Regulation of corpus luteum development and maintenance: specific roles of angiogenesis and action of prostaglandin $F_{2\alpha}$

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Development of the corpus luteum (CL) in ruminants occurs in a rapid and time-dependent manner within 1 week after ovulation, with morphologic and biochemical changes in the cells of the theca interna and granulosa cells of the preovulatory follicle. These changes involve luteinisation of steroidogenic cells and angiogenesis to establish normal luteal function (progesterone secretion). The CL is composed of a large number of vascular endothelial cells, large and small steroidogenic luteal cells, smooth muscle cells, pericytes, fibrocytes and immune cells, indicating that the CL is a heterogeneous tissue. Moreover, the CL produces and secretes growth factors (fibroblast growth factor, vascular endothelial growth factor and insulin-like growth factor), vasoactive factors (nitric oxide, angiotensin II and endothelin-1), steroids (progesterone is important for its own production), oxytocin and prostaglandins ($PGF_{2\alpha}$ and PGE_2) to regulate luteal formation and development. Clearly, the main function of the CL is to produce progesterone, which is a prerequisite for survival of the embryo, implantation and maintenance of pregnancy. Inadequate luteinisation and angiogenesis during the early luteal phase results in poor progesterone secretion and causes compromised embryo development and reduced fertility. Secretion of adequate amounts of progesterone during luteal development requires "precise luteinisation" of theca and granulosa cells to form luteal cells, neovascularization, and the establishment of a blood supply (angiogenesis). $PGF_{2\alpha}$ in the developing CL acts as a local regulator to enhance progesterone secretion directly and indirectly by stimulating angiogenic factors, VEGF and FGF2. The preceding role of $PGF_{2\alpha}$ may explain why the developing CL does not acquire luteolytic capacity until several days following ovulation. The balance between luteotrophic and luteolytic factors as well as stimulation and inhibition of angiogenic factors during luteal formation, development and maintenance can have a profound effect on the fate of the CL.

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A brief historical overview of corpus luteum research in ruminants

As summarized by McCracken *et al.* (1999), Coiter (1573) described the presence of cavities filled with a yellow solid in the rodent ovary. De Graaf and Mullierum (1943) provided the first definitive description of these structures and noted that the number appeared to be related to the number of fetuses in the uterus. Malpighi (1897) provided an accurate microscopic description of these structures and was the first to name the corpus luteum (CL). Subsequently, Beard (1897) postulated that CL were responsible for the suppression of ovulation and oestrus during pregnancy.

Frenkel (1903) demonstrated that corpora lutea are necessary for implantation and the subsequent maintenance of pregnancy in rabbits. Corner & Allen (1929) prepared a relatively pure alcoholic extract of the CL from sows and demonstrated that the extract could maintain pregnancy in ovariectomized rabbits. In 1934, isolation of the pure crystalline hormone was reported by different groups (Slotta *et al.* 1934; Wintersteiner & Allen 1934). Slotta *et al.* (1934) named the compound progesterone and suggested the structure. In 1960, about 27 papers concerning the CL with only one in ruminants were cited according to PubMed. From 1960 onwards, countless studies have been performed on different aspects of luteal formation, maintenance, function and regression during the oestrous cycle and pregnancy. A critical advance in the field has been development of sophisticated methods for measuring progesterone in tissue, culture supernatants, and peripheral blood. Research activities have been directed towards the topics listed below (Fig. 1).

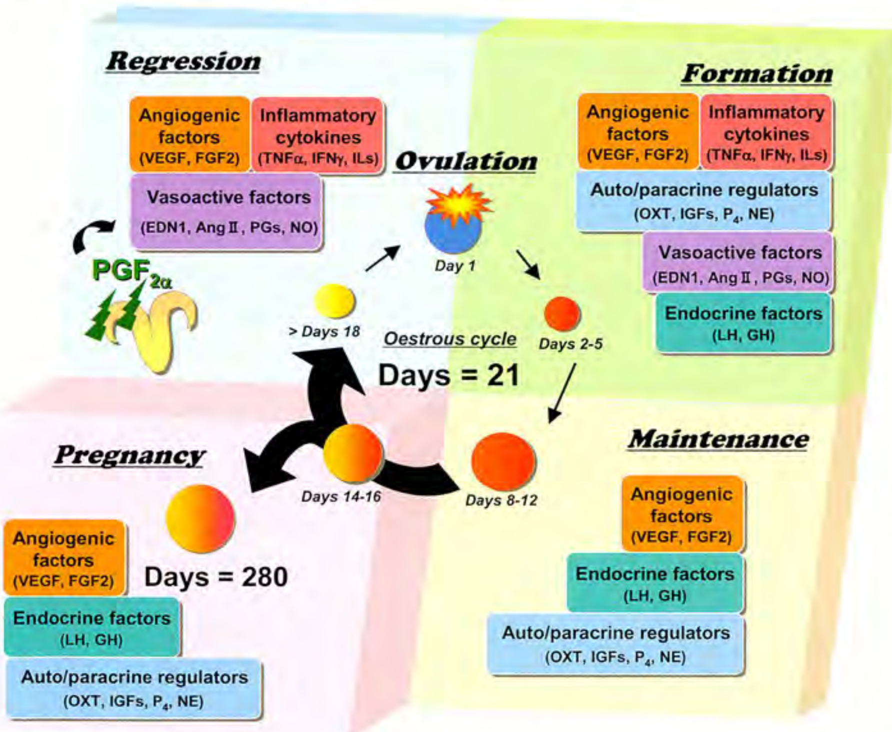

Fig. 1. Schematic presentation of possible involvement of various functional factors during different stages of CL activity. The CL at each stage is regulated by a complex mechanism that consists of endocrine factors, auto/paracrine factors, angiogenic factors, vasoactive factors and inflammatory cytokines. The balance between luteotrophic and luteolytic factors as well as stimulation and inhibition of angiogenic factors is the basic mechanism for CL development, maintenance and regression.

