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Biology of corpus luteum in small ruminants

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Abstract

Corpus luteum (CL) formation is a continuation of the follicular maturation process including cellular hypertrophy, hyperplasia and migration. It is formed by granulosa and theca interna cells along with endothelial cells, pericytes, smooth muscle cells, fibrocytes, macrophages, leucocytes and occasional plasma cells, which vary greatly in number, ultrastructure, chemistry and function during development, differentiation and regression of the CL. Besides progesterone as the principal steroid hormone, the CL also secretes small quantities of prostaglandins, oestradiol-17β and a variety of protein hormones. The cellular and molecular mechanisms involved in the regulation of synthesis, storage, secretion and possible intercellular or paracrine functions of various secretory products of the CL are emphasized. Understanding of these mechanisms may result in the development of improved methodologies for controlling the time of ovulation and (or) increasing pregnancy rates. The gaps in the knowledge have been identified for further investigations. © 2002 Published by Elsevier Science B.V.

Keywords: Corpus luteum; Goat; Ovary; Sheep; Small ruminants

1. Introduction

The corpus luteum (CL) is a transient endocrine gland formed from the wall of the graffian follicle after the release of the egg, by a complex mechanism involving morphological and biochemical changes. It is a dynamic endocrine gland showing variations in size, structure and steroidogenic activities in different stages of the estrous cycle and pregnancy (Fields and Fields, 1996). The CL consists of two types of luteal or steroidogenic cells, viz. granulosa lutein and theca lutein cells and several types of cells such as endothelial cells, pericytes, smooth muscle cells, macrophages, leucocytes and occasional plasma cells (Alila and Hansel, 1984). It secretes progesterone as the principal steroid hormone and small quantities of oestradiol-17β, prostaglandins and a number of peptide hormones such as relaxin, oxytocin, oxytocin-related neurophysin-I, vasopressin and inhibin (Fields, 1991). Information on structure (including ultrastructure), histochemistry, biochemistry, endocrinology, immunology, in vitro manipulation by growth factors and regulatory mechanisms (including apoptosis), has been elaborated especially in rodents and large ruminants (Guraya, 1997a,b, 2000). However, relatively less attention has been paid to the cellular and molecular biology of CL of small ruminants especially goat and sheep which are of great economic importance in the developing countries (Smith et al., 1994). This review intends to integrate information documented on different aspects of CL of small

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ruminants and identify the gaps and propose research to help enhance the productivity of small ruminants.

2. Development and differentiation

The follicle wall after ovulation becomes loose and the vascularization of membrana granulosa occurs simultaneously with the degeneration of basal lamina under the influence of various angiogenic factors (Redmer and Reynolds, 1996; Reynolds and Redmer, 1998). The luteinization of granulosa cells is known to be controlled by luteinizing hormone (LH) and blood vascularity, transporting oxygen, nutrients, hormones and various factors (Niswender and Nett, 1994; Milvae et al., 1996; Guraya, 2000). The sprouting blood capillaries invade the granulosa cells and form an extensive network within CL of the goat (Sharma and Sharma, 1998). The rate of luteal vascular growth is greatest in the early estrous cycle and by midcycle mature CL are highly vascular. The luteinized granulosa cells, the surrounding theca-interstitial cells and invading vasculatures intermingle to form a CL which secretes progesterone during the postovulatory phase. Hypertrophy of luteinized granulosa cells, hyperplasty of fibroblasts of the connective tissues and vascularity contribute to an increase in size of CL. The maximum diameter of CL is reached 6–9 days after ovulation and then regression starts between days 13 and 16 in ewes (Jablonka-Shariff et al., 1993). During the tremendous growth of CL there occur dramatic changes in tissue remodeling, including cell hypertrophy, hyperplasia and migration. These cellular changes occur in unison with changes in extracellular matrix, affecting specific cellular processes such as mitosis, migration, differentiation and gene expression (Getzenberg et al., 1990). Two families of proteins that are involved in extracellular matrix remodeling include metaloproteinases and plasminogen activator/plasmin. Protease inhibitors (TIMP and α_2 macroglobulin) may play a role in regulating the activity of metaloproteinases preceding follicular rupture and in the regulation of tissue remodeling during CL development (Smith et al., 1994). The shape, size and structure of granulosa luteal cells in goats and sheep vary (Brar, 1993). The differences in the degree of hypertrophy or luteinization in different granulosa cells can be attributed to the heterogeneity in granulosa cells of the maturing follicle and granulosa cells surrounding the antrum that possibly do not differentiate (or do not develop sufficient LH receptors to luteinize), immediately after ovulation (Guraya, 2000).

