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Lipid Distribution Variations in Different Stages of Cyclic Corpus Luteum of Indian Buffalo

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ABSTRACT

The present study was conducted on corpus luteum of healthy buffaloes ovaries (n = 24) collected from local slaughter house and were categorized into early (stage I, 1 to 5 days, n=6), mid (stage II, 6 to 11 days, n = 6), late luteal phase (stage III, 12 to 16 days, n = 6) and regressing phase (stage IV, 17 to 20 days, n=6). In the earliest phase i.e., corpus haemorrhagicum, the distribution of total lipids was moderate. However, in the early luteal phase, most of the luteal cells had intense staining for presence of total lipids by Sudan Black B and Oil Red O and phospholipids by Acid Hematin. By the mid luteal phase, fewer luteal cells at this stage showed positive staining for presence of lipids. During the mid luteal phase, the less frequent presence of lipid droplets in luteal cells indicated that cholesterol and its esters present at this stage might have been utilized for active synthesis of progesterone. In late luteal phase, the distribution of lipids increased to depict very intense staining. Moreover, in the regressing phase i.e., corpus albicans, the distribution of lipids increased further and was observed to be both intracellular and extracellular depicting the higher accumulation of lipids in the regressing corpus luteum. The increase in the lipid droplets in luteal cells at this stage indicated the poor mobilization of lipids and thus decline in the progesterone synthesis.

Keywords: Lipids, Phospholipids, 3β-HSD, Corpus Luteum, Buffalo

In bovine ovary, following ovulation under the influence of Luteinizing hormone (LH), the granulosa and thecal cells of the ruptured follicles are luteinized and the follicle is transformed into a corpus luteum. The corpus luteum formed is a dynamic endocrine gland that is composed of steroidogenic and non-steroidogenic cells histologically. The main function of the corpus luteum is the production of progesterone. The steroidogenic cells i.e., small and large luteal cells are associated with the production of steroid hormones principally progesterone (Batra and Sharma, 2014). For any steroid producing cell including luteal cells, the initial step for production of progesterone is to obtain the precursor i.e., cholesterol. However, the luteal cells can also produce cholesterol *de novo*. By default, the major mechanisms for obtaining cholesterol are either the endocytosis of cholesterol rich low density lipoprotein (LDL) or the selective uptake of cholesterol esters from high density lipoprotein (HDL) (Gwynne and Strauss, 1982). Christenson and Devoto (2003) reported that progesterone biosynthesis required two important enzymatic steps and it included, first the conversion of cholesterol to pregnenolone, catalyzed by P450 side chain cleavage (P450 scc) located on the inner mitochondrial membrane. This conversion was followed by succeeding conversion to progesterone that was catalyzed by 3β-hydroxysteroid dehydrogenase (3β-HSD) present in the smooth endoplasmic reticulum (SER). Therefore, the present study was designed to study the distribution of lipid and the localization of the 3β-HSD enzyme in luteal parenchyma and their correlation during different stages of development of corpus luteum i.e., formation and structural regression.

MATERIALS AND METHODS

Collection of samples

The samples of corpus luteum of healthy buffaloes ovaries

(n=24) were collected from local slaughter house and were categorized into early (stage I, 1 to 5 days, n=6), mid (stage II, 6 to 11 days, n=6), late luteal phase (stage III, 12 to 16 days, n=6) and regressing phase (stage IV, 17 to 20 days, n=6), based on their gross morphology (Ireland *et al.,* 1980).

Cryosectioning

The fresh unfixed ovaries from buffaloes having different stages of cyclic corpus luteum were immediately collected immediately after slaughter and stored in liquid nitrogen. These corpus luteum tissues collected from the ovaries was subjected to cryostat sectioning at -20°C with cryostat microtome. The sections of 10-12 µm thickness were obtained on clean glass slides and stained with Sudan Black B, Oil Red O and Acid Hematin to study the distribution pattern of lipids and phospholipids in different stages of cyclic corpus luteum i.e., development and regression. The sections obtained on glass slides were incubated with substrate for 3 β-Hydroxy steroid dehydrogenase (3βHSD) enzyme activity (Nitro BT method; Pearse, 1972) and to study the variation in its distribution pattern according to lipid distribution in different stages of cyclic corpus luteum.