Development of the corpus luteum

An essential step in early CL development is vascularisation of granulosa-lutein cells through angiogenesis. Angiogenesis is defined as the generation of new blood vessels through sprouting from preexisting blood vessels. This process includes breakdown of basement membranes, proliferation and migration of endothelial cells into the extracellular matrix and formation of new capillary lumina along with functional maturation which is regulated by a complex of stimulation and inhibition of angiogenic factors (Schams & Berisha 2004). In cows, sprouting endothelial cells invade the developing CL and continue to grow throughout the first third of the ovarian cycle, and the mature CL is characterized by a dense network of vessels with gradually decreasing vessel density. During luteolysis, all newly formed blood vessels regress, thus luteal angiogenesis is a suitable experimental system to study endothelial cells in sprouting and regressing blood vessels (Augustin *et al.* 1995).

Molecular control of luteal secretion of progesterone

The synthesis and secretion of progesterone are regulated by two morphologically and biochemically distinct steroidogenic cell types in the CL. Small luteal cells respond to luteinising hormone (LH) or cAMP with an increase in secretion of progesterone. Large luteal cells secrete a high basal concentration of progesterone ($\geq 85\%$), do not respond to LH or cAMP and contain receptors for prostaglandin (PG) $F_{2\alpha}$, growth hormone (GH) and other proteins. Progesterone secretion is regulated by two second messenger pathways. Phosphorylation of steroid acute regulatory (StAR) protein by protein kinase A (PKA) stimulates cholesterol transport, whereas phosphorylation by PKC appears to inhibit this process and induces cell death (Niswender *et al.* 2000).

Trophic regulation of luteal function

Both LH and GH are necessary for normal luteal development and function (Niswender *et al.* 2000; Schams & Berisha 2004). Local regulators such as growth factors (insulin-like growth factors, IGFs; fibroblast growth factors, FGFs), ovarian peptides (oxytocin; angiotensin II, Ang II; endothelin-1, EDN1), and steroids (noradrenalin and prostaglandins ($PGF_{2\alpha}$ and PGE_2): Schams & Berisha 2004) play modulatory roles acting in an autocrine/paracrine fashion.

Luteal regression (luteolysis)

After induction of luteolysis by endometrial $PGF_{2\alpha}$, a cascade of events occurs within the CL, leading to functional and structural luteolysis. The acute changes in vasoactive factors suggest that modulation of vascular stability is a critical component of luteolysis (Miyamoto *et al.* 2009). Other cascades after $PGF_{2\alpha}$-induced luteolysis that occur in parallel or subsequent to changes in vascularity include: (i) luteal nitric oxide (NO) release and blood flow, (ii) up-regulation of inflammatory cytokines and luteal cell apoptosis factors, (iii) up-regulation of vasoactive peptides in luteal cells, (iv) extracellular matrix proteases associated with CL tissue remodelling and (v) invasion by immune cells (monocytes, macrophages, T-lymphocytes) from the blood stream after monocyte chemoattractant protein-1 up-regulation.

There are currently (PubMed) about 14,813 papers concerning the CL in different species. About 4,238 of these papers concern domestic ruminants. However, there are a number of important issues relating to luteal function that require further research. For example, a

clearer understanding of how the balance between luteotrophic and luteolytic factors as well as stimulation and inhibition of angiogenic factors affects luteal function is essential. In addition, we need to better understand the role of proteolytic processes (e.g. tissue remodelling), and modulation by the immune system in the regulation of the CL. A schematic of possible involvement of these factors in different stages of the luteal phase is shown in Fig. 1. In this review, we focus on CL development and maintenance in ruminants with specific emphasis on luteal angiogenesis and the action of prostaglandins.

Introduction

Ovulation occurs in response to a cascade of morphological, biochemical, and physiological changes in the Graafian follicle following the LH surge, resulting in expulsion of the matured oocyte and subsequent development of the CL. The bovine CL develops rapidly within 2-3 d after ovulation and is supported by differentiation and hypertrophy of steroidogenic cells, angiogenesis and neovascularisation; it is functional for 17-18 d in the non-pregnant cow. If pregnancy does not occur successfully, the CL must regress within a few days to allow for subsequent ovulation to occur. In non-pregnant cows, luteolysis is caused by pulses of $PGF_{2\alpha}$ that are secreted by the endometrium around d 17-19 of the oestrous cycle (Ginther *et al.* 2009). $PGF_{2\alpha}$ induces a decrease in luteal blood flow which results in a decrease in progesterone released from the CL as well as a decrease in CL volume (Acosta *et al.* 2002; Niswender *et al.* 1976).

