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Angiogenesis in the ovary

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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.

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 most 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, and Folkman and co-workers were the first to demonstrate that recruitment of a blood supply is requisite for sustained growth of tumours (reviewed by Folkman and Klagsbrun, 1987 and Klagsbrun and D'Amore, 1991). In addition, vascular endothelial cells of growing tumours exhibit an extremely high mitotic rate compared with endothelial cells of most normal tissues (Denekamp, 1984). Conversely, insufficient capillary growth occurs in several disease states, including delayed wound healing, nonhealing fractures and chronic varicose ulcers (Folkman and Klagsbrun, 1987).

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; Bassett, 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

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rapidly growing and aggressive tumours (Fig. 1; Denekamp, 1984; Jablonka-Shariff *et al.*, 1993, 1994; Zheng *et al.*, 1994; Christenson and Stouffer, 1995; Nicosia *et al.*, 1995). When fully mature, therefore, the ovarian, uterine and placental tissues are highly vascular and receive some of the greatest rates of blood flow, on a weight-specific basis, of any tissues in the body (Ellinwood *et al.*, 1978; Reynolds *et al.*, 1992, 1994; Reynolds and Redmer, 1995). The extremely high vascularity and rate of blood flow of these tissues are reflected by their high metabolic rates (Swann and Bruce, 1987; Reynolds and Redmer, 1995). Because the tissues of the female reproductive system are so dynamic, they provide a unique model for studying the regulation of angiogenesis during growth, differentiation and regression of normal adult tissues.

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 vascularization 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 thecal endothelial cells, associated with a decrease in thecal vascularity, soon after the onset of atresia in bovine, ovine and porcine follicles (Jablonka-Shariff *et al.*, 1994; 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).