RESULTS AND DISCUSSION

The lipid distribution was analyzed histochemically in different stages of development and regression of cyclic corpus luteum. The sudanophilic lipid distribution was observed to be in the form of few small droplets in the luteal cells present at periphery only with central cavity in the earliest part of cyclic corpus luteum i.e., corpus haemorrhagicum. Further, when the corpus luteum development progressed, the lipid distribution was established to be moderate in the developing small and large luteal cells that comprised the luteal parenchyma at this stage (Fig. 1A). However, the distribution of lipids was observed to be more in luteal cells present at periphery as compared to centrally located luteal cells. At this stage, the total lipid distribution was observed to have started accumulating as minute droplets within the cytoplasm of the developing luteal cells and was as intensely stained by Oil Red O (Fig. 1B). Similarly, Al-zi abi *et al.* (2002) observed the intense staining for lipid in the early luteal phase by Oil Red O in mare's corpus luteum. The lipid accumulation indicated the beginning of synthesis of cholesterol by the developing luteal cells as it was a requisite precursor for progesterone production further. Thus, this increased deposition of lipid that started at this stage might be the result of biosynthesis of cholesterol esters and triglycerides by the luteal cells or due to an increased uptake of cholesterol and triglycerides from the blood, which were not being utilized for progesterone biosynthesis (Flint and Armstrong, 1972).

The activity of 3 β-Hydroxy steroid dehydrogenase (3βHSD) enzyme was also observed to be weak to moderate in the developing luteal cells (Fig. 1C). The moderate activity of 3βHSD here indicated the fact that less enzyme is available for conversion of precursor to progesterone at this stage as the luteal cells were developing at this stage. The lipid deposition therefore was observed to as moderate accumulations in the cytoplasm of developing luteal cells in the parenchyma. Similar correlating finding was made by Chatterjee and Greenwald (1976) that the highest concentration of 3βHSD was found on day 1 of the cycle with its gradual decline over the next 3 days in hamster corpus luteum.

The activity of phospholipids was observed to be strong in this stage. It was observed as uniform, strong bluish black deposition within the cytoplasm of the developing luteal cells. However, the peripheral luteal cells (Fig. 1D) were larger and had more phospholipids whereas the center had a little lighter staining for phospholipids being bluish within their cytoplasm (Fig. 2). Weinhouse and Brewer (1942) stated that as the corpus luteum developed after ovulation, the phospholipids increased gradually whereas the cholesterol esters remained constant or decreased slightly.

In the mid luteal period, the distribution of lipid was observed to be present throughout the luteal parenchyma uniformly. The sudanophilic lipid droplets were observed to be present abundantly within the cytoplasm of most of the luteal cells. However, the luteal cells observed within the center had more lipid droplets within the cytoplasm as compared to the luteal cells at periphery. Moreover, the connective tissue septa had nil lipid accumulation (Fig. 3A). Similar pattern of fine lipid droplets accumulation was observed within the cytoplasm of most of the luteal cells by the Oil Red O. However, the overall distribution of total lipids in the form of fine lipid droplets stained by