The differentiation of theca and granulosa cells into steroidogenic luteal cells is well accepted. However, the idea that small luteal cells originate exclusively from theca cells and large luteal cells from granulosa cells remains controversial. In domestic ruminants there is morphological and immunological evidence for small theca-derived luteal cells differentiating into large luteal cells (Cran, 1983; Alila and Hansel, 1984; Farin et al., 1988). As stated by O'Shea et al. (1986) there is still no compelling evidence supporting the hypothesis that small luteal cells differentiate into large luteal cells during the mid-luteal phase of the estrous cycle. It is not feasible to obtain large and small luteal cell populations in CL between days 1 and 6 of the estrous cycle in sheep. The size distribution of steroidogenic cells actually overlaps and forms populations (Schwall et al., 1986). The number of steroidogenic cells increases in the first half of the cycle while the number of non-steroidogenic cells tend to increase in the later part of the cycle (Farin et al., 1986).

3. Morphology, ultrastructure and histochemistry

The topography of CL spurium (haemorrhagicum) of the goat reveals crater-like depressions of approximately equal size distributed evenly on its outer contour. It appeared as a sponge-like structure with regular spaces, venous sinuses constituting a system of irregular anastomosing tunnels (Brar, 1993; Sharma and Sharma, 1998), that provide indirect evidence of enhanced nutritional intake and increased hormonal production.

Two types of steroidogenic cells, i.e. small luteal cells or theca luteal cells and large luteal cells or granulosa luteal cells are also differentiated on the basis of the histochemistry and immunocytochemistry of the secretory granules (Fritz and Fitz, 1991; Sharma and Sharma, 1998). The large steroidogenic cells represent approximately 40% of the volume of the CL in the goat, although they constitute only approximately 10% of the total cell number (see Table 1 for details). These cells are larger in size than other luteal

Table 1
Morphological characteristics of the cells in the mid-cycle corpus luteum of domestic ruminants

Cell type	Experimental animal	Size (μm)	% of luteal volume	% of luteal cells	Distinguishing characteristics[a,b,c]
Large	Cow[a]	38	40	3.5	Shape: spherical to polyhedral; nucleus: spherical; rough endoplasmic reticulum: abundant isolated stacks; smooth endoplasmic reticulum: abundant; mitochondria: abundant (∼20% of volume). Secretory granules: abundant (0.2 μm electron dense); plasma membrane: highly folded with microvilli; lipid droplets: rare except during luteolysis (sheep); basal lamina: abundant
	Sheep[b]	26–31	33–38	8–14	
	Goat[c]	22–50	∼40	∼10	
Small	Cow[a]	<23	∼28	26	Shape: spindle; nucleus: irregular with cytoplasmic inclusions; rough endoplasmic reticulum: rare; smooth endoplasmic reticulum: abundant; mitochondria: abundant (∼15% of volume); secretory granules: rare; plasma membrane: few microvilli; lipid droplets: present; basal lamina: not well defined
	Sheep[b]	16–18	18–23	23–26	
	Goat[c]	12–20	∼20	∼25	
Endothelial	Cow[a]	∼11	∼14	∼53	Shape: elongated around blood vessels; nucleus: irregular; prominent aggregates of heterochromatin, large nuclear to cytoplasmic ratio; few organelles in cytoplasm; basal lamina: distinct
	Sheep[b]	10–11	9–10	46–48	
	Goat[c]	∼10		∼50	
Fibroblast	Cow[a]	∼15	∼6	10	Shape: elongated; nucleus: elongated with large amounts of heterochromatin; rough endoplasmic reticulum: continuous with dilated cisternae; smooth endoplasmic reticulum: rare
	Sheep[b]	12–15	9	14–22	
	Goat[c]	∼10			

[a] Fields and Fields (1996).
[b] Farin et al. (1986).
[c] Sharma and Sharma (1998).