The preovulatory follicle is compartmentalised into a highly vascular thecal layer and a non-vascular granulosal layer that are separated by a basal membrane and independently regulated by LH and FSH. The CL is composed of a large number of vascular endothelial cells that can account for up to 50% of all luteal cells. Large and small steroidogenic luteal cells constitute about 30% of all cells of CL (O'Shea *et al.* 1989), and the majority of steroidogenic cells are adjacent to one or more capillaries (Zheng *et al.* 1993). The bovine CL also consists of various cell types such as smooth muscle cells, pericytes, fibrocytes and immune cells, indicating that it is a heterogeneous tissue (Farin *et al.* 1986). The bovine CL produces steroid hormones, PGs (Miyamoto *et al.* 1993; Shemesh & Hansel 1975), a number of angiogenic factors, and other local regulators (Reynolds *et al.* 2000; Schams & Berisha 2004).

Changes in the dominant follicle before and after the LH surge

Preovulatory changes induced by the LH surge

The time between the LH surge and ovulation is a critical period for the rapid start of differentiation of the preovulatory follicle. The CL is formed after ovulation from remaining follicular cells. The preparation of luteal cells for progesterone synthesis begins before ovulation. Luteinisation and secretion of progesterone can occur in the absence of ovulation in cattle (Kesler *et al.* 1981) and sheep (Murdoch & Dunn 1983), indicating that mechanisms associated with luteinisation are not dependent on follicular rupture. Following the preovulatory LH surge but before ovulation, follicular cells begin morphological, endocrinological and biochemical changes associated with luteinisation. The critical roles of PGs in ovulation have been demonstrated by an experiment in which an inhibitor of PG biosynthesis effectively inhibited ovulation in cattle (Algire *et al.* 1992). Injection of an ovulatory dose of hCG induced expression of cyclooxygenase-2 (COX-2) in the granulosa cells of bovine preovulatory follicles (Sirois 1994). The levels of $PGF_{2\alpha}$ and PGE_2 increased by more than 100-fold at 25 h after the endogenous LH surge in bovine preovulatory follicular fluid (Short *et al.* 1995). Additionally, Ang II, a potent

vasoconstrictive factor, as well as $PGF_{2\alpha}$ acutely increased around the time of ovulation in the bovine mature follicle in vivo (Acosta *et al.* 2000). FGF2 protein is up-regulated beginning with the LH surge, and the maximum levels were observed at 20 h after GnRH injection in the bovine follicle (Berisha *et al.* 2006). Interestingly, FGF2 localisation changed dramatically from the theca (cytoplasm of endothelial cells) to the nucleus of granulosa cells after the LH surge, suggesting an essential role for survival of granulosa cells or for transition of granulosa cells to luteal cells (Berisha *et al.* 2006). Oxytocin also plays important autocrine/paracrine roles in the follicular/luteal transition of steroidogenesis from estradiol/androgen to progesterone production. Oxytocin not only stimulates progesterone, but also inhibits oestradiol secretion by granulosa cells before the LH surge, but not after the LH surge (Berndtson *et al.* 1996; Voss & Fortune 1991). In addition, oxytocin stimulates progesterone release from early developing CL as examined with the microdialysis system (Miyamoto & Schams 1991).

Vascular changes are associated with the cyclic remodelling of ovarian tissue that occurs during final stages of follicular growth, ovulation and CL development (Acosta *et al.* 2002; Moor *et al.* 1975). The principal angiogenic factors controlling follicular angiogenesis are FGF2 and VEGFA (Berisha *et al.* 2000b). Systemic administration of a VEGF antagonist prevented the development of preovualtory follicles. This inhibition was associated with a decrease in thecal layer vasculature, granulosa cell proliferation, antral formation and steroidogenesis (Wulff *et al.* 2002). Conversely, ovarian injection of VEGFA gene fragments into gonadotropin-stimulated prepubertal pigs increased thecal vascularisation and the number of preovulatory follicles (Shimizu *et al.* 2003). The follicular VEGFA concentration in the fluid and tissue increased in the bovine follicles during final maturation and was associated with increased vascular density in the thacal layer (Berisha *et al.* 2000b). Vessel permeability increased along with neovascularisation of the follicular periphery, thereby supplying a larger effective dose of LH to that particular follicle. LH stimulated VEGFA expression in bovine cultured granulosa cells in a dose dependent manner (Schams *et al.* 2001). As VEGFA mRNA expression is not altered by the LH surge during the periovulatory interval, LH is thought to act at a post-transcriptional level to regulate VEGFA production (Hazzard *et al.* 1999). Capillaries induced by VEGF develop perforations through which blood cells and platelets escape when ovulation occurs. Shortly before ovulation, blood flow stops in a small area of the ovarian surface overlying the bulging follicles. This area, known as the stigma, then ruptures (Findlay 1986).

Follicular luteinisation after ovulation

After follicular rupture, there is a dramatic invagination of the follicular wall that presumably facilitates migration of fibroblasts, endothelial cells and theca interna cells into the central regions of the developing CL. Tissue remodelling and cellular migration are facilitated by breakdown of the basement membrane that separates the avascular granulosa cell layer from the theca interna layer. Small and large luteal cells are derived exclusively from theca and granulosa cells, respectively. However, some small luteal cells may differentiate into large luteal cells (Alila & Hansel 1984). Conversely, some large luteal cells may differentiate into small luteal cells (Fisch *et al.* 1989). In sheep, small luteal cells can stimulate angiogenesis (Grazul-Bilska *et al.* 1991).

