Angiogenesis in the ovary

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Angiogenesis refers to the formation of new blood vessels, or neovascularization, and is essential for normal tissue growth and development (Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991). The angiogenic process begins with capillary proliferation and culminates in formation of a new microcirculatory bed composed of arterioles, capillaries and venules. The initial component of angiogenesis, capillary proliferation, consists of at least three processes: (1) fragmentation of the basement membrane of the existing vessel, (2) migration of endothelial cells (the primary cell type constituting capillaries) from the existing vessel toward the angiogenic stimulus and (3) proliferation of endothelial cells (Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991). Neovascularization is completed by formation of capillary lumina and differentiation of the newly formed capillaries into arterioles and venules.

In adult tissues, capillary growth occurs only rarely, and the vascular endothelium represents a stable population of cells with a low mitotic rate (Denekamp, 1984). Angiogenesis does, however, occur in adults during tissue growth and repair, such as in healing wounds or fractures (Klagsbrun and D'Amore, 1991). Rampant or persistent capillary growth is associated with numerous pathological conditions, including tumour growth, retinopathies, haemangiomas, fibroses and rheumatoid arthritis in the case of rampant capillary growth, and nonhealing wounds and fractures in the case of inadequate capillary growth. The female reproductive organs exhibit marked, periodic growth and regression, accompanied by equally striking changes in their rates of blood flow. It is not surprising, therefore, that they are some of the few adult tissues in which angiogenesis occurs as a normal process. Ovarian follicles and corpora lutea have been shown to contain and produce angiogenic factors. These angiogenic factors appear to be heparin-binding and to belong to the fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) families of proteins. In addition, factors regulating gap junctional communication may play a critical role in coordinating the interactions between luteal vascular and nonvascular tissues. Further elucidation of the specific physiological roles of these factors in follicular and luteal growth, development and function will ultimately lead to improved methods for regulating fertility in mammals.

In adult tissues, capillary growth (angiogenesis) occurs normally during tissue repair, such as in the healing of wounds and fractures. Inappropriate capillary growth is associated with various pathological conditions, including tumour growth, retinopathies, haemangiomas, fibroses and rheumatoid arthritis in the case of rampant capillary growth, and nonhealing wounds and fractures in the case of inadequate capillary growth. The female reproductive organs exhibit marked, periodic growth and regression, accompanied by equally striking changes in their rates of blood flow. It is not surprising, therefore, that they are some of the few adult tissues in which angiogenesis occurs as a normal process. Ovarian follicles and corpora lutea have been shown to contain and produce angiogenic factors. These angiogenic factors appear to be heparin-binding and to belong to the fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) families of proteins. In addition, factors regulating gap junctional communication may play a critical role in coordinating the interactions between luteal vascular and nonvascular tissues. Further elucidation of the specific physiological roles of these factors in follicular and luteal growth, development and function will ultimately lead to improved methods for regulating fertility in mammals.

On the basis of these observations, angiogenesis in normal adult tissues has been likened to processes such as blood clotting, which must remain in a constant state of readiness yet be held in check for long periods (Folkman and Klagsbrun, 1987). Angiogenesis is, therefore, thought to be regulated by angiogenic and anti-angiogenic factors (Folkman and Klagsbrun, 1987; Klagsbrun and D'Amore, 1991). Because regulation of the angiogenic process could become the primary method for treatment of various disorders, much of the work on angiogenesis and its regulation has focused on pathological processes.

The female reproductive organs (the ovary, uterus and placenta) contain some of the few adult tissues that exhibit periodic and dynamic growth and regression (Reynolds et al., 1992). In addition, growth and regression of these tissues is extremely rapid. The rate of cell proliferation in early cycle corpora lutea, for example, is comparable with or greater than that of rapidly growing tumours or regenerating tissues, and is much greater than that of most adult tissues (Reynolds et al., 1994; Zheng et al., 1994; Christenson and Stouffer, 1995; Nicosia et al., 1995). Uterine and placental tissues also exhibit impressive growth during gestation (Reynolds and Redmer, 1995). It is not surprising, therefore, that angiogenesis occurs as a normal process in the female reproductive tissues (Findlay, 1986; Klagsbrun and D'Amore, 1991; Reynolds et al., 1992). However, in contrast to that observed during pathological tissue growth, vascular development of the female reproductive tissues is limited and, therefore, must be tightly regulated (Reynolds et al., 1992, 1994; Reynolds and Redmer, 1995).