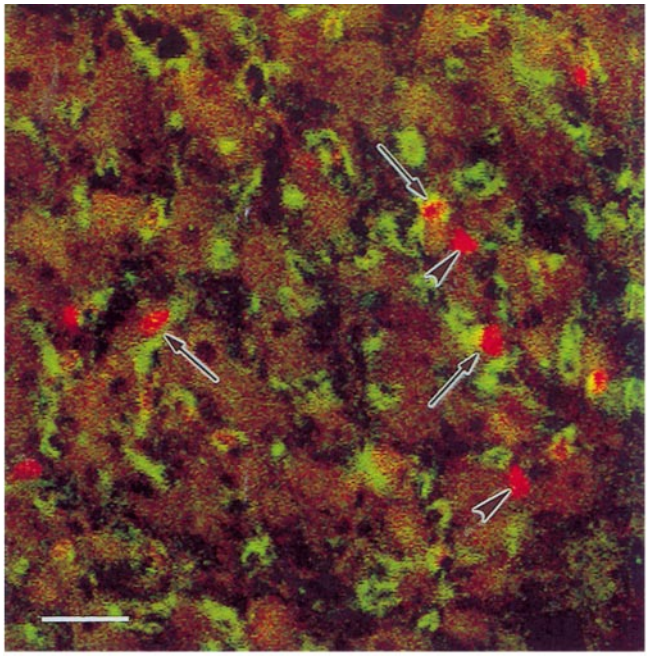
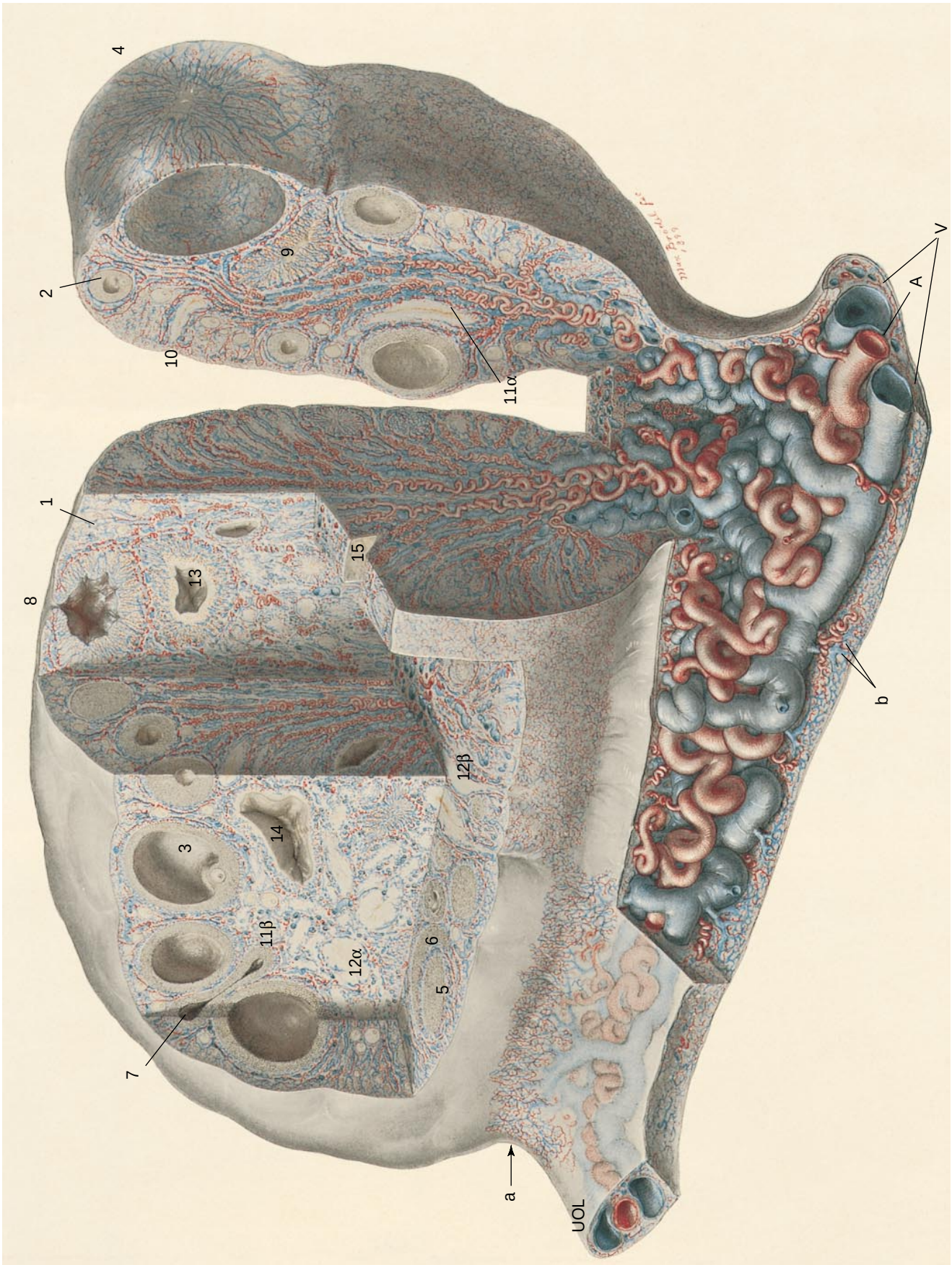


Fig. 1. Dual immunofluorescence localization of factor VIII (a marker for vascular endothelial cells; green fluorescence) and BrdU (a marker for proliferating cells; red fluorescence) in a section of a corpus luteum from a ewe on day 8 of the oestrous cycle. Note that, on the basis of colocalization 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.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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 used 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 thus 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

Fig. 2. The drawing of the vascular anatomy of a mature, functional human ovary represents a reconstruction of serial sections of the ovary after injection of the vasculature with gelatin solution. A: ovarian artery; V: ovarian veins; UOL: utero–ovarian ligament (mesovarium); a: germinal epithelium; b: microvessels supplying the mesovarium; 1–3: progressive stages in follicular development; 4: Graafian follicle with well vascularized theca and granulosa thinned to the point where it is not visible; 5–7: various other developing follicles; 8: corpus haemorrhagicum; 9: corpus luteum; 10–11: regressing corpora lutea; 12: hyaline bodies (*authors note* - probably corpora albicantia); 13–14: cystic follicles; 15: corpus albicans. Reproduced from Clark (1900) with permission.

production of angiogenic factors is probably responsible for the follicular vascularization 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 $F_{2\alpha}$ ($PGF_{2\alpha}$), even though $PGF_{2\alpha}$ has no effect by itself. The effects of LH and $PGF_{2\alpha}$ 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