The differentiation of granulosa and theca cells into large and small luteal cells is characterized by increased progesterone production. The preovulatory LH surge initiates distinct changes in both expression and regulation of steroidogenic enzymes and is a key event in the luteinisation process. Aromatase cytochrome P-450 enzyme (P450arom) is a key enzyme in oestradiol biosynthesis, catalyzing aromatisation of C19 androgens of theca cell origin to C18

oestrogens within granulosa cells. P450arom mRNA decreases in bovine follicles collected at approximately 20 h after the LH surge (Voss and Fortune 1993). Within the bovine CL, 3ß-hydroxysteroid-dehydrogenase (3ß-HSD) mRNA and enzyme activity increases throughout most of oestrus and then decreases during luteolysis (Couet *et al.* 1990). Moreover, 3ß-HSD enzyme activity is greater in the bovine CL than in preovulatory follicles (Couet *et al.* 1990). Thus, 3ß-HSD activity within the CL facilitates high rates of progesterone biosynthesis.

During luteinisation, the changes in gene expression associated with steroid production in steroidogenic cells are regulated by transcription factors such as Ad4BP/SF-1 and DAX-1. The decrease in DAX-1, a suppressor of Ad4BP/SF-1, is involved in acquisition of the ability to produce progesterone when granulosa cells are luteinising (Shimizu *et al.* 2009). However, during luteinisation of theca cells in culture, suppression of CYP 17 genes was induced by an increase in DAX-1 transcription factor (Murayama *et al.* 2008). Thus, DAX-1 likely contributes to the expression of specific genes to shift steroid production during luteinisation of granulosa and theca cells.

Corpus luteum development and angiogenesis

Vasculature of the developing CL

After ovulation, a rich vascular network is established within the CL to support differentiation of follicular cells and progesterone secretion from luteal cells during the early luteal phase. Functionally, luteal blood vessels can be divided roughly into two types of blood vessels. One type is arteriolovenous vessels, i.e., arteriola (diameter: about 40 μm) and venula vessels (diameter: about 30 μm), which have a smooth muscle layer and exhibit a vasorelaxant effect. In the circulation of blood, the arteriola connects to microcapillary vessels followed by venulae (Kashiwagi *et al.* 2002). It has been reported that in the rabbit CL, arteriolovenous vessels exist in the periphery of the CL (Wiltbank *et al.* 1988), and luteal blood flow is observed mainly in the periphery of the bovine CL in color Doppler images (Acosta *et al.* 2002). The other types are capillary vessels having no smooth muscle layer and thus exhibit little vasorelaxant effect. In the bovine and ovine CL, the number of arteriolovenous and microcapillary vessels drastically increases from the early to mid-luteal phase (Bauer *et al.* 2003; Hojo, *et al.* 2009; Shirasuna *et al.* 2010a), indicating active angiogenesis during this period. A recent study indicated that further angiogenesis does not occur throughout the period of early maternal recognition in the cow (Beindorff *et al.* 2010).

Role of angiogenic factors in the developing CL

Angiogenesis is a critical component of normal luteal function. In 1906, Loeb (1906) indicated that the CL closely resembles "transitory tumors", and the rate of luteal growth is equivalent to the fastest growing tumors. Therefore, the growth of blood vessels and establishment of a blood supply are essential during early luteal development. Indeed, the mature CL has the densest capillary network system in the whole body and each luteal cell is adjacent to one or more capillaries.

One of the major angiogenic factors, FGF2, is generally involved in cell growth, differentiation, transformation and angiogenesis. Gospodarowicz *et al*,. (1985) showed that FGF2 is produced in the bovine CL and stimulates neovascularization and proliferation of a variety of cells such as vascular smooth muscle cells, granulosa cells and endothelial cells. The mRNA expression of FGF2 and its receptor is highest during the early luteal phase (Berisha *et al.*

2000a; Schams *et al.* 1994). FGF2 concentrations in CL tissue are high during the early luteal phase, and decrease significantly in the mid-luteal phase during the oestrous cycle in the cow (Schams *et al.* 1994). FGF2 stimulates progesterone secretion from the early bovine CL as determined using a microdialysis system (Miyamoto *et al.* 1992). In a recent study investigating the impact of FGF2 on bovine CL development and function, an FGF2 antibody was injected directly into the CL (Yamashita *et al.* 2008). This treatment markedly suppressed CL volume and progesterone synthesis (plasma progesterone concentrations and mRNA expression of StAR) as well as decreased mRNA expression of key factors related to angiogenesis ($VEGF_{120}$, FGF2, FGFR-1) and increased the ratio of angiopoietin-2/angiopoietin-1 (an index of instability of vessels), all of which promote angiolysis (Yamashita *et al.* 2008). This suggests that FGF2 promotes the establishment of a new vascular network and luteal function during CL formation in the cow.

