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REVIEW

Angiogenesis in the Corpus Luteum

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The ovarian corpus luteum plays a critical role in reproduction because it is the primary source of circulating progesterone. After ovulation, as the corpus luteum forms from the wall of the ruptured follicle, it grows and vascularizes extremely rapidly. In fact, the rates of tissue growth and angiogenesis in the corpus luteum rival those of even the fastest growing tumors. Thus, the corpus luteum provides an outstanding model for studying the factors that regulate the angiogenic process, which is critical for normal tissue growth, development, and function. In agreement with data from other tissues, vascular endothelial growth factors (VEGF) seem to be a major angiogenic factor responsible for vascularization of the developing corpus luteum. Recent data suggest that luteal expression of VEGF occurs primarily in specific perivascular cells, including arteriolar smooth muscle and capillary pericytes, and is regulated primarily by oxygen levels. In addition, soon after ovulation, pericytes derived from the thecal compartment appear to be the first vascular cells to invade the developing luteal parenchyma. The granulosa-derived cells produce a factor that stimulates pericyte migration. Moreover, nitric oxide (NO), which is a potent vasodilator and can stimulate VEGF production and angiogenesis, is expressed in endothelial cells of luteal arterioles and capillaries, often in association with expression of VEGF by luteal perivascular cells. Thus, we have proposed a model for the initial process of luteal vascularization in which hypoxia plays a major role. In this model, which we believe will apply to other tissues as well, a paracrine loop exists between the vascular endothelial cells, which produce NO, and the peri-endothelial cells (vascular smooth muscle and pericytes), which produce VEGF, to ensure coordinate regulation of luteal vasodilation and angiogenesis.

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"Capillary growth is rarely observed in normal adult tissues except in tissues that show cyclical growth such as the corpus luteum in the ovary, the endometrium, hair follicles and deer antlers." O. Hudlická, 1984.

Introduction

As recognized by Hudlická *(1)*, capillary growth in normal adult tissues is indeed a rare event, and the endothelium of most tissues represents an extremely stable population of cells with a low mitotic activity *(2)*. This observation is not surprising, because physiological angiogenesis is normally associated only with tissue growth or repair, and most adult tissues are relatively stable *(1,3)*.

On the other hand, rampant or persistent capillary growth is associated with numerous pathological conditions, including tumor growth, retinopathies, hemangiomas, fibroses, and rheumatoid arthritis *(3,4)*. For example, recruitment of a blood supply is requisite for sustained growth of tumors *(4)*. In addition, vascular endothelial cells of growing tumors exhibit an extremely high mitotic rate compared with endothelial cells of most normal tissues *(2)*. Conversely, insufficient capillary growth occurs in several disease states, including delayed wound healing, nonhealing fractures, and chronic varicose ulcers *(4)*.

By contrast, the tissues of the adult female reproductive system, including the uterus, placenta, and ovary, grow extremely rapid and at regular intervals *(5–8)*. For example, during its growth phase, which lasts about 8–10 d in large mammals, the ovarian corpus luteum doubles in size and cell numbers, every 60–70 h *(6)*. This phenomenal growth rate is associated with an extremely high rate of cell proliferation and is equaled only by the fastest growing tumors *(6,9)*. Unlike that of tumors, however, growth of the female reproductive tissues is normally a self-limiting and highly ordered process *(6)*.

To support the phenomenal rate of tissue growth, microvascular growth and development also are extremely rapid in the female reproductive tissues, these tissues are

highly vascular when mature *(5–8,10)*. For example, most (≈50–85%) of luteal cell proliferation occurs in the microvascular compartment *(6,8,11–13)*. As a result, in the mature corpus luteum microvascular pericytes and endothelial cells comprise 50–70% of the total cell population *(14,15)*. In association with their high vascularity, the female reproductive tissues, and especially the corpus luteum, also receive some of the greatest rates of blood flow, per unit of tissue, of any adult organ and exhibit a high metabolic rate *(7,8,10,16–18)*.

