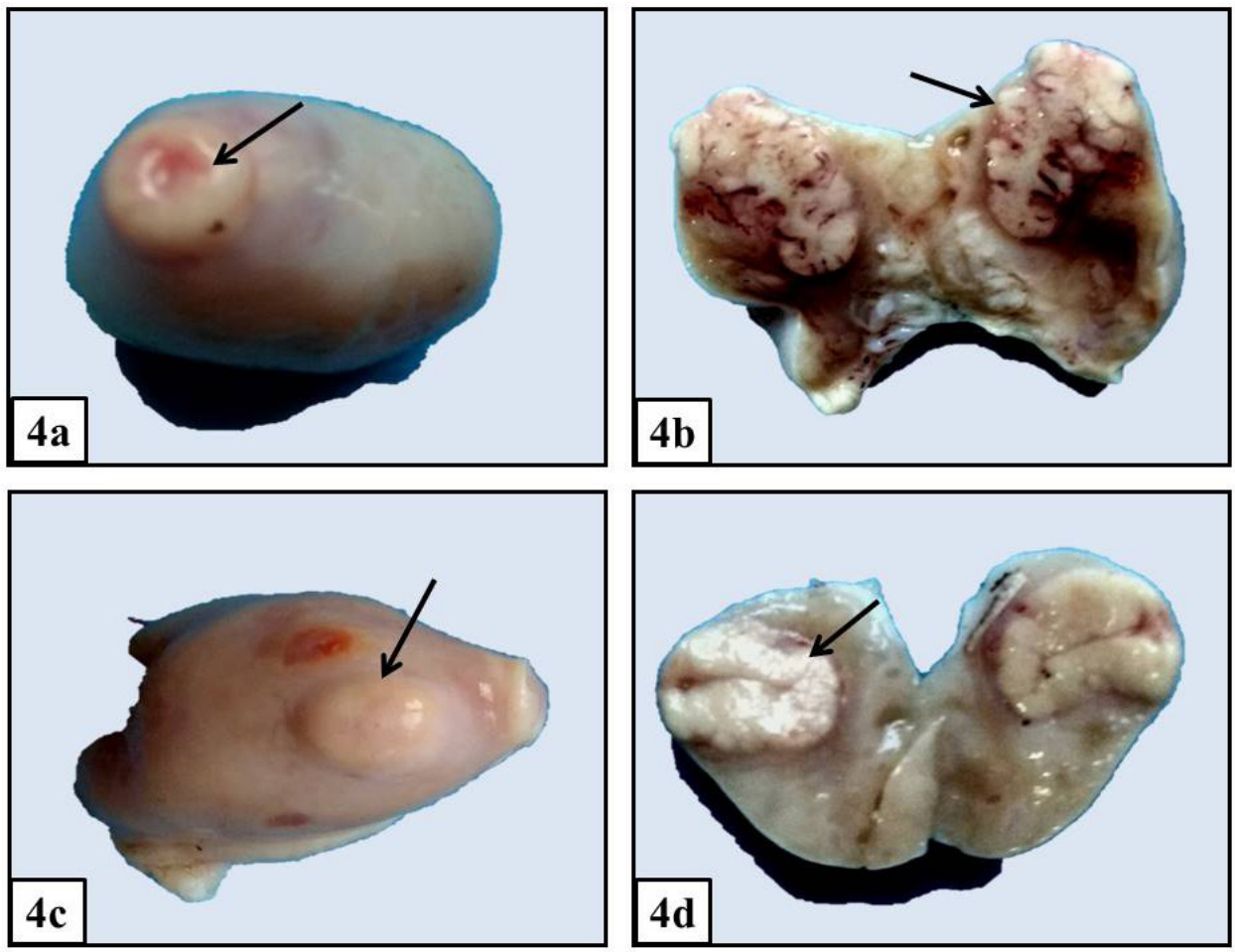

Figure 4. Showing (a) late luteal phase CL with pale yellow to creamish luteal parenchyma shrunken to great extent with central cavity in some (arrow) (b) firm consistency with slight lobulations still visible grossly (Longitudinal cut section) in same phase CL (c) corpus albicans phase CL with firm texture and shrunken parenchyma condensed into small whitish area (arrow) (d) compact and regressed white area (arrow) due to complete loss of vascularity forming scar like tissue in cut section.