The greatest mRNA expression for VEGFA and VEGFR-2 in the CL was detected during the early luteal phase followed by a significant decrease in expression during the mid and late-luteal phase, with further decreases after luteal regression in the cow (Berisha *et al.* 2000b). In contrast, VEGFR-1 mRNA expression did not change during the oestrous cycle (Berisha *et al.* 2000b). The concentration of VEGF protein was increased during the early luteal phase and then decreased, especially in the regressing CL (Berisha *et al.* 2000b). VEGFA can stimulate progesterone secretion from the bovine CL (Kobayashi *et al.* 2001). An injection of VEGF antibody into an intact bovine CL suppressed the increase in CL volume and progesterone synthesis in the early CL (Yamashita *et al.* 2008). These findings are in agreement with the previous report that neutralisation of VEGF using an antibody during the early luteal phase in marmoset monkeys inhibits proliferation of endothelial cells and plasma progesterone concentrations (Fraser *et al.* 2000).

Robinson *et al*,. (2008) investigated the angiogenic and luteotropic roles of FGF2 and VEGF in the bovine CL using an in vitro culture system for luteal angiogenesis. Luteal FGF2 concentration was highest from d 1-2 compared with d 3-4, 5-6 and 8-12 of the oestrous cycle, while luteal VEGF concentration gradually increased from the early to midcycle CL, and then decreased in the regressing CL in the cow (Robinson *et al.* 2009). In a luteal angiogenesis culture system (includes luteal cells, endothelial cells and smooth muscle cells), a physiological dose (1 ng/ml) of FGF2 and VEGF stimulated the extent of the endothelial cell network (Fig. 2; Robinson *et al.* 2008). Using this culture system, a VEGFR2 inhibitor resulted in lower numbers of endothelial networks compared with the control (60% of the control; Woad *et al.* 2009). However, even in the presence of VEGF, a FGFR1 inhibitor drastically reduced the number of vascular networks by more than 90%, suggesting that FGF2 is more crucial than VEGF for forming luteal vascular networks (Fig. 2; Woad *et al.* 2009). The preceding findings indicate that FGF2 plays a key role in the initiation of angiogenesis during very early luteal development in the cow.

In the early CL, FGF2 was localised to endothelial cells but the localization changed to the cytoplasm of luteal cells after 5 to 7 d (Schams *et al.* 1994). VEGFA (mainly localised in the cytoplasm of luteal cells) plays a fundamental role in maintenance of the luteal vasculature following cessation of luteal growth when active angiogenesis is no longer occurring. Indeed, VEGF enhanced endothelial platelet-derived growth factor (PDGF)-BB, a potent stimulator of neovascularisation; whereas, FGF2 enhanced PDGF receptor expression in pericytes and smooth muscle cells surrounding vasculature (Kano *et al.* 2005). Stimulation with VEGF and FGF2 caused a significant pericyte/muscle cell recruitment and formation of vasculature compared with single-agent stimulation, indicating a different contribution of both factors in neovascularisation (Kano *et al.* 2005).

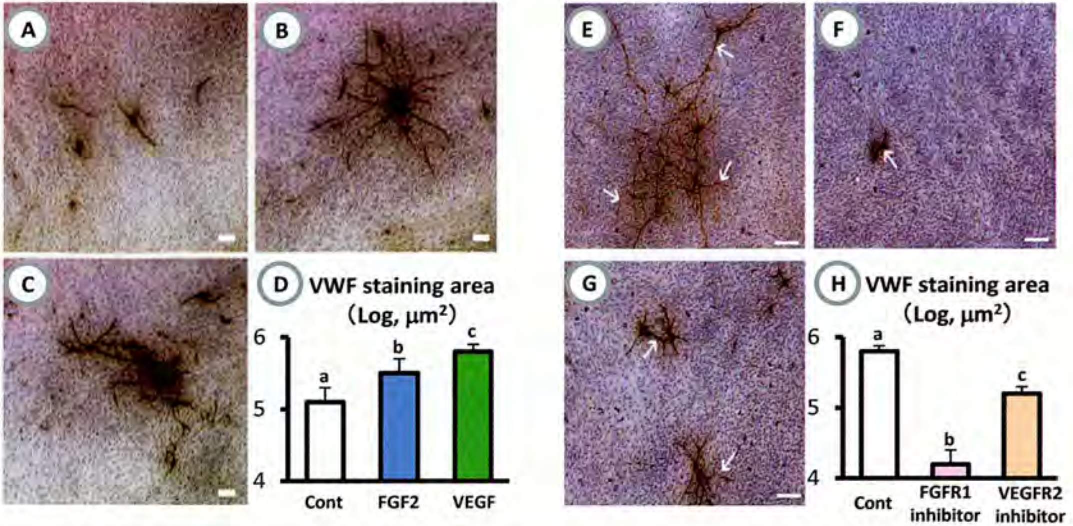

Fig. 2. The effect of FGF2, VEGFA and inhibitors of angiogenesis on the development of the luteal endothelial network in vitro (modified from Robinson *et al.*, 2009; Woad *et al.*, 2009). Luteal cells were dispersed from early CLs. In Figure 2D, luteal cells were treated with control (open bar, Fig. 2A), 1 ng/ml FGF2 (blue bar, Fig. 2B), and 1 ng/ml VEGFA (green bar, Fig. 2C) for 9 days. In Figure 2H, the luteal cells were treated either with control medium containing 1 ng/ml FGF2 and VEGFA (Cont, open bar), plus 1 μM FGFR1 inhibitor (SU5402, pink bar) and 2 μM VEGFR2 inhibitor (SU1498, orange bar) for 9 days. The endothelial cells were immunostained (brown) with von Willbrand Factor (VWF). The scale bar represents 50 μm. Following image analysis and quantification of VWF immunostaining, the effects of angiogenic factors and inhibitors of angiogenesis are shown on total area of VWF in Fig. 2D and 2H. The significant differences ($P < 0.05$) between treatment groups are indicated by different letters and the values are meant + S.E.M.

