



MORPHOLOGICAL AND BIOCHEMICAL VARIATIONS OF NORMAL AND REGRESSING CORPORA LUTEA OF GOAT

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ABSTRACT

Corpora lutea of normal (11-15 days) and regressing category (16-21 days) of goat were analysed for morphological and biochemical variations. For biochemical studies, total lipids cholesterol content and proteins were estimated. Corpus luteum was comprised of two types of steroidogenic cells viz Granulosa luteal cells and Theca luteal cells. Ultrastructurally, regressing corpus luteum revealed disorganization of luteal cells with shrunken cytoplasm, uneven nuclear membrane, increased number of lipid droplets and lysosomes, apoptotic granules, disintegrating and vacuolated rough endoplasmic reticulum and swollen mitochondria as compared to normal corpus luteum. The total lipid content was less in normal corpus luteum (1.840 ± 0.52 mg/100 mg) and more in regressing corpus luteum (3.108 ± 0.84 mg/100 mg). The value of cholesterol was more in regressing (2.683 ± 0.31 μ g/100 mg) as compared to large category ($.315 \pm 0.04$ μ g/100 mg). Whereas the value of protein content of normal corpus luteum was found to be more (19.55 ± 3.45 mg/g wt. of the tissue) as compared to regressing corpus luteum (17.46 ± 1.70 mg/g wt. of the tissue). Ultrastructurally presence of lysosomes during regression corresponds to the autophagocytotic bodies with minimum progesterone level. During regression maximum amount of lipids and cholesterol shows less progesterone production as compared to normal corpus luteum whereas lesser content of protein during regression is related to lesser production of steroidogenic enzymes.

Keywords: Corpus Luteum, Goat Ovary, Lipids, Cholesterol, Proteins.

INTRODUCTION

The corpus luteum of mammalian species is a transient ovarian endocrine gland formed from the wall of the graafian follicle after the release of the ova (Guraya, 1985). It secretes progesterone essential for maintaining pregnancy. In case pregnancy fails, the corpus luteum regresses to surrounding stromal tissue (Sangha *et al.*, 2002; Tomec *et al.*, 2011). Prostaglandins induces this regression through apoptosis (Ford *et al.*, 1998). On the contrary, cellular sources of this progesterone are steroidogenic cells of corpus luteum. The non steroidogenic cells also play an important role in the functioning of this gland (Plant and Zeleznik, 2014). Although morphology of regressing corpus luteum of ewe has been documented (Al-zi-abi *et al.*, 2002). But no morphological and biochemical study have been made so far on this important aspect of goat.

Keeping this in view the present investigation was undertaken to analyse the variations in morphology and biochemical contents (total lipids, cholesterol and proteins) during normal and regressing corpora lutea.

MATERIALS AND METHODS

Goat (*Capra hircus*) ovaries were procured from the slaughterhouse of Delhi and brought to the laboratory in ice bucket at 0°C. Corpora lutea were dissected out and classified into seven categories (Table 1). On the basis of its morphology out of seven categories, corpora lutea of normal and regressing categories were subjected to ultrastructural studies and for biochemical analysis, corpora lutea were weighed and stored at 0°C.

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Table 1. Classification of corpus luteum.

Sr.No.	Size (mm) and colour	Name of category		Stage
1.	<2 Pink with red blood clot	Small	I	1-5 days
2.	2-5 Pink	Medium	II	6-10 days
3.	>5 Red	Large	III	11-15 days
4.	2-4 Brown	Regressing	IV	16-21 days
5.	≈2 Yellow	Previous	V	Pr
6.	<2 White	Penultimate	VI	Pn
7.	>6 Dark red	Pregnancy	VII	<30 days

For ultrastructural studies, corpus luteum was fixed in Karnovsky fixative at 0.1. M phosphate buffer (PH 7.2 to 7.4) at 4°C for 24 hours. The tissue was processed (Zamboni, 1976). The sections were studied under electron microscope, CM-10 Philips installed at All India Institute of Medical Sciences, New Delhi.

For extraction and estimation of total lipids, a known weight of corpora lutea was crushed in a pestle mortar in the presence of 5 grams of anhydrous sodium sulphate till a homogenous powder was obtained. The total lipids were extracted and estimated by the method of Folch *et al.*, (1957) whereas total cholesterol content was estimated following the method of Stadman (1957). Proteins were extracted by following the method of Adams (1980) and estimated by the method of Lowry *et al.*, (1951).