cells and have exceptional steroidogenic and protein-secreting capacity. Besides the usual organelles specific to steroidogenic cells, granulosa luteal cells show lysosomes, multivesicular bodies and peroxisomes (Meyer, 1991). In the goat, and sheep the dense secretory granules have been reported in the luteal cells of normally cycling and pregnant animals (Gemmell et al., 1974, 1977). The large cells in ovine CL are characterized by a centrally placed nucleus with a prominent nucleolus and euchromatic nucleoplasm (Hoyer et al., 1988). The small steroidogenic cells represent approximately 20% of the CL volume and approximately 25% of the cell number (Table 1). The nucleus of the small luteal cell is convoluted with an eccentrically placed prominent nucleolus and areas of peripheral heterochromatin. The small cells have abundant smooth endoplasmic reticulum and mitochondria, which is consistent with their steroidogenic capacity, but seem to lack rough endoplasmic reticulum and secretory granules, suggesting absence of a protein-secreting function. Occasionally, the nuclei of these cells contain inclusions filled with cytoplasmic material (Rodgers and O'Shea, 1982). In mature CL of sheep, large luteal cells contain a 1.8-fold greater in frequency of mitochondria per unit volume of cytoplasm than small luteal cells indicating higher metabolic investment in development and differentiation (Kenny et al., 1989).

Large and small luteal cells have been clearly identified in ruminants (O'Shea, 1987; Fields and Fields, 1996). The small luteal cells are spindle shaped (12–22 μm) and large luteal cells are spherical (22–50 μm) in goats and sheep (Rodgers and O'Shea, 1982; Singh and Prakash, 1988; Sharma and Sharma, 1998). The mean luteal cell diameter in CL haemorrhagicum of goat has been reported to be 35 ± 2.2 μm. As the animal proceeds further in the luteal phase of the estrous cycle, the mean luteal cell diameter increases up to 49 ± 1.73 μm (Brar, 1993). The volume density of small luteal cells vary from $10 \pm 2.7 \times 10^6$ on day 4 to $49 \pm 13.7 \times 10^6$ on day 16 whereas large luteal cells varied from $12 \pm 1.5 \times 10^6$

on day 4 to $22 \pm 5.1 \times 10^6$ on day 8 of the estrous cycle in ewes (Farin et al., 1986; Rodgers et al., 1984). The variations in the luteal cell dimensions are a direct indication of their steroidogenic activity as is evident from the increase in their diameter from days 6 to 8 which then remains approximately unchanged up to day 15 of the estrous cycle in ewe (Cunningham et al., 1975). After day 16 the regression of the luteal cells sets in and display orderly series of changes in their morphology which are characteristic of apoptosis (Juengel et al., 1993). During pregnancy, the mean luteal cell size increases to 50 ± 1.97 μm, the smallest luteal cells measuring 26 μm in diameter whereas largest luteal cells reaching a diameter of 75 μm (Brar, 1993).

The capillary endothelial cells constitute approximately 10% of the CL volume, but represent approximately 50% of the total cell number (Table 1). After dissociation these cells are approximately 10 μm in diameter. However, in vivo these cells have a long slender shape and line the lumen of the blood vessels in the CL. The fibroblasts infiltrate the CL after breakdown of the basement membrane during ovulation and luteinization and have an unknown luteal function.

During luteinization the intercellular contacts between differentiating granulosa luteal cells get re-established and the follicular epithelium is reorganized. The cells show an increase in number of zeotic blebs and microvilli indicating proliferative activity. Gonadotrophic hormones are considered to amplify and modulate rather than to induce the development of gap junctions (Niswender and Nett, 1994; Guraya, 2000). The intercellular interacting structures are present between the small luteal cells and large luteal cells of goat (Brar, 1993). The data obtained by Grazul-Bilska et al. (1996) have demonstrated that the gap junctions may be important for the regulation of luteal growth, differentiation and regression in the cow. The role of gap junctions and surface components of cells need to be defined more precisely in the CL as it consists of various types of cells and tissues (Pate, 1996).