Rapid growth and regression of the female reproductive tissues are accompanied by equally rapid changes in their vascular beds (Clark, 1900; Andersen, 1926; Basset, 1943; Burr and Davies, 1951; Zheng et al., 1993; Reynolds and Redmer, 1995). During growth of these tissues, their vascular endothelial cells exhibit mitotic rates equal to or greater than those of the most...
Ovarian vascular growth and development

Earlier in this century, several investigators noted the tremendous vascularity of ovarian follicles and corpora lutea and recognized the importance of the ovarian vasculature in supporting growth and development of these tissues (Clark, 1900; Andersen, 1926; Bassett, 1943; Burr and Davies, 1951). In his classic treatise, Clark (1900) evaluated vascular development of the human ovary from the fetal stage through senescence and provided the first clear picture of the vascular architecture of the various ovarian tissues (Fig. 2).

After evaluating vascular anatomy throughout follicular development, Clark (1900) concluded that “…the vital impulse to growth in the theca interna depends not on a maintenance of its primitive blood supply, but upon a decided increase of that supply.” More recent studies have confirmed that dominant follicles have not only a more vascular theca, but also an increased uptake of serum gonadotrophins compared with other antral follicles (McNatty et al., 1981; Zeleznik et al., 1981). Thus, increased vascularity may be a primary determinant of follicular dominance.

The early investigators also observed the heterogeneity in follicular vasculation and suggested its importance in determining whether follicles remain healthy or become atretic (Clark, 1900; Andersen, 1926; Bassett, 1943; Burr and Davies, 1951). Subsequent work has supported the contention that maintenance of the follicular vasculature is important for maintaining follicular health. For example, early atretic ovine follicles will regenerate when placed in vitro, suggesting that decreased vascularity may limit access of atretic follicles to nutrients, substrates and trophic hormones in vivo (Moor and Seamark, 1986). More recently, Greenwald (1989) found that reduced DNA synthesis of follicular endothelial cells was associated with reduced follicular vascularity and was one of the earliest signs of atresia. Similarly, we have observed a cessation in proliferation of theca luteal cells, associated with a decrease in thecal vascularity, soon after the onset of atresia in bovine, ovine and porcine follicles (Jablonka-Shariff et al., 1993; P. M. Fricke, J. J. Ford, D. A. Redmer and L. P. Reynolds, unpublished).

Vascular development of the ovarian follicle becomes even more impressive after ovulation, in association with the development of the corpus luteum (Clark, 1900; Andersen, 1926; Bassett, 1943). The capillary network of the mature corpus luteum is so extensive that the majority of parenchymal (steroidogenic) cells are adjacent to one or more capillaries (Fig. 3; Dharmarajan et al., 1985; Zheng et al., 1993), which is not surprising, since in several mammals most (up to 85%) of the cells that are proliferating during growth of the corpus luteum are endothelial cells (Jablonka-Shariff et al., 1993; Reynolds et al., 1994; Christenson and Stouffer, 1995; Nicosia et al., 1995). In addition, morphometric studies in several species have shown that endothelial cells probably constitute about 50% of the cells in the mature corpus luteum (Farin et al., 1986; Lei et al., 1991). The mature corpus luteum also receives most of the ovarian blood supply, and ovarian blood flow is highly correlated with progesterone secretion (Niswender and Nett, 1988; Reynolds et al., 1994).

Conversely, inadequate luteal function has been associated with decreased luteal vascularization, and several investigators have suggested that reduced ovarian blood flow plays a critical role in luteal regression (Niswender and Nett, 1988; Reynolds et al., 1992, 1994). It is interesting to note that during luteal regression, while a portion of the capillary bed regresses, the larger microvessels appear to increase in abundance and probably play an important role in resorption of the luteal tissue (Zheng et al., 1993; W. A. Ricke, D. A. Redmer, and L. P. Reynolds, unpublished).