Potential interaction between cytokines and angiogenesis in the developing CL

The early CL produces angiogenic and luteotropic factors. Tumor necrosis factor-α (TNFα) is recognised as a tumoricidal factor produced by activated macrophages and luteal cells; TNFα and its receptors are detected in the early CL (Sakumoto *et al.* 2000). TNFα is a representative angiogenic factor regulating VEGF and FGF2 in bovine retinal cells (Yoshida *et al.* 2004). Interleukin (IL)-1 and IL-8, mainly produced by activated monocytes and macrophages, are also good candidates as angiogenic factors. Indeed, IL-1α and IL-1β can up-regulate VEGF expression in endometriotic stromal cells (Li *et al.* 1995). Koch *et al.,* (1992) indicated that IL-8 can induce the same levels of chemotaxis, proliferation and angiogenesis in human endothelial cells as FGF2. These findings lead to the hypothesis that various cytokines effectively coordinate to promote angiogenesis and ensure luteal development and function.

Extracellular matrix (ECM) is crucial for angiogenesis and tissue remodeling. Matrix metalloproteinases (MMPs) cleave specific components of the ECM and are inhibited by tissue inhibitors of metalloproteinases (TIMPs). MMPs and TIMPs play a major role in the process of follicular development and atresia, ovulation and CL development, maintenance and regression. A detailed mechanism of MMP-TIMP system in the ruminant CL was nicely reviewed previously (Curry & Smith 2006; Smith *et al.* 1999).

Distinct regulatory mechanisms of $PGF_{2\alpha}$ in the early and midcycle CL

An intriguing question concerning bovine CL function in recent years is "Why is the developing CL (d 1 to 5 of the oestrous cycle) resistant to the luteolytic action of $PGF_{2\alpha}$; whereas, after d 5 exogenous $PGF_{2\alpha}$ can induce rapid luteolysis?" (Henricks *et al.* 1974; Levy *et al.* 2000). To investigate the mechanisms underlying the different actions of $PGF_{2\alpha}$ in the early CL ($PGF_{2\alpha}$-resistant) versus midcycle CL ($PGF_{2\alpha}$-responsive) in domestic animals, several studies have focused on potential differences in steroidogenesis (Tsai & Wiltbank 1998), prostaglandin synthesis (Silva *et al.* 2000; Tsai & Wiltbank 1997b), immune function (Tsai *et al.* 1997a) and vasoactive factors (Choudhary *et al.* 2004; Levy *et al.* 2000; Wright *et al.* 2001). Although $PGF_{2\alpha}$ acutely decreased mRNA expression of 3β-HSD in both the early and midcycle CL, $PGF_{2\alpha}$ decreased StAR mRNA expression in the midcycle CL but not in the early CL (Tsai & Wiltbank 1998), indicating that StAR may be a key factor in reduced steroidogenesis following $PGF_{2\alpha}$ administration in the cow.

Prostaglandin $F_{2\alpha}$ receptor (FPr) mRNA is expressed in the CL at high levels throughout the oestrous cycle (Sakamoto *et al.* 1995; Wiltbank *et al.* 1995). Shirasuna et al., (2008) showed that FPr was localised to luteal cells but also to large blood vessels (mainly endothelial cells) in the periphery of the early and midcycle CL. The presence of FPr in the early CL can induce a specific tissue response (Levy *et al.* 2000; Tsai & Wiltbank 1998). Indeed, these previous reports indicated that FPr mRNA expression was decreased by $PGF_{2\alpha}$ administration at both stages (Levy *et al.* 2000; Tsai & Wiltbank 1998). Thus, the refractoriness of the early CL to the luteolytic effect of $PGF_{2\alpha}$ is not caused by a lack of the FPr.