Histochemical analysis of the CL of the goat revealed the sudanophilic nature of the granulosa lutein cells that may be related to the synthesis and secretion of progesterone (Brar, 1993). During the luteal phase and early pregnancy, the luteal cell cytoplasm gave a weak to moderate reaction while during the follicular phase (estrous) a slight increase in lipid content, cholesterol and its esters were observed (Miyamoto et al., 1984). In the CL vernum sudanophilic lipids stained intensely (Singh and Prakash, 1988; Brar, 1993). However, only trace amounts of lipids are observed on day 14 of the cycle and day 25 of pregnancy in sheep (Dingle et al., 1968). During the luteal regression, lipid droplets increase in size and number, and become a major component of corpus albicans (Brar, 1993). In the ovary of pregnant sheep, a positive staining was observed for mucopolysaccharides, glycogen and lipids in luteal cells (Roy and Saigal, 1986). The granulosa lutein cells develop a moderate Δ5-3β hydroxy steroid dehydrogenase (3β-HSD) activity within a few hours of corpus haemorrhagicum formation (Hay and Moor, 1975). Both large and small luteal cells of pregnant sheep CL gave a strong reaction for succinate dehydrogenase (SDH) (Roy and Saigal, 1985). Miyamoto et al. (1984) reported a strong activity of glucose-6-phosphate dehydrogenase (G-6-PDH) and isocitrate dehydrogenase and moderate activity of SDH and malate dehydrogenase (MDH) in CL of pregnant goats. The alkaline phosphatase (AKP) was higher than acid phosphatase (ACP). However, Bhattacharya and Saigal (1990) have reported a high activity of phosphatases and non-specific estrases in luteal cells of CL vernum as compared to CL spurium. Brar (1993) observed that in goats a moderate ACP activity in CL spurium and vernum increased in the regressing CL, and the activities of G-6-PDH, SDH and AKP were higher during pregnancy and the active secretory period as compared to the regressing CL.

Luteal regression in the goat is associated with regressing blood vessels, an increase in the number of lipid bodies, lysosomes, emergence of autophagocytic bodies and shrinking luteal cell mass (Brar, 1993; Pate, 1994). In sheep, these changes are marked by the appearance of autophagic vacuoles in the luteal cells that increased with advancement of cycle. The mitochondria clump together with autophagic and ACP positive vacuoles containing cellular organelles at various stages of degeneration (Gemmell et al., 1976; Stacy et al., 1976). Both small and large luteal cells contain a number of lipid droplets. The luteolytic changes in the vascular tissue appear on day 14 of the estrous cycle in ewe and include infolding of basal

lamina, increase in the number of gaps in the endothelium, increased endothelial cell projections into the lumen, inclusion of hetero and/or autophagus vacuoles in the cytoplasm of many endothelial cells and degeneration of endothelial cells leading to ultimate disintegration of affected vessels (O'Shea et al., 1986).

4. Biochemistry

The total protein content increased in the developing and mature CL as compared to corpus haemorrhagicum in the goat. The CL of pregnancy had maximum protein concentration. In the regressing CL, the protein content decreased with the lowest amount in corpus albicans (Brar, 1993). Rao et al. (1989) attributed the increase in the structural proteins to the increasing volume of the luteal cells in CL of sheep. The variations in the concentrations of various minerals such as copper, zinc, manganese, iron, magnesium, sodium and potassium during the CL formation, maturation and regression in buffalo and goats suggest their direct and indirect roles in the secretory and degenerative processes of CL (Brar, 1993), which remains to be determined in relation to activities of various enzymes of luteal cells and blood vascularity. Luteal cells produce a variety of proteins including peptide growth factors. Some of these proteins enter the blood stream and are targeted to other organs (Schams, 1987), whereas protease inhibitors, (Smith et al., 1993) and angiogenic factors (Grazul-Bilska et al., 1991), probably act locally.

4.1. Steroidogenesis

Steroid hormone biosynthesis is modulated by the availability of cholesterol and expression of specific steroidogenic enzymes (Stocco and Clark, 1997). The preovulatory gonadotropin surge initiates distinct changes in both expression and regulation of steroidogenic enzymes (Fig. 1) and is a key event in the luteinization process (Guraya, 2000). The mRNAs encoding cholesterol side-chain cleavage cytochrome P-450 (P-450$_{SCC}$) enzymes have been detected within stage I, II, and III, bovine CL (early, early-mid- and late-mid-luteal phase). Concentrations of P-450$_{SCC}$ increased at least 12-fold within stage II and III CL (Rodgers et al., 1986). The preovulatory gonadotropin surge results in acquisition of the 3β-HSD enzyme by the granulosa cells and an overall increase in 3β-HSD activity within corpora lutea, which increases progesterone biosynthesis (Hay and Moor, 1975). Another key enzyme, 17α-hydroxylase cytochrome P-450 (P-450$_{17\alpha}$), a microsomal enzyme, (required for the conversion of pregnenolone or progesterone to androgens) was abundant within bovine follicles but virtually non-detectable within stage I–IV CL (Rodgers et al., 1987). This lack of expression of P-450$_{17\alpha}$ gene may explain the absence of significant androgen production by bovine CL (Savard, 1973; Guraya, 1997a). Expression of P-450$_{arom}$ gene within ovarian tissues of domestic ruminants follows a pattern similar to that of P-450$_{17\alpha}$. Their relative amounts increase in preovulatory follicles, and after the preovulatory gonadotropin surge, P-450$_{arom}$ mRNA decreases rapidly. The changes during luteinization inhibit P-450$_{17\alpha}$ and P-450$_{arom}$ gene expression (Smith et al., 1994).

