HISTOCHEMICAL OBSERVATIONS ON CORPUS LUTEUM OF INDIAN BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

Histochemical observations were made on corpora lutea of 20 cyclic and 10 pregnant buffaloes. The luteal cells from fully developed corpus luteum of cycle and that of pregnancy were strongly positive for neutral and acidic mucopolysaccharides (NMPS; AMPS), lipids phospholipids and basic proteins, reflecting a better physiological secretory activity. The NMPS and AMPS and basic proteins were comparatively low in the developing and regressing corpus luteum. Calcium deposits were absent in the luteal cells.

INTRODUCTION

The various histochemical moieties play a definite rôle in the secretory activity of gland. The present study was aimed to elucidate the histochemical characters of corpus luteum of cyclic and pregnant Indian buffaloes and to correlate it with its secretory activity. Scanty information is available on histochemical characters of corpus luteum of buffaloes (Singh *et al.*, 1990) but comprehensive details are lacking.

MATERIALS AND METHODS

The corpora lutea of thirty Indian buffaloes were collected from abbatoir. Twenty animals were cyclic and ten pregnant. The corpora lutea were classified in accordance with Okuda *et al.* (1988) into three types based on its physioanatomy viz. developing (n=6), developed (n=7) and regressing (n=7) cyclic corpus luteum. The tissue samples were also collected in the liquid nitrogen at -190°C for cryomicrotomy. The tissue samples were processed for paraffin block preparation by cedar wood oil schedule (Luna, 1968). The paraffin blocks were serially sectioned at 5-7 μm and these sections were used for histochemical staining alongwith control sections as given in Table 1. Cryostat sections of 10-12 μm were used for demonstration of lipids. The observations were recorded from both the peripheral and central part of corpora lutea.

RESULTS AND DISCUSSION

Polysaccharides

The capsule, septae and stroma in the developing and developed cyclic corpora lutea were moderately PAS positive. However, the activity was weak to moderate in regressing corpus luteum. The capsule and stroma were weakly positive for acidic mucopolysaccharides in all groups. The capsule and stroma of corpus luteum of pregnancy were strongly positive for neutral and acid mucopolysaccharides.

The small luteal cells (SLC) and large luteal cells (LLC) were weak to moderately positive for neutral mucopolysaccharides in developing and developed corpus luteum (Fig.1). The activity was weak in regressing corpus luteum. The neutral mucopolysaccharide content of SLC was less as compared to LLC. Acidic mucopolysaccharide activity was weak in luteal cells of all reproductive phases.

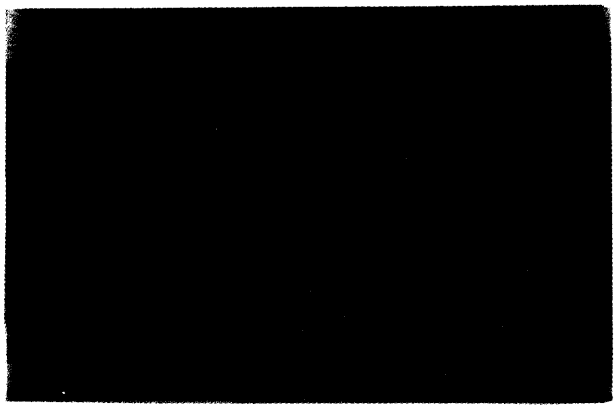

Fig. 1: Developed cyclic corpus luteum of buffalo showing PAS positive reaction in large luteal cell (LLC), small luteal cell (SLC) and blood vessels (BV). PAS Stain, 140 X.

The SLC and LLC were moderately positive for neutral mucopolysaccharides in corpus luteum of

Table 1: Histochemical techniques used in paraffin/cryostat sections of buffalo corpus luteum

Cytoplasmic Inclusion	Method	Source
Neutral mucopolysaccharides (NMPS)	Periodic-Acid Schiff	Sheehan and Hrapchak (1973)
Glycogen	PAS with diastase digestion at pH 6.0	Luna (1968)
Acidic mucopolysaccharides (AMPS)	Alcian blue at pH 2.5	Luna (1968)
Lipids	a. Sudan Black B b. Oil Red O	Chayed *et al.* (1969)
Phospholipids	Acid Haematin	Chayen *et al.* (1969)
Basic proteins	Mercuric Bromphenol blue	Chayen *et al.* (1969)
Calcium	Von Kossa	Luna (1968)

Rable 2: Histochemical observations on cropus luteum of buffalo

Cytoplasmic Inclusion	Stage of corpus luteum							
	Developing		Developed		Regressing		CL of pregnancy	
	SLC	LLC	SLC	LLC	SLC	LLC	SLC	LLC
Neutral mucopolysaccharides	+/++	+/++	+/+	++/+++	+	+	++	++
Glycogen	+/++	+/++	+/+	++/+++	+	+	++	++
Acid mucopolysaccharides	-	+	-	+	+	+	+	+
Lipids	++	++	+++	+++	++++	++++	+++	+++
Phospholipids	+++	+++	+++	+++	++	++	+++	+++
Proteins	+	+	++/+++	++/+++	+	+	++/+++	++/+++