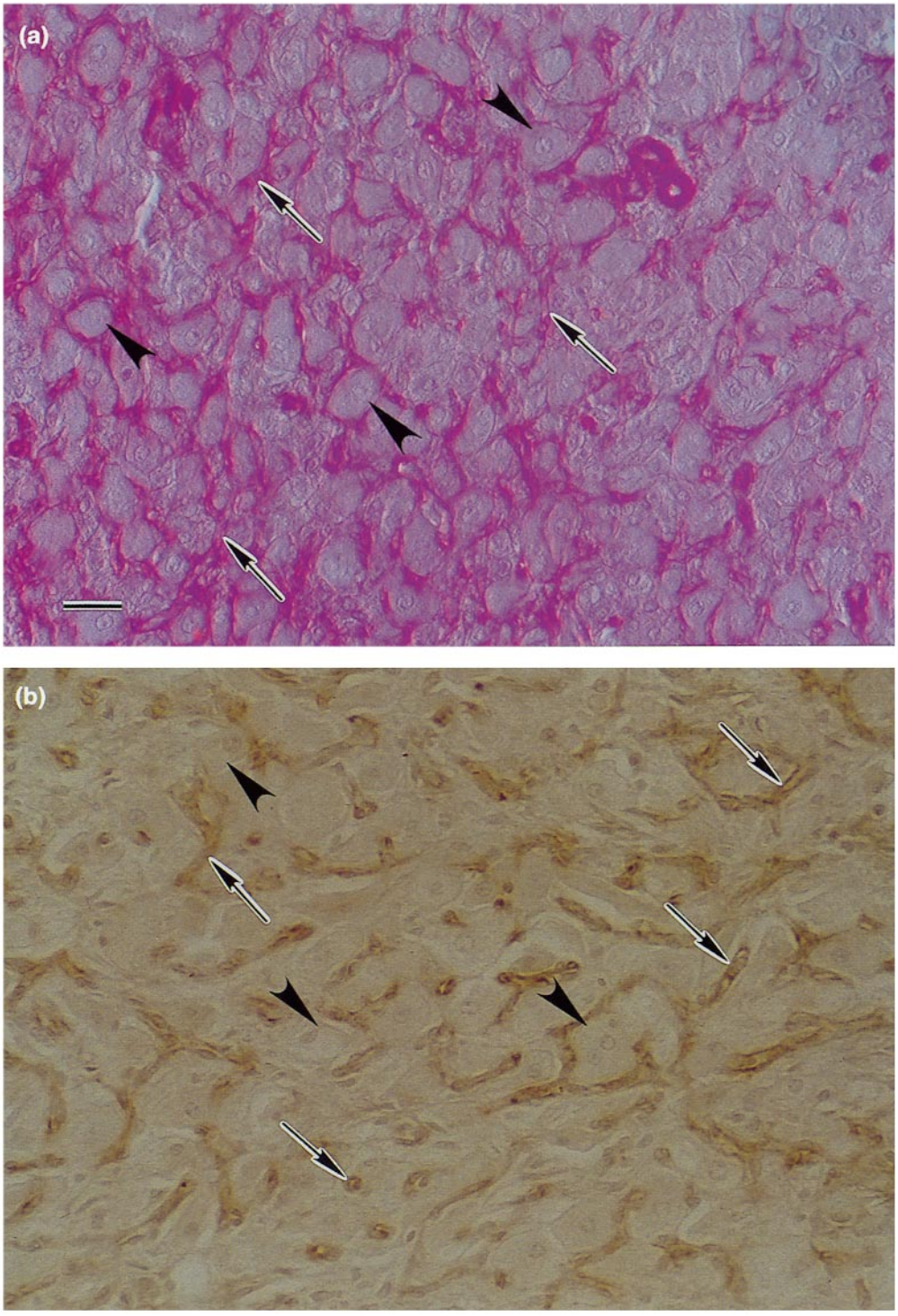

Fig. 3. Histological sections of mid-luteal bovine corpora lutea stained (a) histochemically with haematoxylin and periodic acid–Schiff's reagent and (b) immunohistochemically for Factor VIII (von Willebrand factor), both of which have been used to localize capillaries (Reynolds *et al.*, 1992; Zheng *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).