There are differences in the ability of small and large luteal cells to secrete progesterone in the presence of LH. In cattle (Hansel et al., 1991), sheep (Fitz et al., 1982), and pigs (Lemon and Loir, 1977), basal secretion of progesterone in vitro was less in small cells relative to large cells; however, the magnitude of LH-stimulated progesterone secretion was greater in small cells. Small and large luteal cells may synergize to increase secretion of progesterone (Lemon and Mauleon, 1982; Hansel et al., 1991). However, Rodgers et al. (1985) reported no such interaction between the two steroidogenic cell populations. Approximately 80% of the progesterone secreted in vivo by ovine corpora lutea is believed to come from large luteal cells (Niswender et al., 1985). Two of the intracellular effector systems that regulate luteal progesterone involve protein kinase A and protein kinase C. These two enzymes are present in both luteal cell types, however, with opposing effects. Protein kinase A is a potent stimulator of progesterone production in small luteal cells and protein kinase C is a potent inhibitor of progesterone production in large luteal cells (Guraya, 1997a).

The quantitative analysis of lipids has revealed that the concentration of phospholipids of regressing CL decreased whereas total glycerides and total cholesterol increased significantly (Brar, 1993). Waterman (1988) has attributed the dynamic alterations in lipid content and composition in sheep CL to the loss of

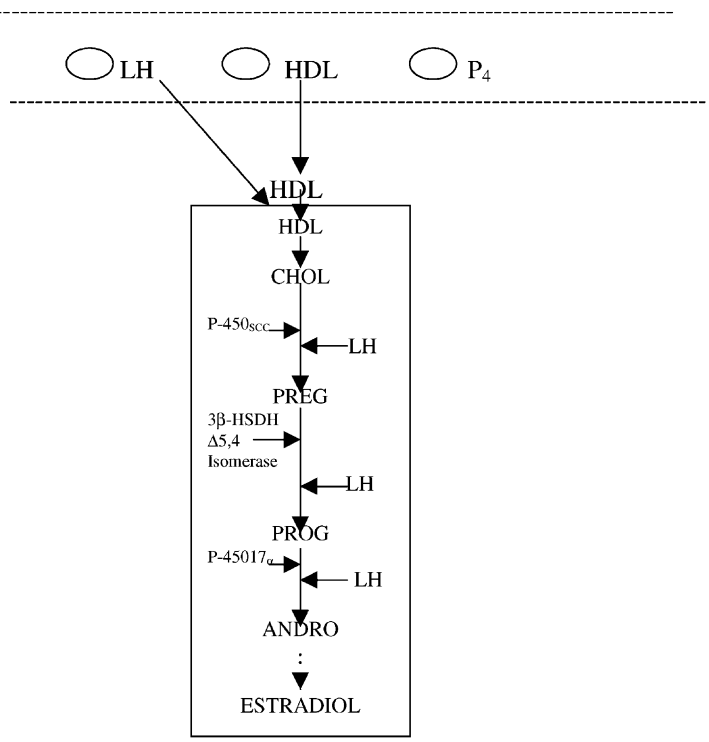

Fig. 1. Schematic model of steroidogenic pathway and enzymes for the luteal cells in mature corpus luteum. Dotted lines indicate the absence of reaction. At the top is shown a single capillary, which delivers nutrients, hormones and lipoprotein (HDL) to the luteal cell. CHOL: cholesterol; PREG: pregnenolone; PROG: progesterone; ANDRO: androgen; SCC: mitochondrial side-chain cleavage enzyme; 3β-HSD: 3β-hydroxysteroid dehydrogenase.

luteal function. Blood neutral lipids are believed to be the source of the fatty acid infiltration within the regressing luteal tissue which result in the increased percentage of arachidonic acid from days 14 to 16 in ewes.