Fig. 1. Dual immunofluorescence localization of factor VIII (a marker for vascular endothelial cells; green fluorescence) and BrdU (a marker for proliferating cells; red fluorescence) in a section of a corpus luteum from a ewe on day 8 of the oestrous cycle. Note that, on the basis of colocalization of factor VIII and BrdU (arrows), a large proportion of the proliferating cells appears to be endothelial cells. However, a few of the proliferating cells do not colocalize with factor VIII (arrowheads). Luteal parenchymal cells (faint greenish brown) can be seen in the background. The size of the field is 216 μm x 216 μm; scale bar represents 25 μm. Reproduced from Jablonka-Shariff et al. (1993) with permission.
Ovarian angiogenic factors

General

The development of in vivo and in vitro assays within the last two decades has made possible the isolation and characterization of angiogenic and anti-angiogenic factors from numerous tissues (Folkman and Klagsbrun, 1987; Klagsbrun and D’Amore, 1991). The in vivo methods have been utilized to evaluate the ability of a factor to influence neovascularization, that is, to influence the entire process of angiogenesis (Folkman and Klagsbrun, 1987; Klagsbrun and D’Amore, 1991). The most commonly used of these techniques are the corneal pocket assay, which permits linear measurement of capillary growth induced by a substance implanted onto the cornea of a rabbit, mouse or rat, and the chorioallantoic membrane (CAM) assay, which evaluates the ability of a substance to stimulate or inhibit neovascularization when placed onto the CAM of a chicken embryo (Folkman and Klagsbrun, 1987; Klagsbrun and D’Amore, 1991). A significant observation is that tissues from tumours, corpora lutea, uteri and placentae induce a neovascular response in the CAM assay, whereas most other fetal or adult tissues do not (Redmer et al., 1988; Klagsbrun and D’Amore, 1991; Reynolds et al., 1992).

In contrast to in vivo techniques, in vitro assays evaluate the ability of a factor to influence one of the individual components of the angiogenic process. These assays test the ability of a substance to influence: (1) production of proteases by endothelial cells, (2) migration of endothelial cells, or (3) proliferation of endothelial cells (Folkman and Klagsbrun, 1987). Factors identified with these in vitro bioassays are likely to have similar effects in vivo, since close agreement has been shown among in vivo and in vitro assays for angiogenic factors (Folkman and Klagsbrun, 1987; Redmer et al., 1988; Reynolds et al., 1992). Nevertheless, the angiogenic activity of factors identified by using the in vitro assays need to be confirmed with one of the in vivo bioassays described above (Klagsbrun and D’Amore, 1991; Reynolds et al., 1992).

During the last decade, many growth factors and cytokines have been found in luteal tissues, luteal cells, or ‘luteinized’ granulosa cells in culture (reviewed by Reynolds et al., 1994). These include epidermal growth factor, insulin-like growth factors, nerve growth factor, transforming growth factors and tumour necrosis factor α. In addition, numerous studies have shown both positive and negative effects of these factors on luteal steroidogenesis (Reynolds et al., 1994). In contrast, relatively few studies have evaluated the effects of these growth factors and cytokines on proliferation of luteal cells. In our studies, we have focused on evaluating angiogenic growth factors produced by the corpus luteum during its rapid growth phase, as well as by the mature corpus luteum during the oestrous cycle and pregnancy (Reynolds et al., 1994).

In the course of these studies, as summarized below, a major finding was that the angiogenic activity present in media conditioned by bovine follicles and bovine and ovine corpora lutea binds relatively strongly to heparin-affinity columns (Grazul-Bilska et al., 1992, 1993, 1995; Reynolds et al., 1992, 1994). This observation has led us to hypothesize that these ovarian angiogenic factors belong to one of the families of heparin-binding angiogenic factors, namely the fibroblast growth factors (FGF) or the vascular endothelial growth factors (VEGF), rather than to one of the other families of growth factors found in the ovary (Reynolds et al., 1992, 1994). This hypothesis is consistent with the suggestion that FGF and VEGF are probably key mediators of the angiogenic process in a variety of tissues (Klagsbrun and D’Amore, 1991; Reynolds et al., 1992, 1994; Ferrara, 1993; Redmer et al., 1996).