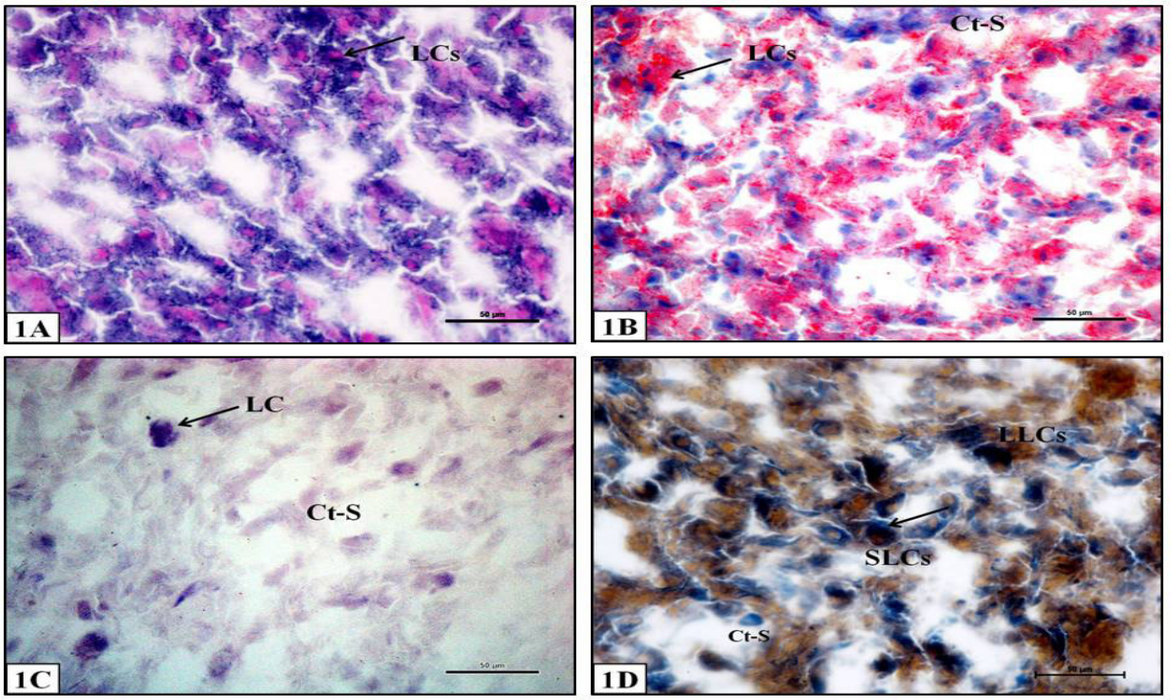

Fig. 1: Photomicrograph of corpus haemorrhagicum showing, (A) moderate lipid accumulations in cytoplasm of developing small and large luteal cells (LCs, arrow). Sudan Black B X400; (B) accumulation of minute lipid droplets (arrow) within the cytoplasm of the developing luteal cells and no lipid in connective tissue septa (Ct-S). Oil Red O X400; (C) weak to moderate 3βHSD activity (arrow) in developing luteal cells (LCs) and weak in connective tissue septa (Ct-S). Nitro BT method X400; (D) bluish black phospholipids deposition within the cytoplasm of the developing small (SLCs) and large luteal cells (LCs, arrow) at periphery. Acid Hematin X400.

Oil Red O was observed to be in lesser luteal cells and was mainly observed in the large luteal cells.

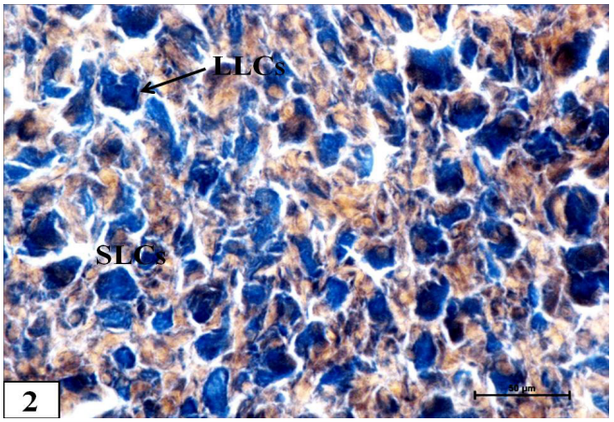

Fig. 2: Photomicrograph of corpus haemorrhagicum showing phospholipids distribution in the luteal cells (arrow) in center of the parenchyma. Acid Hematin X400.

Conversely, the connective tissue elements were devoid of the lipid accumulations (Fig. 3B). Guraya (1966) stated that when the amount of lipid was less, the release of hormone progesterone was occurring.

The localization of 3βHSD enzyme was observed to be strong within the cytoplasm of luteal cells at this stage. The enzyme was observed to be present strongly within the luteal cells located at periphery (Fig. 3C). However, the luteal cells at the center had activity for this enzyme but it was observed to be weak to moderate (Fig. 3D). The expression of 3βHSD enzyme was inversely related to the distribution of total lipids i.e., periphery had more enzyme localization and less lipid distribution and center had lesser enzyme expression and more lipid distribution.