4.2. Peptide hormones

A number of luteal peptides regulate luteal physiology in autocrine or paracrine mode (Guraya, 1997a, 2000). The major peptide hormones are relaxin, oxytocin, inhibin and vasopressin. Relaxin stimulates the production of different types of ovarian cells and modulates follicular growth and ovulation locally (Fields, 1991). In the goat, a positive immunostaining for relaxin has been observed in both small and large luteal cells in CL, which decreased gradually in the mature CL and CL of early pregnancy. However, the luteal cells in regressing CL exhibit an increased immunostaining for relaxin (Brar, 1993). In cycling ewes an episodic release has been reported but there are no conclusive localization studies (Sherwood, 1988). Only limited evidence for relaxin in pregnant ewe CL is available, and low levels have been reported in ovarian extracts.

Oxytocin has been immunolocalized in the CL of many mammalian species (Fields, 1991). In ewes, the circulating levels of oxytocin vary synchronously with changes in progesterone concentrations (Sheldrick and Flint, 1981; Mitchell et al., 1982). The luteal oxytocin binds to the endometrial oxytocin receptors and releases uterine $PGF_{2\alpha}$ which further induces the release of oxytocin leading to luteolysis (Schams, 1989). Luteal oxytocin is produced mainly by the large luteal cells although some form of intercellular communication between small and large luteal cells in the secretion of progesterone and oxytocin have been recorded (Schams, 1987; Rodgers, 1990). The presence of high concentrations of oxytocin is reported in CL of the non-pregnant sheep and goat (Flint and

Sheldrick, 1983; Brar et al., 1994). After luteinization of the granulosa cells in response to LH and FSH, the production of oxytocin is increased. Besides the gonadotropins, insulin-like growth factors also form a potent stimulus for oxytocin secretion (Schams, 1987). The low oxytocin secretion from the CL in early gestation allows the luteal maintenance in pregnant ewes and goats (Sheldrick and Flint, 1983). The molecular mechanisms involved in the regulatory process need to be investigated further to establish the specific functions in chronological order.

Luteal cells of CL have been identified as the source of inhibin where it may have some autocrine, paracrine or systemic effect involving cellular growth, differentiation and function (Rodgers, 1990). Inhibin with low molecular weight (10.5 kDa) showed a progressively increasing immunostaining in the CL during growth and development with the mature CL of goats showing the highest immunostaining while inhibin with high molecular weight (13 kDa) was detected only during a period of maximal development of CL in goats (Brar, 1993). The sheep CL have been shown to secrete inhibin into ovarian venous blood (Tsonis et al., 1988). However, Mann et al. (1989) reported that ovine CL does not secrete any inhibin and the major source of ovarian inhibin secretion is large follicles. There is no report of immunolocalization of inhibin in sheep CL. Further in situ hybridization studies of Rodgers et al. (1989) showed the absence of α and β inhibin subunits mRNAs in the luteal cells of mature ovine CL as well as in CL of pregnancy. The precise physiological significance of these results needs to be determined in relation to the autocrine and paracrine role of inhibin in the biology of CL.

Vasopressin has been reported from CL of the non-pregnant cow (Wathes et al., 1983, 1984). Its concentration is highest during the mid-luteal phase, decreased thereafter and was not detectable during pregnancy, following a trend similar to that of oxytocin. Ivell and Richter (1984) demonstrated 1000 times less vasopressin mRNA than oxytocin in bovine luteal tissue. No information regarding vasopressin mRNA is available in goats and sheep.

The functional significance of luteal neurophysin is still not known. During the oestrous cycle, luteal oxytocin and neurophysin mRNAs show fluctuations, reaching maximum levels on day 3 that are 250 times higher than seen in the hypothalamus of the cow (Ivell and Richter, 1984). Low levels of immunoreactive oxytocin and neurophysin in the CL of non-pregnant and pregnant sheep have been reported (Watkin, 1983; Theodosis et al., 1986; Sawyer et al., 1986). Fields et al. (1987) suggested that it might have a very significant role in the three-dimensional folding of the prohormone that protects the number of peptide from premature protease degradation.