Because they exhibit regular intervals of dramatic tissue growth and angiogenesis, we have proposed that the tissues of the female reproductive system can serve as models to study not only reproductive function but also tissue growth in general. If we can understand regulation of the angiogenic process in these tissues, we should gain a better understanding of angiogenesis that occurs during normal tissue growth as well as abnormalities of the angiogenic process that occur in various pathological conditions.

Ovarian Angiogenesis

During the first half of the twentieth century, numerous investigators noticed the high vascularity of the ovarian tissues and concluded that angiogenesis must be a critical component of follicular and luteal function (for a review, *see* ref. *7*). In evaluating vascular development in the human ovary, Clark *(19)* 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." Similarly, in their classic study of the vascular system of the chicken ovary, Nalbandov and James *(20)* observed that "the spatial relationship of the growing follicles to the surrounding blood vessels is such that it is logical to conclude that an adequate vascular system is essential for the growth of a follicle."

More recent work has supported the concept that maintenance of the follicular vasculature is important for maintaining follicular health. For example, dominant follicles of sheep and monkeys not only have more vascular thecae, but also an increased uptake of serum gonadotropins compared with other antral follicles, whereas vascularity of atretic follicles is reduced *(21–24)*. Moor and Seamark *(25)* observed that early atretic follicles of sheep will regenerate when placed in vitro, and concluded that in vivo, atresia was caused by decreased vascularity, which resulted in limited access of the follicles to nutrients, substrates, and tropic hormones resulting from decreased vascularity. Greenwald *(26)* found that one of the earliest signs of atresia in hamsters was reduced DNA synthesis of follicular endothelial cells, which was associated with reduced follicular vascularity. 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 ([*27*]; P.M. Fricke, J. J. Ford, D.A. Redmer, and L.P. Reynolds, unpublished observations).

Thus, increased thecal vascularity may be a primary determinant of follicular dominance and, conversely, reduced thecal vascularity appears to be an important component of follicular atresia. In support of these concepts, although thecal tissues of sheep and cows produce angiogenic activity regardless of follicular status, only the granulosa cells of the estrogen-active, presumably healthy, preovulatory follicles produce angiogenic activity, whereas those of estrogen-inactive follicles do not *(28,29)*. Based on these observations, we concluded that while the theca of all antral follicles produces angiogenic activity, production of angiogenic factors by the avascular granulosa cells probably contributes to maintaining thecal vascularity and thus the health of the preovulatory follicles *(28,29)*.

Microvascular development of the follicular wall becomes even more extensive after ovulation, in association with vascularization of the corpus luteum *(7,8)*. The corpus luteum becomes so vascular, in fact, that the majority of the parenchymal (steroidogenic) cells of the mature corpus luteum are in contact with one or more capillaries *(5,7,8,30)*. In addition, the mature corpus luteum also receives most of the ovarian blood supply, and ovarian blood flow is highly correlated with the rate of progesterone secretion *(6,16,31)*.

Conversely, inadequate or abnormal luteal function, which occurs in domestic animals during various physiological conditions, including puberty, resumption of ovarian cyclicity postpartum, the beginning of the breeding season, and after induced ovulation, has been suggested to result from inadequate luteal vascularization *(32–37)*. Similarly, inadequate luteal function in humans, which occurs relatively frequent and is recognized as a luteal phase defect or deficiency, is associated with reduced vascularity of the corpus luteum *(38–41)*.

Luteal Angiogenic Factors

Previous Studies

For more than 20 yr, corpora lutea have been known to contain angiogenic activity (for a review, *see* ref. *7*). In fact, the corpus luteum is one of the most angiogenic tissues known, and stimulates angiogenesis in a variety of in vitro and in vivo assays (Fig. 1, Table 1) *(7,42,43)*. A bewildering array of potential angiogenic growth factors and their receptors are present in the corpus luteum, including angiopoietins, epidermal growth factor, fibroblast growth factors (FGFs), insulin-like growth factors, nerve growth factor, transforming growth factors, tumor necrosis factors, and vascular endothelial growth factors (VEGFs) *(6,7,44–46)*.