Follicular angiogenic factors

Initially, it was reported that follicular tissue from rabbits treated with pregnant mares’ serum gonadotrophin (PMSG) had no influence on angiogenesis in the corneal pocket assay (Gospodarowicz and Thakral, 1978). Ovarian tissue from PMSG-treated rats, however, caused an angiogenic response in the CAM assay (Koos and LeMaire, 1983). Makris et al. (1984) reported subsequently that extracts of the thecal layer, but not the granulosa cell layer of pig follicles stimulated migration and proliferation of endothelial cells in vitro. This observation was potentially significant, since only the thecal layer of the ovarian follicle is well vascularized, whereas the granulosa layer is avascular (Moor and Seamark, 1986). Later reports indicated that media conditioned by granulosa cells stimulated migration (Redmer et al., 1985a) and proliferation (Koos, 1986) of endothelial cells.

Thus, the initial reports on angiogenic activity of ovarian follicles were inconclusive, and the relationships among stage of follicular development, gonadotrophin treatment, and follicular angiogenic activity were unclear. To evaluate these relationships, we stimulated preovulatory follicular growth in ewes either by inducing luteal regression or by gonadotrophin treatment, and thereby obtained follicles at known stages of development (Taraska et al., 1989). Media conditioned by thecal tissues stimulated proliferation of endothelial cells regardless of the stage of follicular development. However, granulosa cells produced an endothelial mitogen only when they were obtained from non-atretic follicles just before ovulation. We made similar observations for bovine preovulatory follicles (Redmer et al., 1991a) and concluded that production of angiogenic factors by granulosa cells may help maintain the vasculature and thereby the health of the preovulatory follicle, and may also participate in recruitment of blood vessels into the early corpus luteum (Redmer et al., 1991a; Zheng et al., 1993). However, thecal...
production of angiogenic factors is probably responsible for the follicular vasculization that supports growth and development of the follicle (Clark, 1900; Andersen, 1926; Taraska et al., 1989; Redmer et al., 1991a).

The endothelial mitogen produced by preovulatory bovine follicles is retained on heparin affinity columns and elutes with high-salt (3.0 mol NaCl l⁻¹) buffer (Fricke, 1991). As mentioned above, this observation is significant because previously identified heparin-binding angiogenic factors include FGF and VEGF, both of which are potent angiogenic factors that appear to play major roles in angiogenesis in numerous tissues (Folkman and Klagsbrun, 1987; Klagsbrun and D’Amore, 1991; Ferrara, 1993). In addition, mRNA encoding FGF-2 (basic FGF) has been found in extracts of bovine, but not rat, granulosa cells (Neufeld et al., 1987; Koos and Olson, 1989), and mRNA encoding VEGF is present in preovulatory follicles of monkeys and rats (Ravindranath et al., 1992; Koos, 1995). Determining the specific roles of these growth factors in follicular angiogenesis, however, will require further investigation.

Luteal angiogenic factors

Luteal tissues, tissue extracts and conditioned media from several mammals stimulate angiogenesis in the in vivo bioassays as well as the in vitro (endothelial cell migration and proliferation) bioassays (Redmer et al., 1985b; Reynolds et al., 1992). Bovine, ovine and porcine corpora lutea produce angiogenic factors throughout the oestrous cycle and early pregnancy (Reynolds et al., 1992, 1994; Ricke et al., 1995), indicating that corpora lutea produce an angiogenic factor(s) at all stages of luteal development. Although the significance of this observation remains unclear, we have hypothesized that angiogenic factors are involved not only in luteal vascular development but also in maintaining vascular beds of mature corpora lutea (Grazul-Bilska et al., 1992, 1995). Angiogenic factors could have other roles in differentiation and function of the corpus luteum (Reynolds et al., 1992, 1994), a possibility which we will address later in this review.