5. Regulatory mechanisms

The intricate luteal function is controlled by the interplay of various luteotrophic factors that include gonadotropins (LH, hCG, prolactin), $PGF_{2\alpha}$ and oestrogen which maintain the function of CL. If fertilization occurs, the conceptus secretes a myriad of products that maintain the CL. The primary luteotrophic hormone in most species is LH. Obviously, the gonadotropin surge is essential for inducing ovulation and initiating the luteinization process. The withdrawal of LH support under experimental conditions invariably results in luteal demise. However, in ewes, the luteotropin-deprived CL does not cease functioning but ceases its normal growth and development pattern (Denamur et al., 1973; Farin et al., 1990; Baird, 1992). There is extensive evidence that at least some of the progesterone secretion from the ruminant CL seems to be autonomous from LH stimulation. Data using an antagonist to GnRH in sheep (McNeilly et al., 1992) are consistent with the idea that much of the luteal progesterone is secreted by LH-independent mechanisms probably by the differentiation of the highly steroidogenic large luteal cells. The underlying mechanisms responsible for this LH-independent secretion of progesterone are not yet defined. One possible explanation for high basal production in large luteal cells is that luteotrophins other than LH are tonically stimulating progesterone secretion from large luteal cells. Two hormones that have been found to have stimulatory effects on mixed or large luteal cells are $PGE_{2\alpha}$ (Fitz et al., 1984) and insulin (Sauerwein et al., 1992).

A number of growth factors, including epidermal growth factor, basic fibroblast growth factor, and insulin-like growth factors are produced and have stimulatory effects on the CL (Guraya, 2000). Garverick and Smith (1986) observed that high concentrations

of intrafollicular oestradiol-17β and adequate thecal vascularization may be important determinants of luteal function, which may also be influenced by LH-binding inhibitor (LH-BI).

Mechanisms controlling progesterone production by the CL of pregnancy in the goat and pig have not been fully elucidated. However, luteal secretion of progesterone in the sheep is controlled largely by maternal pituitary gonadotropins (Denamur, 1974) and removal of the pituitary results in abortion. At later stages of gestation, however, the sheep tolerates hypophysectomy without pregnancy being disturbed whereas the goat still responds by aborting (Cowie et al., 1963). According to Currie (1977), goat placental lactogen contributes to the regulation of progesterone secretion by the CL. Malecki et al. (1987) suggested that the goat does not require maternal pituitary LH to maintain pregnancy between days 50 and 130 of gestation and concluded that CL function may be maintained by luteotrophins or an antiluteolytic agent produced by the uterine components. The goat CL may be capable of producing progesterone independently of luteotrophins (Rothchild, 1981). White et al. (1985) observed lower oestradiol in follicles destined to give rise to subfunctional corpora lutea as a result of prior removal of ovulatory follicles in ewes during the breeding season. The results of these investigations further support that more receptors for LH and/or greater secretion of oestradiol are required if the follicle is to become a fully functional CL with a normal life span. Whether the follicular role in luteal span is determined by follicular competence per se possibly through effects of higher secretion of oestrogen on uterine receptors for progesterone permitting the luteolysis to be programmed as suggested for the normal ovine cycle (Ottobre et al., 1984; Garverick and Smith, 1986), remains to be determined. Mechanisms resulting in subnormal luteal function still remains to be determined more precisely.

Besides these, numerous neuropeptides produced by ovarian cell types are known to exert autocrine and paracrine effects on the preovulatory follicles (McDonald et al., 1987; Guraya, 1997a, 2000). The possibility that a GnRH-like peptide plays a physiological role in regulating rat CL function (Tsafriri and Adashi, 1994) remains to be tested for CL of ruminants and humans. Catecholamine stimulation of CL progesterone production is observed in cows and a modulatory role of adrenergic agents in CL function has been suggested (Godkin et al., 1977).

Recent correlative, physiological and biochemical investigations clearly indicate that the regression of CL in many domestic animals is due to uterine release of $PGF_{2\alpha}$ (Inskeep, 1973; Pate, 1994). Progesterone is believed to delay the production of prostaglandins which are well known to induce regression of luteal cell mass. The prostaglandins possibly reduce the potential ability of CL to produce progesterone by acting directly on luteal cells or indirectly through their effects on ovarian vasculature (Knickerbrocker et al., 1988). The best evidence that $PGF_{2\alpha}$ is uterine luteolytic factor is produced by the fact that spontaneous luteal regression can be prevented by active or passive immunization of ewes (Fairclough et al., 1976; Scaramuzzi and Baird, 1976). Different mechanisms proposed to explain the luteolytic effects of $PGF_{2\alpha}$ include a fast and dramatic decrease in luteal blood flow, a decrease in the numbers of LH receptors, an uncoupling of the LH receptors from adenylate cyclase, and a cytotoxic effect (Guraya, 1997a).