SLC = Small luteal cells - = Negative ++ = Moderate
LLC = Large luteal cells + = Weak +++ = Strong ++++ = Intense

pregnancy whereas some of LLC were weakly positive for acidic mucopolysaccharides (Table 2). The neutral mucopolysaccharide contents of SLC and LLC were confirmed to be glycogen which might be required for energy in precess of synthesis of hormones as suggested by Singh (1975) in cow. Some of the regressed luteal cells showed abundant of granular neutral mucopolysaccharides as reported earlier in luteal cells of buffalo (Goswami, 1985). However, the role of glycogen in regressing corpus luteum could not be ascertained.

Lipids

The Sudan black B and oil red O stained cryostat sections showed that connective tissue components were devoid of any lipid in cyclic as well as in corpus luteum of pregnancy. In the developing corpus luteum it was uniformly distributed in perinuclear area and droplets were fewer and of moderate intensity, whereas SLC and LLC were strongly positive for lipids in developed corpus luteum (Fig. 2 & 3). The lipids droplets were fine to medium, coarse and evenly distributed. The regressing corpus luteum showed increased amount of lipids in luteal cells. The lipid droplets were much coarser and increased in amount than earlier groups.

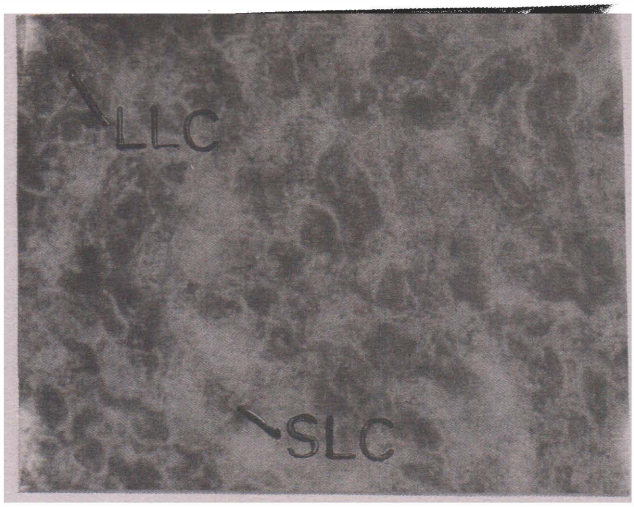

Fig. 2: Cryostat section of developing cyclic corpus luteum of buffalo showing lipid in large luteal cell (LLC) and small luteal cell (SLC). Sudan Black B, 140 X.

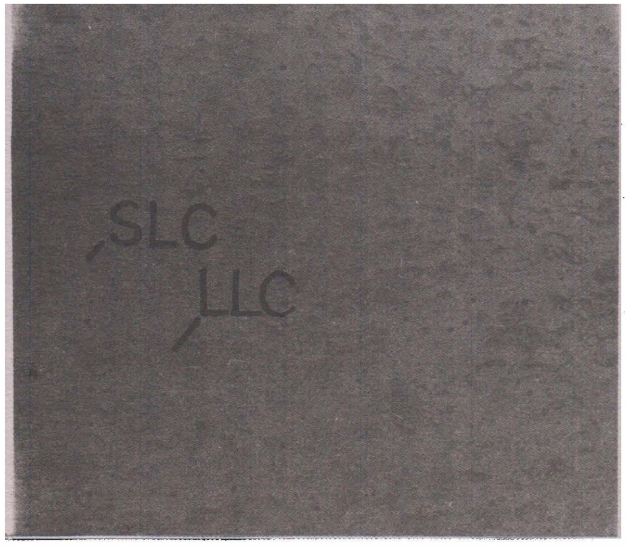

Fig. 3: Cryostat section of corpus luteum of pregnancy showing fine lipid droplets in small luteal cell (SLC) and large luteal cell (LLC). Oil red O, 140 X.

In the corpus luteum of pregnancy large amount of coarse lipid droplets were seen in the perinuclear area of lutein cells. The higher lipids content in developed corpus luteum and that of pregnancy may be related with steroidogenesis whereas the increased accumulation of lipids in regressed luteal cells was mainly due to reduced utilization of cholesterol for steroid hormone synthesis (Dellmann. 1993).

Fields *et al.* (1985) and Singh *et al.* (1990) also reported a loss of secretory granules and increased lipid during pregnancy in cattle and buffalo respectively. Weber *et al.* (1987) observed similar changes in later stages of estrus cycle.

Phospholipids

The capsule and stromal tissue were devoid of phospholipids both in cyclic as well as pregnant buffalo. SLC and LLC were strongly positive for phospholipids in developing, developed and corpus luteum of pregnancy whereas moderately positive in regressing (Fig.4).

Basic Proteins

The developing and regressing luteal cells showed weak reaction for basic proteins in SLC and LLC whereas a strong reaction observed in developed cyclic and pregnant animals appears to be suggestive of high secretory activity. The capsule and stroma were weakly positive (Fig. 5).