Luteinizing hormone (LH) stimulates production of an endothelial chemoattractant by corpora lutea from nonpregnant cows (Redmer et al., 1988). This effect of LH is blocked by the luteolytic hormone prostaglandin F₂α (PGF₂α), even though PGF₂α has no effect by itself. The effects of LH and PGF₂α on luteal production of angiogenic factors are similar to the effects of these hormones on expression of mRNA for FGF-2 (Stirling et al., 1991). These findings emphasize the potential importance of systemic hormones in regulating luteal vascular growth and development by affecting production of local angiogenic factors.

The angiogenic activities produced by bovine, ovine and porcine corpora lutea can be partially immunoneutralized with antibody against FGF-2 but not with anti-FGF-1 antibody (Doraiswamy et al., 1995a; Grazul-Bilska et al., 1995; Ricke et al., 1995). FGF-2 also has been detected in bovine, ovine and porcine luteal tissues and conditioned media (Grazul-Bilska et al., 1992; Zheng et al., 1993; Schams et al., 1994; Doraiswamy et al., 1995a; Ricke et al., 1995). During the oestrous cycle, expression of FGF-2 in bovine corpora lutea follows a pattern similar to that of angiogenic activity (Redmer et al., 1988; Stirling et al., 1991; Schams et al., 1994). Makris et al. (1989) reported the presence of at least four heparin-binding proteins, which were immunoreactive with antibodies against FGF-1 and FGF-2, in pooled homogenates of pig corpora lutea. In addition, the two major peaks of heparin-binding angiogenic activity produced by bovine and ovine corpora lutea elute from heparin-affinity columns at the approximate salt concentrations that would be expected for FGF-1 (acidic FGF) and FGF-2, respectively (Fig. 4; Grazul-Bilska et al., 1993, 1995). It seems likely, therefore, that FGF-1 and FGF-2 are major angiogenic factors in corpora lutea.

We have found that bovine corpora lutea from the oestrous cycle and ovine corpora lutea from early pregnancy each produce at least three heparin-binding endothelial mitogens, some of which do not appear to be FGF (Grazul-Bilska et al., 1993, 1995). The affinity of these factors for heparin is strikingly similar across the two species (Fig. 4). There is also good evidence that endothelial chemoattractants are present in bovine, ovine and porcine luteal-conditioned media (Redmer et al., 1988; Doraiswamy et al., 1995a; Grazul-Bilska et al., 1995; W.A. Ricke, L.P. Reynolds, and D.A. Redmer, unpublished). In addition, Rone and Goodman (1990) reported that a factor present in rat luteal-conditioned media is chemotactic but not mitogenic for endothelial cells and is probably not an FGF. It is therefore apparent that multiple angiogenic factors, some of which are distinct from known FGFs, are produced by corpora lutea. Thus, as in other tissues, the coordinated growth of vascular and nonvascular compartments of the developing corpus luteum is probably regulated by many factors (Folkman and Klagsbrun, 1987).

As for follicles, VEGF is an attractive candidate for one of these luteal angiogenic factors because it binds to heparin with an affinity similar to that of one of the angiogenic peaks identified (peak 2, Fig. 4), and is a specific growth factor for endothelial cells (Ferrara, 1993). We have shown that an antibody against VEGF will immunoneutralize the endothelial chemoattractant activity produced by early cycle but not mid- or late cycle ovine corpora lutea (Doraiswamy et al., 1995a). In addition, a portion (approximately 40%) of the endothelial mitogenic activity produced by pig corpora lutea was neutralized by VEGF antibody (Ricke et al., 1995). We have also shown by western analysis that VEGF is present in ovine luteal homogenates (Redmer et al., 1996). Moreover, we have shown by ribonuclease protection assay that amounts of mRNA encoding VEGF in ovine corpora lutea are two- to threefold greater early in the oestrous cycle (days 2–4) compared with mid- or late cycle (days 8–15; Redmer et al., 1996). More recently, we have localized VEGF to perivascular cells of the developing as well as the mature corpus luteum (Fig. 5). Taken together, these observations suggest that VEGF may play a major role in vascularization of the developing corpus luteum.