Substances like $PGF_{2\alpha,}$ oxytocin and neurophysin inhibit progesterone secretion directly in porcine CL, which is negated by their stimulatory action of estradiol release in the CL. The estradiol stimulation has a powerful effect on progesterone production. This effect dominates in developing CL (Fig. 2). In aged CL, macrophages release cytokines like tumor necrosis factor (TNF) that inhibit estradiol release, thus the inhibitory effects of $PGF_{2\alpha}$ and oxytocin become operative and their function becomes luteolytic (Wuttke et al., 1993). Although many factors like $PGF_{2\alpha}$, oxytocin, TNF are involved, the chronology of events of luteolysis is not clear. Among the most accepted molecular mechanisms of luteolysis is apoptosis (Sawyer et al., 1990; Juengel et al., 1993). It involves the activation of Mg^{2+} and Ca^{2+}-dependent restriction endonucleases within the cells that cleave the nuclear DNA into 185 bp oligonucleotides (Arends et al., 1990; Sharma, 2000). In cattle apoptosis occurs during both spontaneous and $PGF_{2\alpha}$-induced luteal regression. The accompanying decline in progesterone production and oligonucleotide formation suggest the role of apoptosis in structural regression (Juengel et al., 1993). Further studies are required to define the precise role of apoptosis in luteolysis in domestic ruminants.

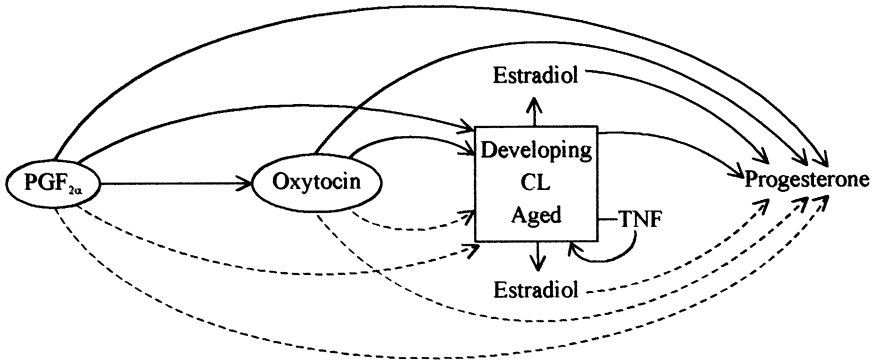

Fig. 2. Schematic representation of luteotrophic role of $PGF_{2\alpha}$ and oxytocin in progesterone production in young, maturing (developing) CL and luteolytic role of the same on aged. Dark arrows represent the stimulatory effect and dotted arrows show the inhibitory effect.

6. Conclusions and perspectives

The CL is a dynamic entity that secretes progesterone as a principal hormone and estrogen, relaxin, oxytocin, neurophysin-I, inhibin and vasopressin in minute quantities. The luteal cells are derived from theca and granulosa cells that differentiate into Small luteal cells and large luteal cells. The transmutation of these cells with regard to their function has been observed in some species. The small luteal cells are ultrastructurally most equipped for steroidogenic functions, while large luteal cells secrete both steroids and the regulatory peptides. Which factors induce variations in their morphology and functions need to be analyzed. A host of steroidogenic and metabolic enzymes along with rich vascularity present testify to the secretory importance of CL in estrous cycle and in pregnancy. Biochemically, variations in proteins, lipids, hormones such as progesterone and estrogen, and peptides like relaxin, inhibin, vasopressin, oxytocin and neurophysin-I revealed a positive correlation with its functions. Role of oxytocin synthesis and release is both stimulatory and inhibitory depending upon the estrogen synthetic potential of the CL. However, relaxin, vasopressin, inhibin and neurophysin-I need to be further investigated to establish their specific physiological obligations in CL functions. Although the role of uterine $PGF_{2\alpha}$ in CL regression is established, more information about peptide hormones especially growth factors need to be analyzed to establish their regulatory significance. The molecular mechanism and the regulatory proteins of the process of apoptosis need to be analyzed in the light of available information on various interacting factors as how specific apoptotic genes get stimulated and which specific enzymatic or apoptotic pathway CL regression follows. Broad-based cellular and molecular studies are still required to explain the increase in luteal insufficiency which is impairing animal production. In this regard, it will be rewarding to study the effects of various xenobiotic factors.

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