Calcium

Calcium deposits could not be demonstrated in any of luteal cells at any stage, however, except a very weak reaction in lumen of blood vessels.

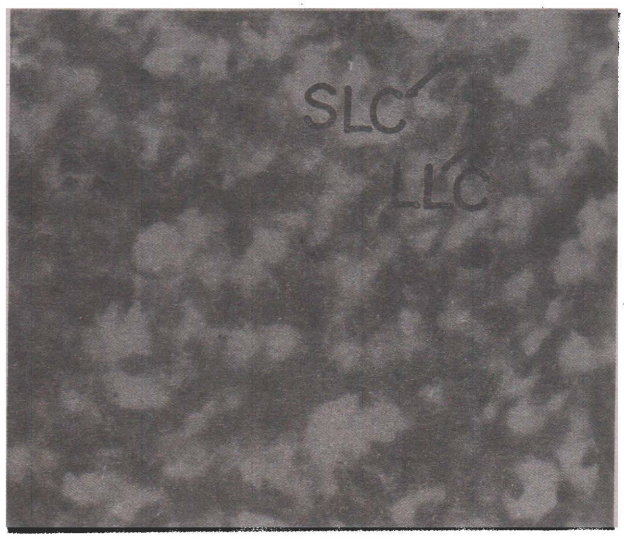

Fig. 4: Phospholipids in small luteal cell (SLC) and large luteal cell (LLC) of developing cyclic corpus luteum in cryostat section. Baker's method, 140 X.

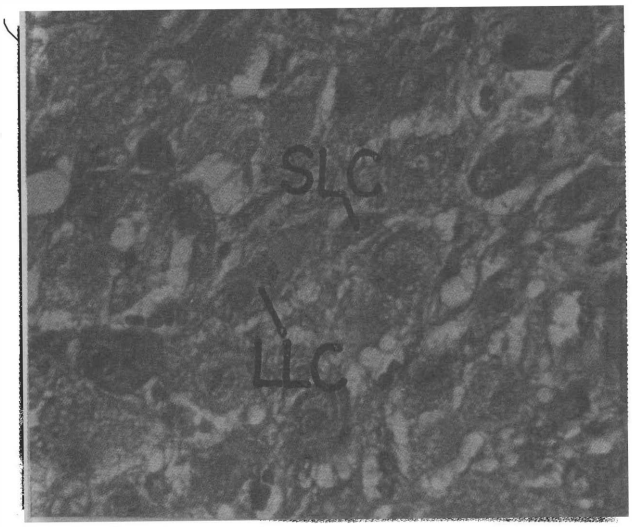

Fig. 5: Basic proteins in small luteal cell (SLC) and large luteal cell (LLC) of developed cyclic corpus luteum. Bromphenol Blue, 280 X.

ACKNOWLEDGEMENTS

Authors are thankful to Dean, College of Veterinary Science, PAU, Ludhiana for providing facilities. Typing by Shri Dalbir Chand and laboratory help by Shri Yashbir Singh is duly acknowledged.

REFERENCES

Chayan, J., R.G. Butcher, L. Bitensky and L.W. Poulter, 1969. A guide to Practical Histochemistry. Oliver and Boyd, Edinburg. pp: 83-174.

Dellmann, H.D., 1993. Text book of Veterinary Histology. Lea and Febiger, Philadelphia, pp: 239.

Fields, M.J., W. Dubois, P.A. Fields, 1985. Dynamic features of luteal secretory granules: Ultrastructural changes during course of pregnancy in cows. Endocrinology, 117: 1675-1682.

Goswami, R.K., 1985. Histomorphological and histochemical observations on ovary in Murrah buffaloes. M.V.Sc. Thesis, Haryana Agriculture University, Hisar, India.

Luna, L.G., 1968. Manual of Histological Staining Methods of Armed Forces Institute of Pathology. 3rd ed. McGraw Hill Book Company, New York, pp: 34-157.

Okuda, K., S. Kito, N. Sumi, K. Sato, 1988. A study of the central cavity in the bovine corpus luteum. J. Vet. Rec., 123: 180-183.

Sheehan, D.C. and B.B. Hrapchak, 1973. Theory and Practice of Histotechnology. The C.V.Mosby Co., Saint Louis. pp: 89-115.

Singh, U.B., 1975. Structural changes in the granulosa lutein cells of pregnant cows between 60 and 245 days. Acta Anat., 93: 447-457.

Singh, U.B., S. Sulochana and G.P. Sharma, 1990. Histological changes in the corpus luteum of buffaloes from 30-150 days of pregnancy. Indian J. Anim. Reprod., 11: 28-30.

Weber, D.M., P.A. Fields, L.J. Bomrell., S. Tumwasorn, B.A. Ball, M. Drost, M.J. Fields, 1987. Functional difference between small and large luteal cells of the late pregnant Vs nonpregnant cow. Biol. Reprod., 37: 685-697.