In monkey corpora lutea, mRNA encoding VEGF is present in greatest amounts in the early luteal compared with the late luteal phase, and is reduced by treatment with a GnRH antagonist (Ravindranath et al., 1992). In addition, hCG or LH can induce VEGF transcription in preovulatory rat follicles and in bovine granulosa cells undergoing luteinization (Garrido et al., 1993; Koos, 1995). Phillips et al. (1990) also have found mRNA encoding VEGF in rat corpora lutea, and others have demonstrated its presence in luteinized human granulosa cells (Yan et al., 1993; Kamat et al., 1995). These observations provide
Fig. 3. Histological sections of mid-luteal bovine corpora lutea stained (a) histochemically with haematotoxylin and periodic acid–Schiff’s reagent and (b) immunohistochemically for Factor VIII (von Willebrand factor), both of which have been used to localize capillaries (Reynolds et al., 1992; Zheng et al., 1993). In (a) and (b), note that most of the parenchymal cells (a few are identified with arrowheads) are in contact with one or more capillaries (a few are identified with arrows). There is intense staining of the walls of the larger microvessels in the upper right corner of (a). Both micrographs are at the same magnification; scale bar represents 25 µm. Adapted from Zheng et al. (1993).
further support for the hypothesis that VEGF is an important regulator of ovarian vascular growth, especially in the developing corpus luteum.

Additional considerations
On the basis of the observation that some luteal angiogenic factors are produced throughout the lifespan of the corpus luteum, we have suggested that they play additional roles in luteal differentiation and function (Reynolds et al., 1992, 1994). This proposal seems reasonable because numerous angiogenic factors, including the FGF and VEGF, have other effects on cell differentiation and function (Klagsbrun and D’Amore, 1991). For example, VEGF was formerly known as vascular permeability factor, or VPF, because of its ability to increase vascular permeability in several tissues (Klagsbrun and D’Amore, 1991; Ferrara, 1993). VEGF could play a similar role in the developing corpus luteum. A possibility for FGFs is that

Fig. 4. Heparin–agarose affinity chromatograms of luteal explant-conditioned media from (a) ewes on day 24 of pregnancy and (b) nonpregnant cows during the mid-luteal phase. After the explant-conditioned media were applied to the heparin-affinity column, fractions were eluted with a salt (NaCl) gradient (○), and each fraction was evaluated for its mitogenic effects on endothelial cells (●) and also for its protein concentration (★). Note that peaks 2 and 3 elute at salt molarities that would be expected for fibroblast growth factor-1 (FGF-1) (or vascular endothelial growth factor) and FGF-2 (approximately 1.2 and 2.0 mol NaCl l⁻¹, respectively). (a) and (b) reproduced with permission from Grazul-Bilska et al. (1995) and (1993), respectively.
they protect luteal cells from undergoing cell death, as has been reported for other cell types (Tilly et al., 1992; Yasuda et al., 1995).

FGF receptor 1 (FGFR-1) is present at high concentrations in luteal parenchymal cells during early to mid-cycle but is reduced markedly during luteal regression (Fig. 6; Doraiswamy et al., 1995b). In contrast, FGFR-1 is present in luteal endothelial cells at all stages, and is especially abundant in those of the larger luteal microvessels late in the oestrous cycle (Fig. 6). Although luteal FGFR-2 is present in parenchymal cells at all stages, it is present in the vasculature only late in the oestrous cycle, and then only in the larger microvessels (Fig. 6). As mentioned previously, the larger microvessels of the corpus luteum are maintained or may even increase in abundance late in the cycle. If FGF inhibits luteal cell death, a selective increase in FGFR in the endothelial cells of the larger microvessels would explain how they are maintained while the other luteal tissues regress. We have therefore hypothesized that FGF may affect not only luteal cell proliferation but also luteal cell death, thereby regulating luteal cell turnover and function.

As mentioned earlier, growth and vascular development of the ovarian tissues are extremely rapid but limited, and therefore must be tightly regulated. In addition, growth of the vascular beds must be closely coordinated with tissue growth to ensure normal function of the mature ovarian tissues (Klagsbrun and D’Amore, 1991; Reynolds et al., 1992). So far we have discussed coordination of ovarian vascular growth by systemic and local humoral factors. Another important means of coordinating function among populations of ovarian cells, however, is by contact-dependent mechanisms, which involve direct coupling of the cells via gap junctions (Redmer et al., 1991b; Grazul-Bilska et al., 1994).