This pattern of expression of 3βHSD and lipids was correlated with its physiological role and thus indicated that lipid distribution was lesser at periphery probably because more 3βHSD enzyme present there converted

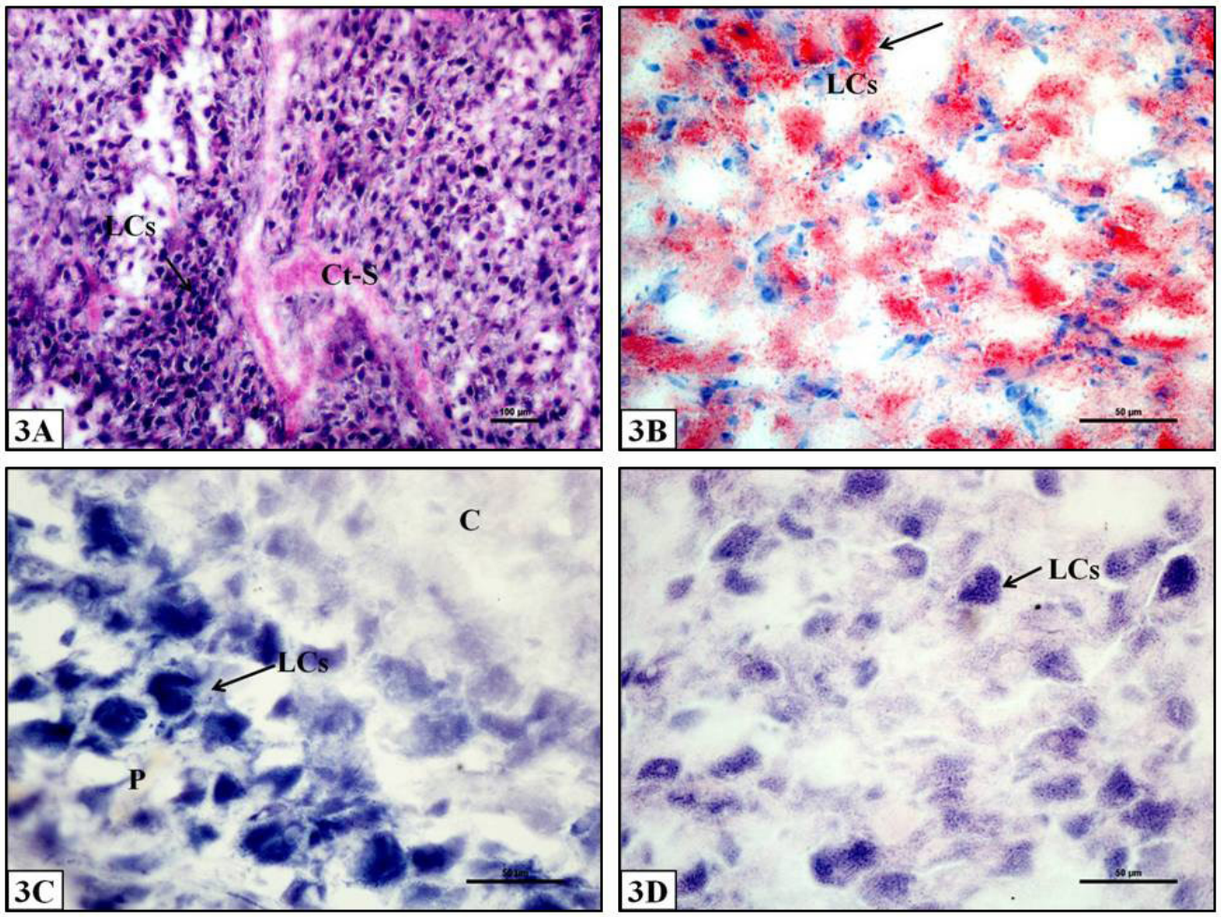

Fig. 3: Photomicrograph of mid luteal corpus luteum showing, (A) abundant sudanophilic lipid accumulated within the cytoplasm of the luteal cells (LCs, arrow) in center as compared to the luteal cells at periphery and nil in septa (Ct-S). Sudan Black B X100. (B) More abundant and fine lipid droplets (arrow) within the luteal cell (LCs) cytoplasm. Oil Red O X400; (C) strong 3βHSD within the luteal cells (LCs, arrow) at periphery (P) and weak in center (C). Nitro BT method X400; (D) weak to moderate 3βHSD within the luteal cells (LCs, arrow) at center. Nitro BT method X400.