Angiogenic factors

In a study focusing on the response of angiogenic factors to $PGF_{2\alpha}$ administration, $PGF_{2\alpha}$ injection down-regulated mRNA expression of VEGFA and FGF2 in the midcycle CL (Shirasuna *et al.* 2010b), suggesting that inhibiting angiogenesis may initiate or at least contribute to luteolysis. $PGF_{2\alpha}$ administration drastically decreased VEGF protein expression, in the bovine midcycle CL, after 30 min (Berisha *et al.* 2010). Interestingly, in the early CL (d 4 of the oestrous cycle), $PGF_{2\alpha}$ stimulated mRNA expression of VEGFs and FGF2 (Shirasuna *et al.* 2010b) and $PGF_{2\alpha}$ up-regulated VEGF transcription in human cancer cells (Sales *et al.* 2005). Moreover, both the production of $PGF_{2\alpha}$ and mRNA expression of COX-2 were higher in the early CL compared to other stages of the luteal phase (Kobayashi *et al.* 2001; Milvae & Hansel 1983). Importantly, in the bovine early CL, $PGF_{2\alpha}$ clearly stimulated progesterone secretion (Miyamoto *et al.* 1993; Okuda *et al.* 1998), and VEGF and FGF2 stimulated $PGF_{2\alpha}$ and progesterone secretion (Kobayashi *et al.* 2001). Consequently, it is proposed that $PGF_{2\alpha}$, in the early CL, acts as a local regulator to enhance progesterone secretion directly and also indirectly by stimulating VEGF and FGF2 (Fig. 3). The preceding mechanism may be one of the main reasons why the early CL is resistant to the luteolytic effects of $PGF_{2\alpha}$ (Miyamoto *et al.* 2009).

Insulin-like growth factors

The insulin-like growth factor (IGF) system is essential for support of progesterone secretion from luteal cells. The bovine and ovine CL have been identified as sites of IGF-I and IGF-II mRNA expression as well as peptide production and action throughout the luteal phase (Einspanier *et al.* 1990; Khan-Dawood *et al.* 1994; Sauerwein *et al.* 1992). The mRNA expression of IGF-I

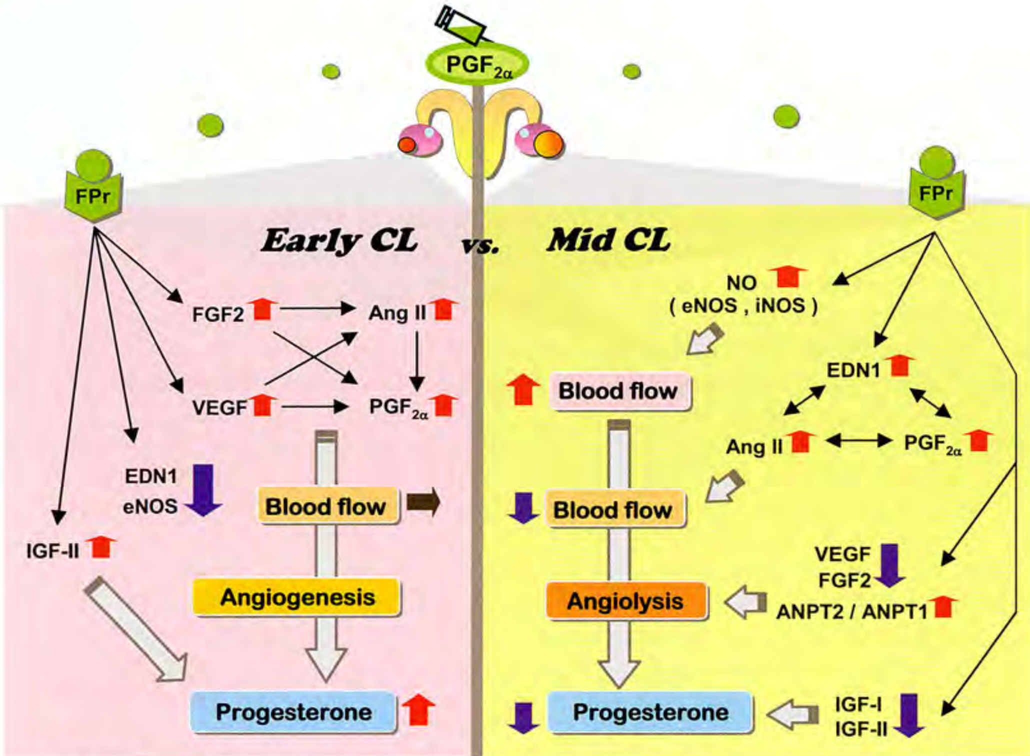

Fig. 3. Proposed model for the differential response of the early and mid-cycle CL to PGF$_{2\alpha}$ administration in the cow. After PGF$_{2\alpha}$ injection, PGF$_{2\alpha}$ enters into the CL via bloodstream. In the early CL, PGF$_{2\alpha}$ acutely stimulates VEGF, FGF2 and IGF-II expression in the bovine CL. VEGF and FGF2 stimulate luteal Ang II and PGF$_{2\alpha}$, and Ang II stimulates luteal PGF$_{2\alpha}$. This local positive feedback system between angiogenic and vasoactive factors supports angiogenesis and progesterone secretion within the CL; consequently, the early CL exhibits resistance to the luteolytic effect of PGF$_{2\alpha}$. Additionally, PGF$_{2\alpha}$ acutely suppresses EDN1 and eNOS expression in the bovine early CL, probably contributing to no response in blood flow.