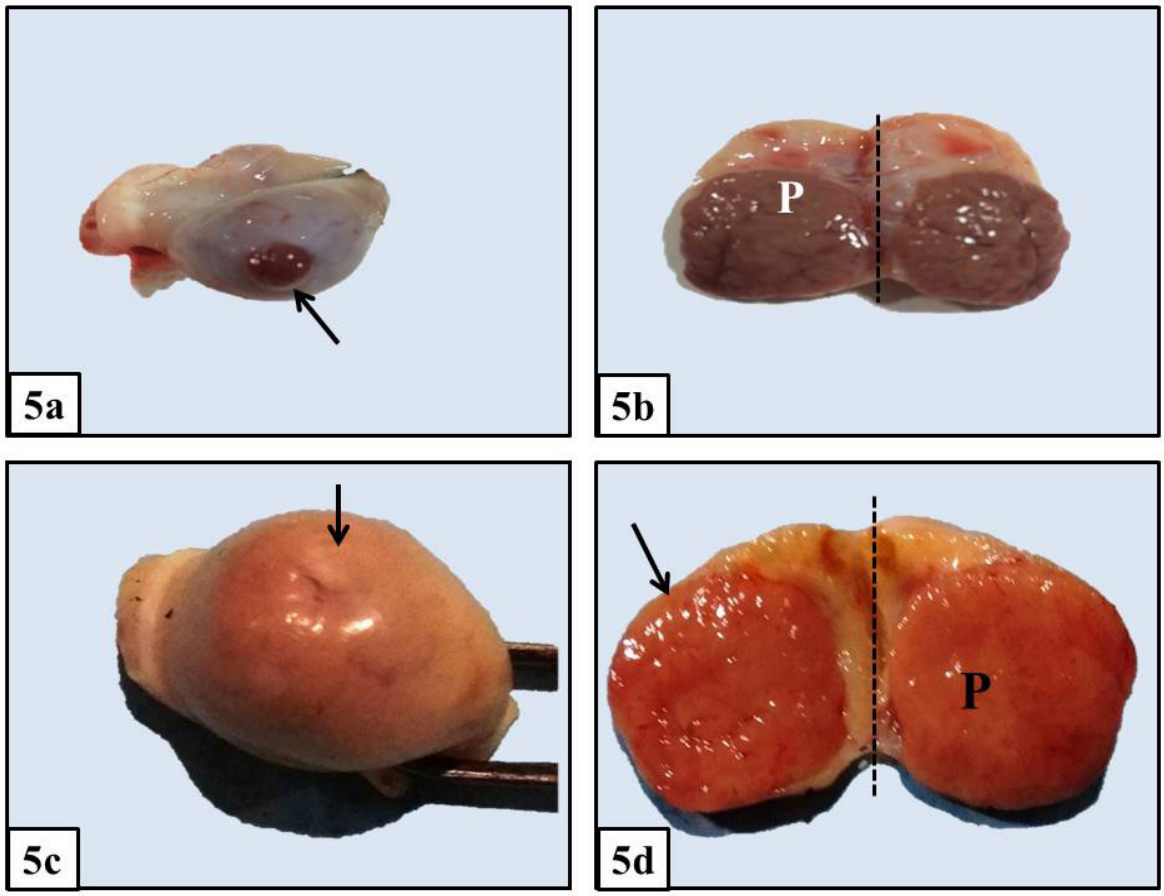

Figure 5. Corpus luteum of pregnancy showing (a) early pregnancy CL with slight protrusion from the surface and most of the body of CL or parenchyma was embedded within the ovary (b) (c) mid pregnancy CL with completely embedded parenchyma within the ovary (arrow) (d) longitudinal section of mid pregnancy CL with its luteal parenchyma (P) occupying almost whole of the ovary.