We have shown that connexin 43, a major gap junctional protein, is present in the developing vasculature as well as the parenchyma of the early cycle corpus luteum (Fig. 7). Luteal parenchymal cells can communicate with endothelial cells via gap junctions, and the rate of communication between these cells can be regulated by luteotrophic and luteolytic hormones (Redmer et al., 1991c). These observations are intriguing because during neovascularization, including that of the developing corpus luteum, capillary sprouts migrate through the basement membrane of the existing blood vessels into the surrounding tissues and initially form naked capillary tubes that are devoid of basement membranes (Folkman and Klagsbrun, 1987; Zheng et al., 1993). Thus, during early luteal development, direct contact between parenchymal cells and endothelial cells is likely to occur, which would make it possible to coordinate their growth and development via gap junctional communication.

In contrast to the developing corpus luteum, the microvasculature of the mature corpus luteum displays a well-established basement membrane that separates the endothelial cells from the surrounding parenchymal cells (Zheng et al., 1993). However, the capillary basement membranes of some mature adult tissues have pores large enough to allow contact between endothelial cells and the surrounding cells (Carlson, 1989). Although direct cell–cell contact across luteal capillary basement membranes has not been reported, the microvasculature of the mature corpus luteum displays an extremely high permeability, which is thought to result from large pores or ‘gaps’ present in the luteal capillaries (Morris and Sass, 1966;
Fig. 6. Immunohistochemical localization of fibroblast growth factor receptor 1 (FGFR-1) (flg; a–c) and FGFR-2 (bek; d–f) in ovine corpora lutea from the early (a and d), mid- (b and e), and late (c and f) luteal stages of the oestrous cycle. In (a–c) note the intense localization of FGFR-1 in smaller (arrows) and larger (*) microvessels at all stages; also note the intense staining for FGFR-1 in parenchymal cells (arrowheads) of early and mid-luteal corpora lutea (a and b), but not in those of the late luteal stage (c). In (d–f) note the staining for FGFR-2 in parenchymal cells (arrowheads) at all stages; also note the localization of FGFR-2 in larger microvessels (*) only in late luteal corpora lutea (f). All micrographs are at the same magnification; scale bar represents 25 µm. Unpublished observations of V. Doraiswamy, L.P. Reynolds and D.A. Redmer.
ment and differentiated function of luteal vascular and non-vascular elements. Note the relative abundance of Cx43 staining in cells of the interstitial areas (arrows) and potential gap junctional connections between interstitial cells and parenchymal cells (arrowheads). Scale bar represents 25 μm. Unpublished observations of A.T. Grazul-Bilska, J. Bilski, L.P. Reynolds and D.A. Redmer.

Ellinwood et al., 1978). We and others have observed that the intercellular space separating luteal endothelial cells from the surrounding parenchyma is unusually narrow (about 70 nm; Morris and Sass, 1966; D.A. Redmer, E.C. Carlson and L.P. Reynolds, unpublished). Moreover, we have demonstrated gap junction-mediated intercellular communication between parenchymal and endothelial cells of mature bovine and ovine corpora lutea (Redmer et al., 1991b, c; Grazul-Bilska et al., 1994). Thus, contact-dependent communication via gap junctions may be an important mechanism for coordinating growth, development and differentiated function of luteal vascular and non-vascular tissues.

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References

Key references are identified by asterisks.

Andersen DH (1926) Lymphatics and blood vessels of the ovary of the sow Contributions to Embryology 17 107–123


Bassett DL (1943) The changes in the vascular pattern of the ovary of the albino rat during the estrous cycle American Journal of Anatomy 73 251–291

Burr JH and Davies JH (1951) The vascular system of the rabbit ovary and its relationship to ovulation Anatomical Record 111 273–297


Clark JG (1980) The origin, development and degeneration of the blood vessels of the human ovary Johns Hopkins Hospital Report 9 593–676


Dharmarajan AM, Bruce NW and Meyer GT (1985) Qualitative ultrastructural characteristics relating to transport between luteal cell cytoplasm and blood in the corpus luteum of the pregnant rat American Journal of Anatomy 172 87–99