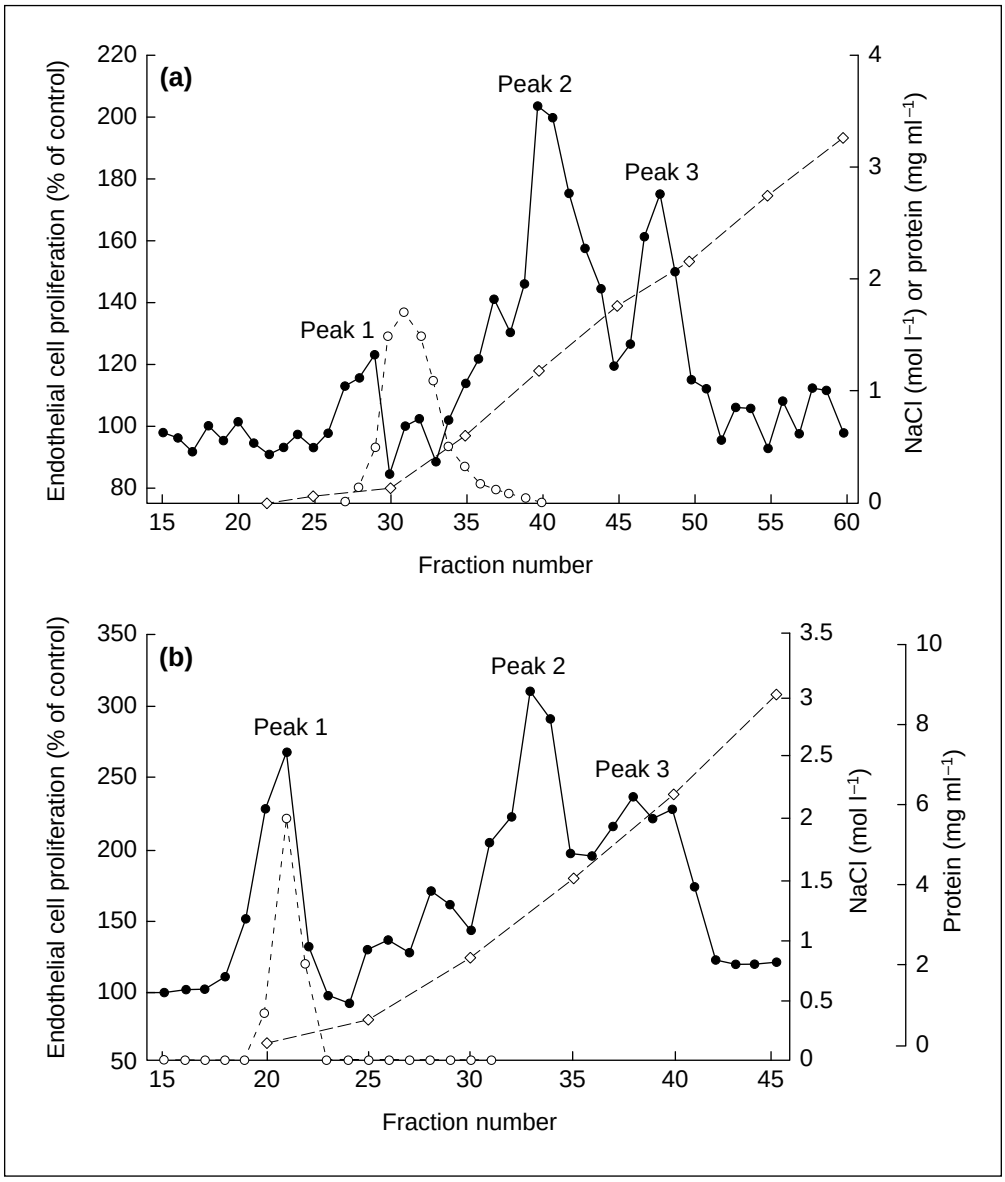

Fig. 4. Heparin–agarose affinity chromatograms of luteal explant-conditioned media from (a) ewes on day 24 of pregnancy and (b) nonpregnant cows during the mid-luteal phase. After the explant-conditioned media were applied to the heparin-affinity column, fractions were eluted with a salt (NaCl) gradient (◇), 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.