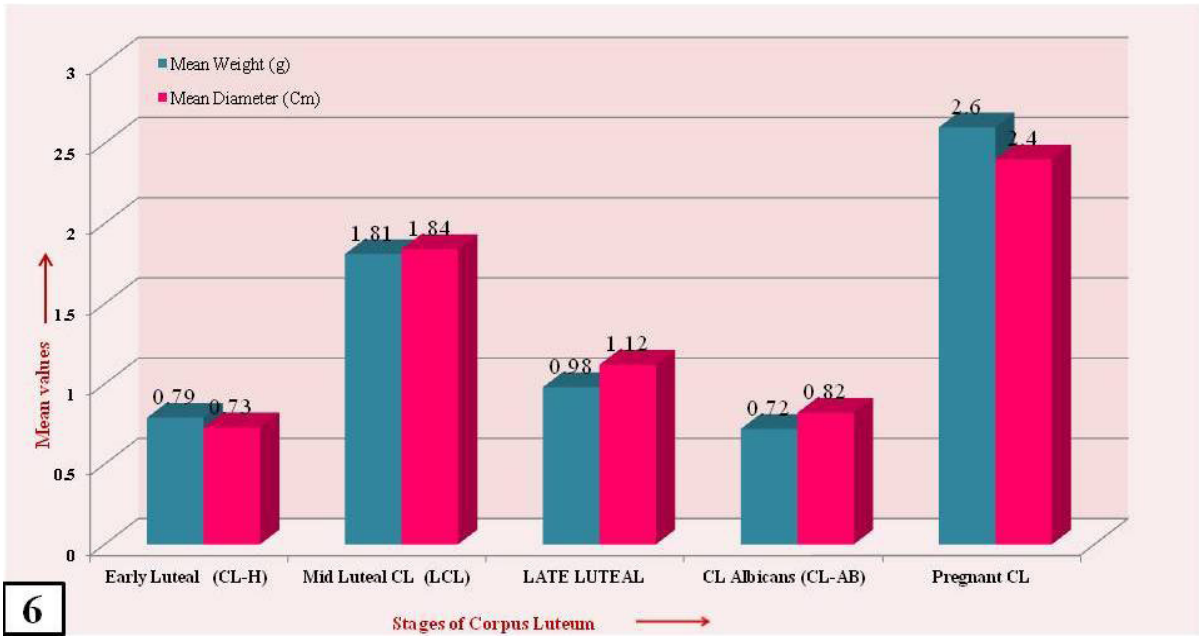

Figure 6. Showing mean weight (g) and diameter (cm) of corpus luteum (CL) at different stages of development in buffalo.

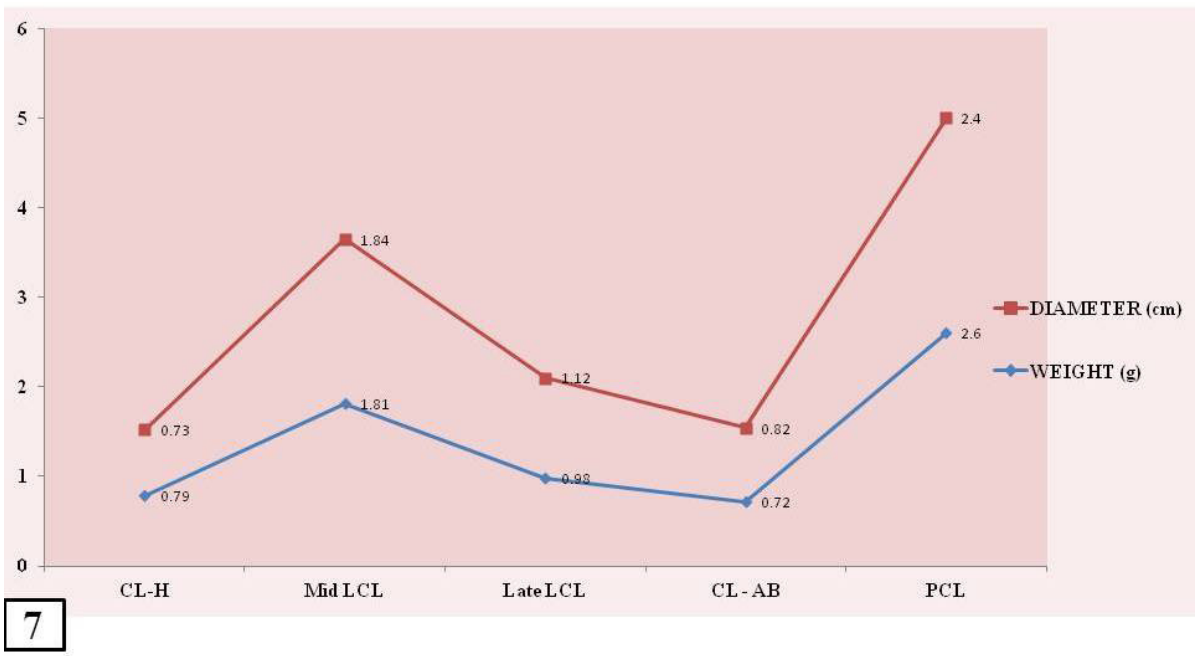

Figure 7. Showing relationship trend between mean weight (g) and diameter (cm) of corpus luteum (CL) at different stages of development.

and IV of luteal phase 1.62±0.11 g and 0.97±0.05 g, respectively.

The quantitative analysis of buffalo CL further revealed that there was a direct positive correlation between size and weight of CL in different phases of its cyclicity and CL of pregnancy as well (Figure 7).

CONCLUSION

The present qualitative analysis revealed that development of CL initiated as an extremely vascular structure due to intense angiogenesis and it progressed to luteal phase which eventually gets regressed to corpus albicans in absence of conception. Most of the changes in the dynamic structure of CL were visible grossly. The quantitative analysis further revealed that there was direct positive correlation between size and weight of CL in different phases of its cyclicity. However, buffalo CL was observed with a peculiarity in CL of pregnancy as it was completely embedded with the parenchyma of ovary. Therefore, these characteristics of buffalo CL make this study imperative for any further studies involving the CL of buffalo.

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