the lipids in the form of cholesterol within luteal cells to progesterone. The process of progesterone biosynthesis required two enzymatic steps: firstly the conversion of cholesterol to pregnenolone which was catalyzed by P450 side chain cleavage (P450scc) located on the inner mitochondrial membrane and subsequently the conversion of pregnenolone to progesterone that was catalyzed by 3β-hydroxysteroid dehydrogenase (3β-HSD) present in the smooth endoplasmic reticulum (Fig 4). Similar observations were made by Christenson and Devoto (2003).

The distribution of phospholipids that stained intensely with Acid Hematin increased within the cytoplasm of the luteal cells that constituted the parenchyma in the mid luteal phase. It was observed to be present as strong bluish black fine granules of phospholipids within the cytoplasm of luteal cells present in this phase. However, the phospholipid accumulation was strong both at periphery and center as well (Fig. 5). Similarly, Guraya (1968) reported that the cytoplasm of luteal cells in corpus luteum of American opossum was shown to contain phospholipids in the luteal phase and it was closely associated with the synthesis of steroid hormones.

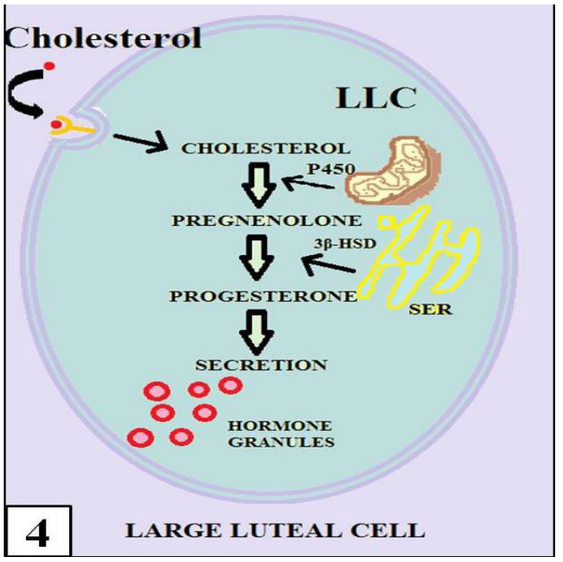

Fig. 4: Schematic diagram showing utilization of lipids within the luteal cell cytoplasm for biosynthesis of hormone progesterone and involvement of the enzyme 3β-HSD in the process of its conversion (*created by first author).

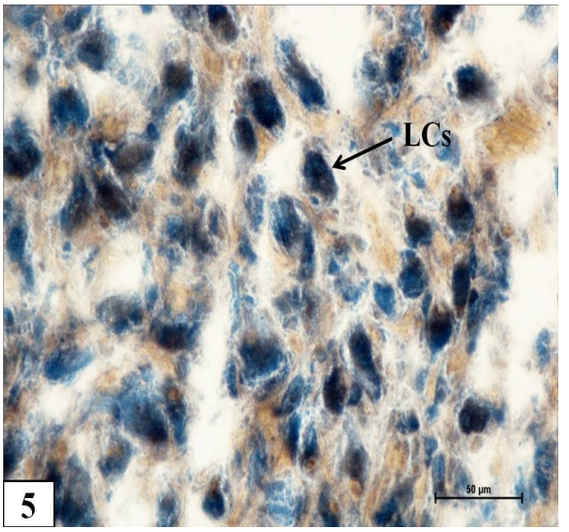

Fig. 5: Photomicrograph of mid luteal phase corpus luteum showing strong phospholipids distribution within the cytoplasm of luteal cells (LCs, arrow) throughout the parenchyma. Acid Hematin X400.