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

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;

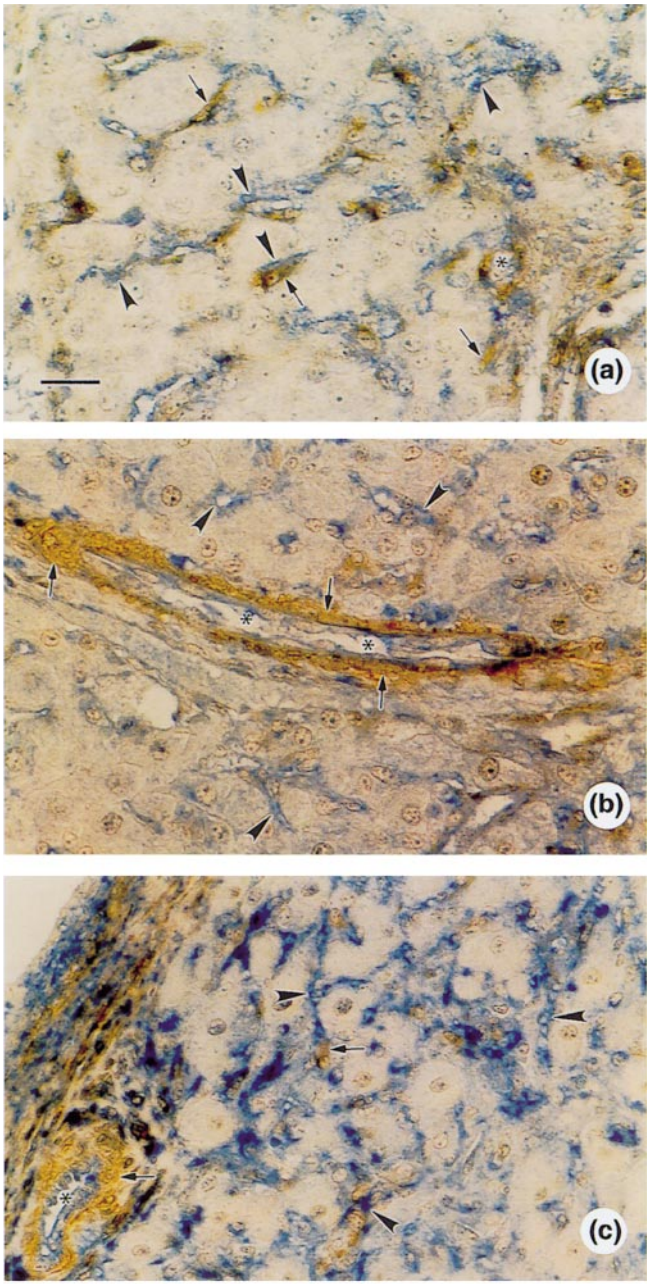

Fig. 5. Immunohistochemical localization of VEGF (vascular endothelial growth factor, brownish staining) and lectin BS-1 (marker for endothelial cells, bluish staining; Augustin *et al.*, 1995) in ovine corpora lutea from early (a, day 2; b, day 4) and mid- (c, day 10) luteal stages of the oestrous cycle. In (a–c) for the most part, the brownish cytoplasmic staining for VEGF (arrows) does not correspond to the bluish lectin staining for endothelial cells (arrowheads), but rather seems to be primarily perivascular. This perivascular staining for VEGF is most apparent in the larger microvessels (*). The relative abundance of VEGF stained cells appears to decrease, whereas that of lectin stained endothelial cells appears to increase from the early (a and b) to the mid-luteal (c) stage. All micrographs are at the same magnification; scale bar represents 25 μm. Unpublished observations of V. Doraiswamy, A.T. Grazul-Bilska, R.M. Moor, L.P. Reynolds and D.A. Redmer.