RESULTS

The small theca luteal cell of large category was characterized by the presence of abundant smooth endoplasmic reticulum, mitochondria, secretory granules, uneven cell membrane and a very few number of lipid droplets. The nucleus was oval in shape and possessed electron dense, heterogenous and eccentrically placed

nucleolus. The nucleosol was clear and possessed a number of darkly stained electron dense patches of chromatin material of diffused nature (Figure 1). During regression, disorganization of granulosa luteal cell with uneven plasma membrane, elongated nucleus, undulating and dispersed nuclear membrane were observed. The clumps of vacuolated and deeply condensed chromatin material was seen adhering to the nuclear membrane and was heterogenously dispersed within the nucleoplasm (Figure 2). Degenerating swollen rough endoplasmic reticulum and golgi apparatus were irregularly dispersed in the cytoplasm (Figure 3). Vacuolated mitochondria with reduced number of cristae and lysosomes were observed (Figure 4).

The total lipid content and cholesterol content were estimated in normal and regressing corpora lutea of goat. The minimum total lipid content was recorded in large category (1.840 ± 0.52 mg/100 mg wt. of the tissue) whereas during regression, it was 3.108 ± 0.84 mg/100mg wt. of the tissue. The cholesterol content during regression was more (2.683 ± 0.31 µg/100mg) as compared to large category (0.315 ± 0.04 µg/100mg) (Table 2). The total protein content in large category was found to be more than regressing category (Table 2).

Table 2. Variations in the amount of total lipids, cholesterol and proteins of normal and regressing caprine corpora lutea.

Biochemical Parameters	Categories of Corpus Luteum	
	Large/Normal (11-15 days)	Regressing (16-21 days)
Total Lipids mg/100mg	1.840 ± 0.52 (1.315 – 2.365)	3.108 ± 0.84 (2.173 – 4.210)
Cholesterol (µg/100mg)	0.315 ± 0.04 (0.266 – 0.363)	2.683 ± 0.31 (2.250 – 3.000)
Proteins (mg/g)	19.55 ± 3.45 (16.090 – 23.000)	17.46 ± 1.70 (15.758 – 19.166)

(Range in Parenthesis).



Figure 1. Small theca luteal cell of large category (11-15 days) of corpus luteum revealing abundant smooth endoplasmic reticulum, mitochondria, secretory granules and very few lipid droplets (X 20160).

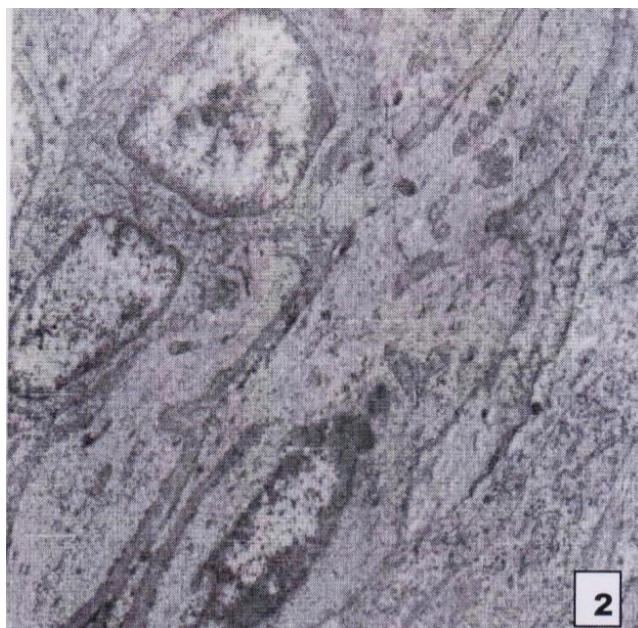


Figure 2. Electronmicrograph of corpus luteum of regressing category (16-21 days) showing disorganization of granulosa luteal cell with uneven plasma membrane, elongated nucleus and undulating nuclear membrane (X 11130).

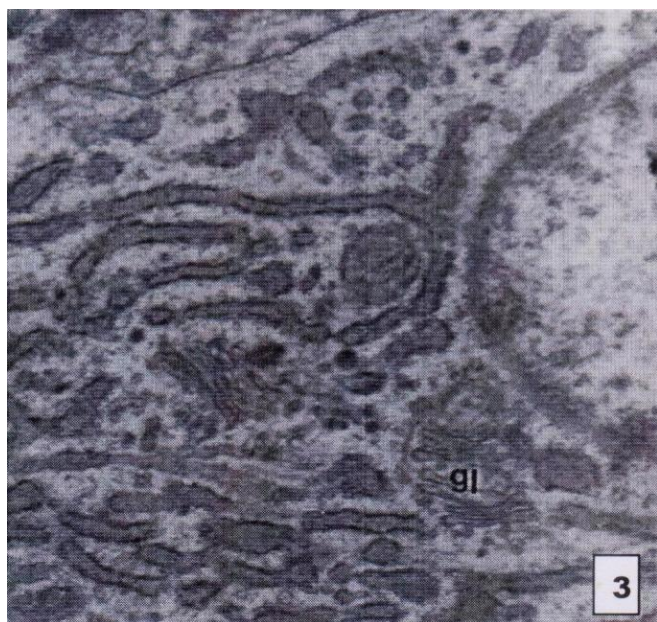


Figure 3. Electronmicrograph revealing fine morphology of granulosa luteal cell of corpus luteum of regressing category (16-21 days) showing Golgi apparatus (gl) and degenerating swollen rough endoplasmic reticulum (X 34440).