Doraiswamy V, Grazul-Bilska AT, Ricke WA, Redmer DA and Reynolds LP (1995a) Immunoneutralization of angiogenic activity from ovine corpora lutea (CL) with antibodies against fibroblast growth factor (FGF)-2 and vascular endothelial growth factor (VEGF) Biology of Reproduction 52 (Supplement 1) 112


Farin CE, Moeller CL, Sawyer HR, Gamboni F and Niswender GD (1986) Morphometric analysis of cell types in the ovine corpus luteum throughout the estrous cycle Biology of Reproduction 35 1299–1308


Gospodarowicz D and Thakral KK (1978) Production of a corpus luteum angiogenic factor responsible for proliferation of capillaries and neovascularization of the corpus luteum Proceedings of the National Academy of Sciences USA 75 847–851

Grazul-Bilska AT, Redmer DA, Killilea SD, Kraft KC and Reynolds LP (1992) Production of mitogenic factors(s) by ovine corpora lutea throughout the estrous cycle Endocrinology 130 3625–3632


Greenwald GS (1989) Temporal and topographic changes in DNA synthesis after induced follicular atresia Biology of Reproduction 41 175–181

Jablonka-Shariff A, Grazul-Bilska AT, Redmer DA and Reynolds LP (1993) Growth and cellular proliferation of ovine corpus lutea throughout the estrous cycle Endocrinology 133 1871–1879


Koos, RD (1986) Stimulation of endothelial cell proliferation by rat granulosa cell-conditioned media Endocrinology 119 481–489


Fig. 7. Immunohistochemical localization of connexin (Cx) 43 in an ovine corpus luteum from the early luteal stage (day 4) of the oestrous cycle. Note the relative abundance of Cx43 staining in cells of the interstitial areas (arrows) and potential gap junctional connections between interstitial cells and parenchymal cells (arrowheads). Scale bar represents 25 μm. Unpublished observations of A.T. Grazul-Bilska, J. Bilski, L.P. Reynolds and D.A. Redmer.

Koos RD and Olsen CE (1989) Expression of basic fibroblast growth factor in the rat ovary: detection of mRNA using reverse transcription-polymerase chain reaction amplification Molecular Endocrinology 3 2041–2048


Redmer DA, Rone JD and Goodman AL (1985a) Detection of angiotropic activity from primate dominant follicles 67th Annual Meeting of the Endocrine Society p 151, Abstract 604


Redmer DA, Grazul-Bilska AT and Reynolds LP (1991b) Contact-dependent intercellular communication of bovine luteal cells in culture Endocrinology 129 2757–2766

Redmer DA, Grazul-Bilska AT and Reynolds LP (1991c) Contact-dependent intercellular communication between bovine luteal cells and endothelial cells in culture Biology of Reproduction 44 (Supplement 1) 110


*Reynolds LP, Killilea SD and Redmer DA (1992) Angiogenesis in the female reproductive system FASEB Journal 6 886–892

*Reynolds LP, Killilea SD, Grazul-Bilska AT and Redmer DA (1994) Mitogenic factors of corpora lutea Progress in Growth Factor Research 5 159–175

Ricke WA, Redmer DA and Reynolds LP (1995) Initial characterization of mitogenic factors produced by porcine corpora lutea throughout the estrous cycle Biology of Reproduction 52 (Supplement 1) 112

Rone JD and Goodman AL (1990) Preliminary characterization of angiogenic activity in media conditioned by cells from luteinized rat ovaries Endocrinology 127 2821–2828


Swann RT and Bruce NW (1987) Oxygen consumption, carbon dioxide production and progesterone secretion in the intact ovary of the day-16 pregnant rat Journal of Reproduction and Fertility 80 599–605


Tilly JI, Billig H, Kowalski KI and Hsuheh AJW (1992) Epidermal growth factor and basic fibroblast growth factor suppress the spontaneous onset of atrophy in cultured rat ovarian granulosa cells and follicles by a tyrosine kinase-dependent mechanism Molecular Endocrinology 6 1942–1950