In the late luteal phase, the luteal cells were observed to be degenerating and the lipid accumulation in the form of coarse sudanophillic lipid droplets increased within the degenerating luteal cells (Fig. 6A). The intense staining for sudanophillic lipid droplets was observed both intracellularly and extracellularly in luteal parenchyma at this stage. The intense lipid accumulation in was observed in the increased vacoulations that developed within the regressing luteal cells at this stage. However, no lipid deposition was observed within the endothelial cells of thicker capillaries with increased smooth muscle around them (Fig. 6B). However, the connective tissue septa present at this stage also had mild deposition of lipids within the fibers. The total lipid distribution stained with Oil Red O increased significantly and observed to be present around the lumen of regressing capillaries present at this stage (Fig. 6C). The progressive increase in lipid droplets at this stage indicated that it might be due to increase in cholesterol content that corresponds to its reduced utilization for progesterone synthesis and thus increased accumulation (Quirke *et al*., 2001 and Logan *et al*., 2002).

However, the phospholipids distribution within the luteal cells was mildly reduced in the parenchyma at this stage. Also, the activity of 3β-HSD was observed to be reduced considerably at this stage. Deane *et al.* (1966) reported that there was a decline in of 3β-HSD enzyme activity that could be responsible for elevation in lipid content at this stage. This observation was correlated by the fact that the absence of this enzyme at this stage consequently inhibited the production of progesterone from cholesterol and thus corresponds to the increased level of lipids.

In the corpus albicans phase, the parenchyma was considerably shrunken and the cellularity was reduced. Most of the parenchyma was occupied by intense staining total lipids present at this stage in the form of coarse lipid granule accumulations. The sudanophilic lipid accumulations increased sharply at this stage to such an extent that it formed several small nodular accumulations within the parenchyma around regressing luteal cells and mainly around regressing capillaries and thicker blood vessels (Fig. 6D). Subsequently, moving further the parenchyma was constituted of mainly deposits of lipid accumulations and regressed thicker blood vessels.