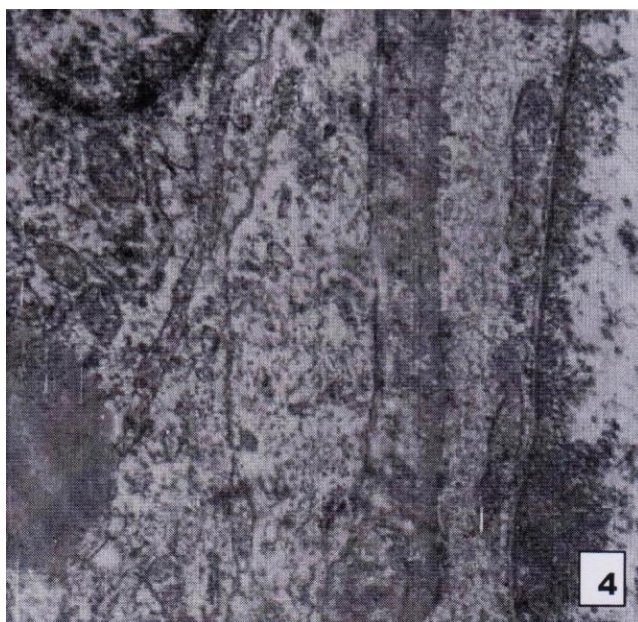


Figure 4. Fine morphology of theca luteal cell of corpus luteum of regressing category (16-21 days) showing a small number of mitochondria, secretory granules and lysosomes (X 35280).

DISCUSSION

The presence of abundant smooth endoplasmic reticulum and mitochondria in addition to usual cell organelles in small luteal cells indicate their higher steroidogenic potential as in other bovine species (Brar, 1993; Meyer, 1991). During regression steroidogenic cells of goat corpus luteum revealed apoptotic specific degenerating features similar to those reported in cow (Brar, 1993; Juengel *et al.*, 1993; Pate, 1994; Sharma and Batra, 2008). A similar pattern of changes during luteal regression was reported in ewe also (Gemmell *et al.*, 1976). A large number of lipid droplets and lysosomes were observed in regressing steroidogenic cells. The presence of lysosomes is positively correlated to the appearance of autophagocytotic bodies with the declining level of progesterone as observed in most of the bovine species (Khalid and Haresign, 1996; Palta *et al.*, 1996).

The goat corpus luteum revealed minimum amount of total lipids and cholesterol on day 11-15 of large category and maximum amount during regression (16-21 days). In bovine corpus luteum very little lipids were observed on day 10 to 13, their amount increased during regression (Dean *et al.*, 1966; Guraya, 2000; Sangha *et al.*, 2002). A similar trend of quantitative analysis of lipids were reported in buffalo, cow, ovine and goat (Brar, 1993; Sharma *et al.*, 1996; Waterman, 1988). The increase in the lipid droplets and vesicles during regression is because of poor mobilization of lipids and decline in progesterone synthesis (Brar, 1993; Guraya, 2000; Singh and Prakash, 1988). Membranes of agranular endoplasmic reticulum besides acting as a site for enzymes that are involved in steroidogenesis, also accumulate and store as a constituent of their lipid component like cholesterol which act as a precursor for steroidogenesis (Juengel *et al.*, 1998; O'Shaughnessy and Wathes, 1985; Pate and Condon, 1982). During regression, activity of enzymes involved in steroidogenesis have been correspondingly decreased which may be responsible for elevation in lipid content (Guraya, 1995). In goat luteal cells, the presence of rough endoplasmic reticulum and free ribosomes indicate that they are actively involved in the synthesis of protein which may include steroidogenic enzymes. Smooth endoplasmic reticulum may also be synthesized from rough endoplasmic reticulum (Gemmell *et al.*, 1974, 1977; Meyer, 1991).

CONCLUSIONS

During regression apoptosis, vacuolated mitochondria with reduced cristae, swollen and disintegrating rough endoplasmic reticulum, increased number of lipid droplets and lysosomes were observed. The presence of lysosomes is positively correlated to the appearance of autophagocytotic bodies with the declining level of progesterone. During regression maximum level of total lipids and cholesterol are related to the minimum amount of progesterone. Biochemical estimation of proteins during regression was found to be less which is attributed to the lesser production of steroidogenic enzymes.

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