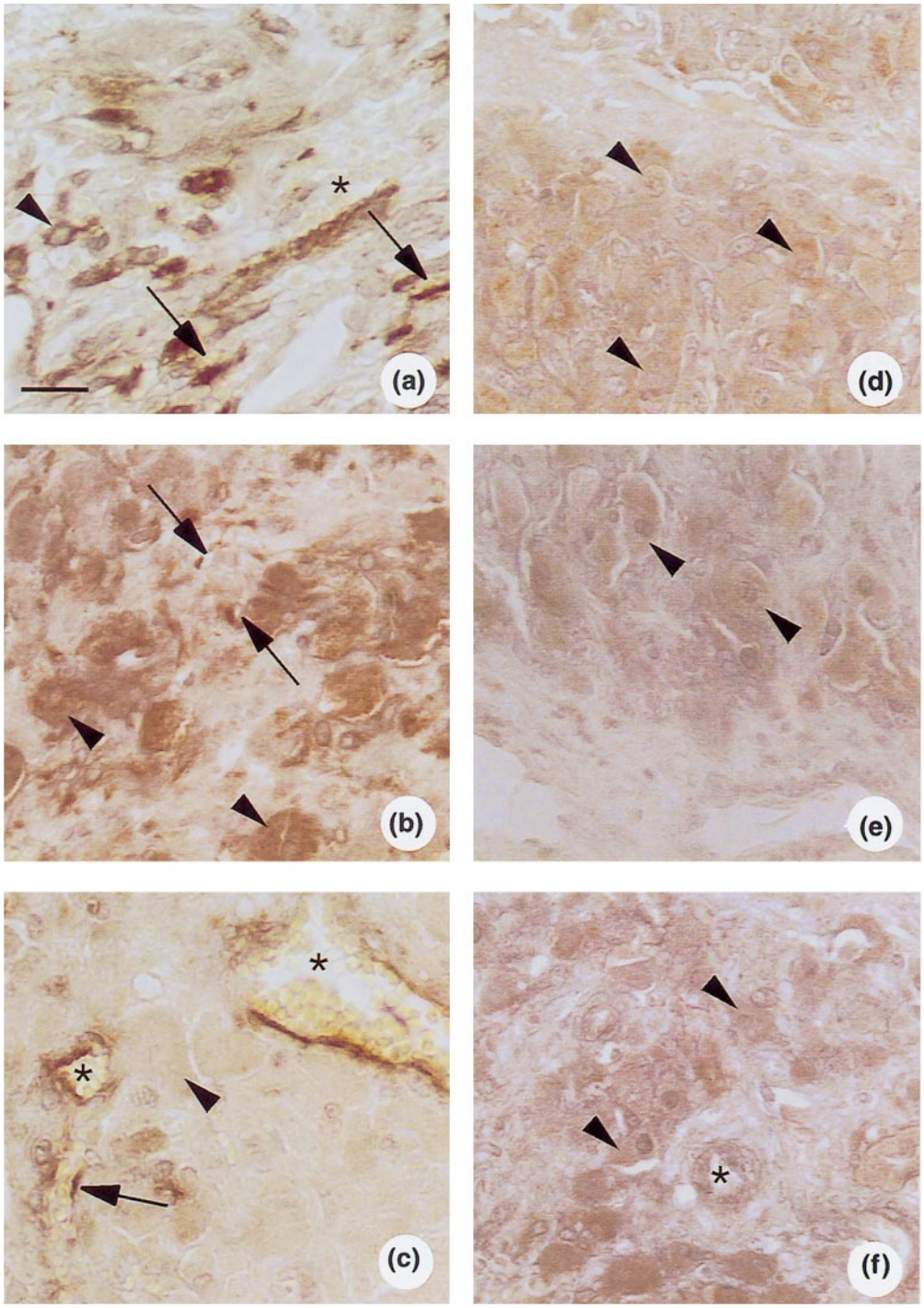

Fig. 6. Immunohistochemical localization of fibroblast growth factor receptor 1 (FGFR-1) (*flg*; a–c) and FGFR-2 (*bek*; d–f) in ovine corpora lutea from the early (a and d), mid- (b and e), and late (c and f) luteal stages of the oestrous cycle. In (a–c) note the intense localization of FGFR-1 in smaller (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.

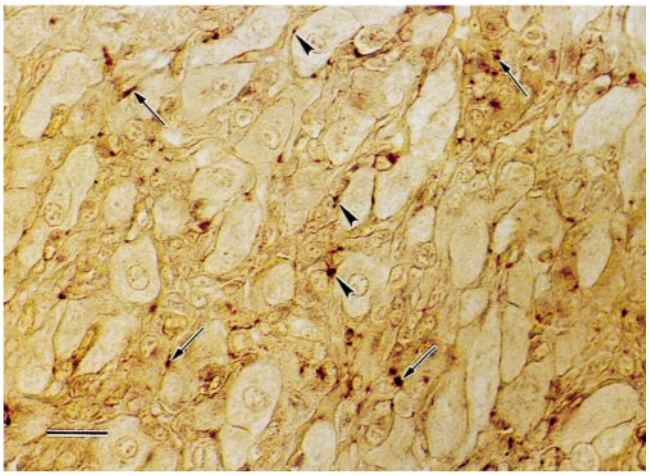

Fig. 7. Immunohistochemical localization of connexin (Cx) 43 in an ovine corpus luteum from the early luteal stage (day 4) of the oestrous cycle. Note the relative abundance of Cx43 staining in cells of the interstitial areas (arrows) and potential gap junctional connections between interstitial cells 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.

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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