In the midcycle CL, PGF$_{2\alpha}$ suppresses VEGF and FGF2 expression and creates the increased ratio of ANPT2/ANPT1 (an index of unstability of vessels), initiating angiolysis within the CL. In addition, PGF$_{2\alpha}$ decreases IGF-I and IGF-II expression directly causing a reduction in progesterone. A luteolytic dose of PGF$_{2\alpha}$ stimulates NO production by eNOS and iNOS expression in the CL, thus the luteal blood flow (one of the earliest physiological signs of the luteolytic cascade in the cow) is acutely increased as a result of vasodilation by NO action. Coincidently, vasoactive factors such as EDN1, Ang II and luteal PGF$_{2\alpha}$ start to increase within the CL to induce severe vasoconstriction. These events are coordinated and induce directly or indirectly the drastic decrease in progesterone secretion.

and IGF-II are higher in the early CL than other stages of the bovine luteal phase (Schams *et al.* 2002). Also, IGF-I was localised to both large and small luteal cells, whereas, IGF-II was localised to the perivascular fibroblasts of large blood vessels and pericytes of capillaries in the bovine CL (Amselgruber *et al.* 1994). Although PGF$_{2\alpha}$ significantly inhibited mRNA expression of both IGF-I and IGF-II in the midcycle CL, IGF-II mRNA expression was stimulated by PGF$_{2\alpha}$ in the early CL (Shirasuna *et al.* 2010b). Importantly, the stimulatory effect on progesterone

secretion of IGF-II was greater than that of IGF-I in bovine CL tissue culture (Green *et al.* 2007). Additionally, IGF-II stimulated the chemotactic motility of endothelial progenitor cells in a dose-dependent manner and increased neovascularisation in the mouse (Maeng *et al.* 2009). These data suggest that $PGF_{2\alpha}$ may have potential as an angiogenic and luteotrophic agent in the early CL.

Vasoactive factors

Vasoactive factors: EDN1, Ang II and NO are involved in the process of luteal regression in ruminants (Acosta *et al.* 2009; Girsh *et al.* 1996a; Hayashi & Miyamoto 1999; Hinckley & Milvae 2001; Miyamoto *et al.* 1997; Skarzynski *et al.* 2000). In fact, EDN1, Ang II and NO can inhibit progesterone secretion in the bovine (Girsh *et al.* 1996b; Miyamoto *et al.* 1997; Stirling *et al.* 1990) and ovine (Hinckley & Milvae 2001) CL *in vitro*. Moreover, $PGF_{2\alpha}$ stimulated biosynthesis of EDN1 (and EDN1 mRNA expression), Ang II (and ACE mRNA expression) and NO (and eNOS/iNOS mRNA expression) *in vivo* and *in vitro* (Acosta *et al.* 2009; Girsh *et al.* 1996a; Hayashi & Miyamoto 1999; Hinckley & Milvae 2001; Miyamoto *et al.* 1997). A recent study indicated that $PGF_{2\alpha}$ stimulated mRNA expression of EDN1, ACE, eNOS and iNOS in the midcycle CL (Shirasuna *et al.* 2010b). Contrary to the midcycle CL, $PGF_{2\alpha}$ significantly decreased EDN1 and eNOS mRNA expression in the early CL (Shirasuna *et al.* 2010b). Therefore, $PGF_{2\alpha}$ likely has a dual function depending on the stage of the luteal phase, acting as a luteotrophic factor (in the early CL) and a luteolytic factor (in the midcycle CL) in the cow (Fig. 3).

Conclusions

Development of the bovine CL occurs rapidly and in a time dependent manner within 1 week after ovulation, with morphologic and biochemical changes in the cells of the theca interna and granulosa cells of the preovulatory follicle. These changes involve luteinisation of steroidogenic cells and angiogenesis to establish luteal function (progesterone secretion). Angiogenic factors such as FGF2 and VEGF have a crucial role in CL development in ruminants. Additionally, the luteolysin $PGF_{2\alpha}$ has a stage-specific action depending on the stage of luteal development (early vs. mid) during the oestrous cycle in the cow. Taken together, $PGF_{2\alpha}$ appears to possess a dual function, acting as a luteotrophic or an anti-luteolytic factor by stimulating angiogenic factors in the early CL but acting essentially as a luteolytic factor by stimulating vasoactive- and PG-related factors and by inhibiting angiogenic factors, in the midcycle CL in the cow.

Clearly, the main function of the CL is to produce progesterone, which is a prerequisite for survival of the embryo, implantation and maintenance of pregnancy. In the cow, maternal concentrations of progesterone have a marked influence on the development of the embryo and its ability to produce interferon τ (IFNτ). Mann *et al.* (1999) indicated that a late rise in progesterone after post-ovulation or inadequate progesterone secretion during the early luteal phase resulted in the development of compromised embryos with limited ability to produce IFNτ during the maternal recognition period (d 16 after insemination). In contrast, d 16 embryos of cows with an earlier rise in progesterone secretion were more elongated and produced large amounts of IFNτ (Mann *et al.* 1999). Moreover, the progesterone concentration in milk of pregnant cows was significantly higher than cows that were mated but not pregnant cows on d 6 after insemination (Mann *et al.* 1999). These findings strongly suggest that an effective and immediate increase in progesterone is critical in stimulating proper embryo development and IFNτ synthesis.

Secretion of adequate amounts of progesterone during luteal development requires proper differentiation and luteinisation of theca and granulosa cells into luteal cells, growth of blood vessels, and the establishment of a blood supply (angiogenesis). Inadequate luteinisation and angiogenesis during the early luteal phase results in poor progesterone secretion causing compromised embryo development and a reduction in fertility. Further investigation into the precise role of luteinisation and angiogenesis during luteal formation and development may provide new insight for better fertility in the cow.

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