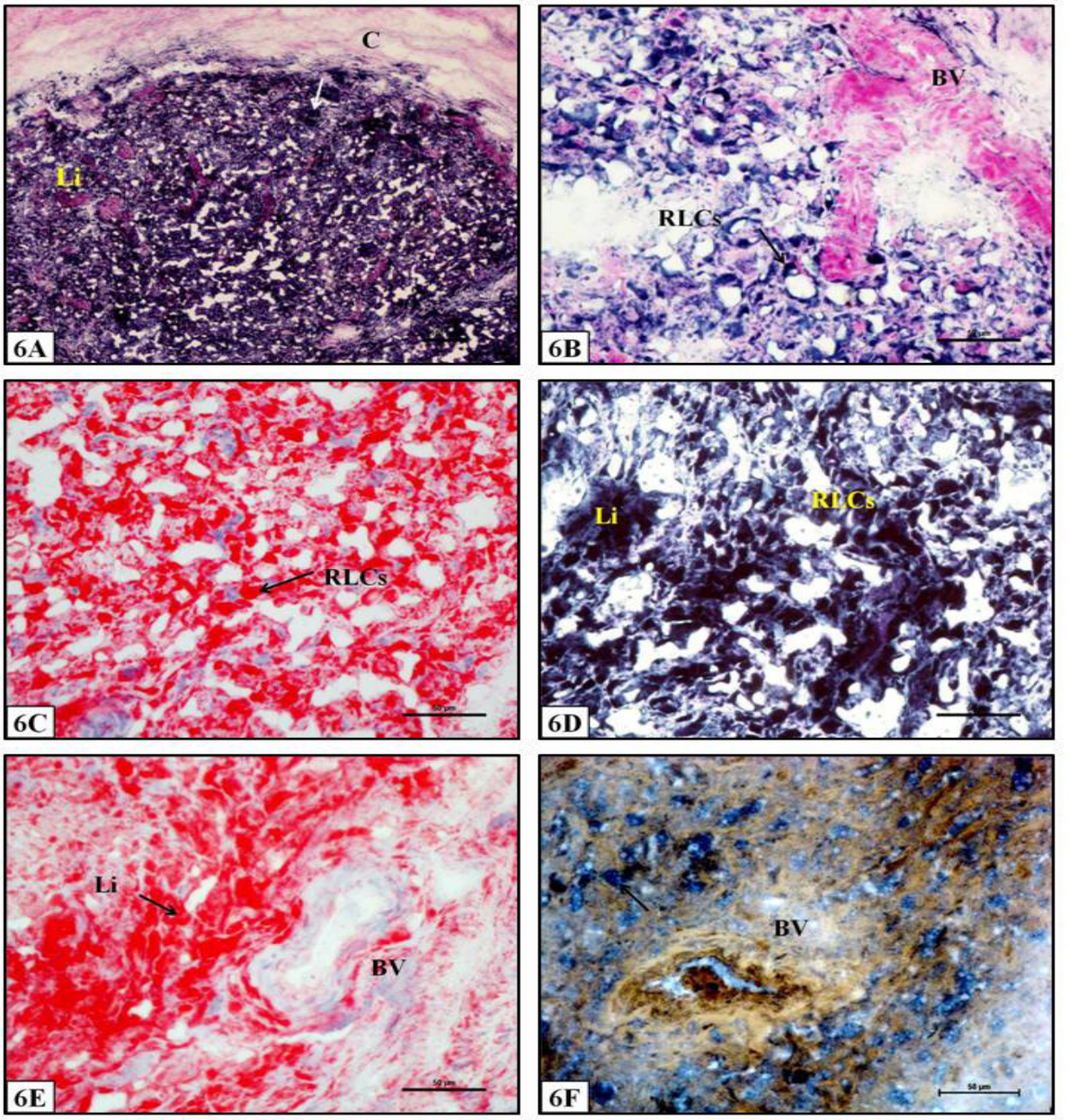

Fig. 6: Photomicrograph of the late luteal phase corpus luteum showing, (A) lipid (Li) accumulation in the form of coarse sudanophillic lipid droplets within the degenerating luteal cells and capsule (C) devoid of it. Sudan Black B X100; (B) intense lipid accumulation in the increased vacoulations (arrow) within the regressing luteal cells (RLCs) and no lipid in thicker capillaries (BV) with increased smooth muscle around them. Sudan Black B X400; (C) abundant lipid droplets (Li, arrow) around the regressing luteal cells (RLCs), lumen of regressing capillaries and mild in connective tissue septa. Oil Red O X400; (D) coarse lipid granules (Li) accumulations around regressing luteal cells (RLCs), capillaries and thicker blood vessels. Sudan Black B X400; (E) Photomicrograph of corpus albicans phase showing intense lipid accumulations (Li, arrow) around prominent blood vessels (BV). Oil Red O X400; (F) reduced phospholipids content in the parenchyma around the regressing luteal cells and around regressing capillaries (BV). Acid Hematin X400.

Moreover, blood vessels had lipid deposits around their outer boundaries (Fig. 6E). Similar observations were made in regressing corpus luteum of sparrow (Guraya and Chalana, 1975), mare (Al-zi abi *et al.,* 2002) and goats (Batra and Sharma, 2014).

The 3β-HSD enzyme activity was observed to be nil in the corpus albicans phase corresponding to its inverse relationship with sharp increase in lipid distribution at this phase. However, the distribution of phospholipids reduced considerably at this stage and was observed to be present in few regressing luteal cells and around few regressing capillaries observed at this stage (Fig. 6F). Several authors stated that with the regression of the corpus luteum, the luteal cells begin to store lipid granules which consisted of cholesterol and cholesterol esters, triglycerides and some phospholipids. Similar distribution of lipids stored in the regressing corpus luteum was reported in corpus luteum of rat (Guraya, 1964) and hamster (Guraya and Greenwald, 1965). The significant increase in accumulation of lipids and decline in progesterone production in regressing corpus luteum was attributed to degeneration in mitochondria and smooth endoplasmic reticulum (SER) (Umo, 1975 and Levine *et al*., 1979). Therefore, the variations in distribution of total lipids and phospholipids were observed within the cyclic corpus luteum during its development and regression and it was inversely related with the activity of 3β-HSD enzyme involved in steroidogenesis.

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