To determine which are the major angiogenic factors of the corpus luteum, we have evaluated in vitro production of angiogenic activity by growing, early cycle corpora lutea as well as mature corpora lutea during the estrous cycle and pregnancy (*see* ref. *7*). Across several of these studies in cows, pigs, and

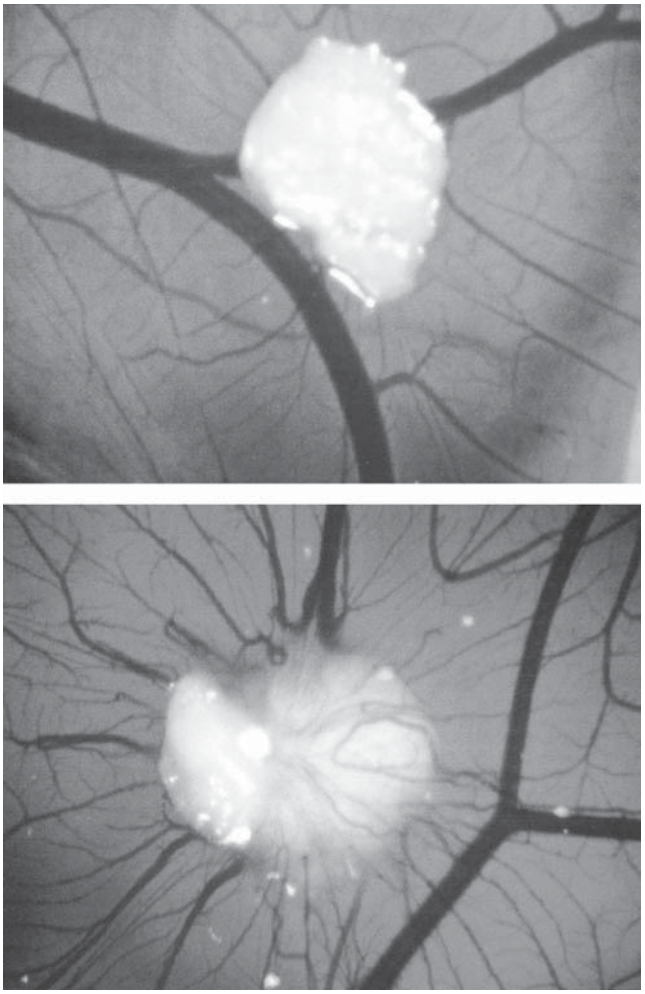

Fig. 1. Vascular response of the chicken chorioallantoic membrane (CAM) to bovine fetal muscle **(top)** and luteal **(bottom)** tissue implants. Note that the luteal tissue stimulates the classic spoke-wheel pattern of vascular invasion, whereas the fetal muscle causes a minimal vascular response (Adapted from ref. *43*; *see* Table 1.)

Table 1
Angiogenic Activity of Bovine Corpora Lutea from Several Stages of Estrous Cycle[a]

Assay[b]	Stage of estrous cycle		
	Early	Mid	Late
Endothelial cell proliferation (% of controls)	136 ± 14	160 ± 8	197 ± 15
Endothelial cell migration (% of controls)	176 ± 6	173 ± 8	204 ± 14
CAM assay (graded response)	1.9 ± 0.2	2.4 ± 0.2	3.0 ± 0.1

[a] Data are taken from ref. *43* and represent the mean ± SE for $n = 9$ to 10 cows per stage.

[b] Data for endothelial cell proliferation and migration assays represent the response of the cells to conditioned media from luteal tissue explant cultures and are expressed as percentage of controls (unconditioned media). Data for the CAM assay, which is an in vivo assay for angiogenesis, are expressed as a graded response on a scale from 0 (no response) to 4 (an exceptional response), with control tissue (fetal muscle) causing a minimal response (0.5 ± 0.2, $n = 5$; *see* Fig. 1).

sheep, a major finding was that all of the activity present in media conditioned by luteal tissues binds quite strongly to heparin-affinity columns (Fig. 2) *(5,7)*. Based on this observation, we hypothesized that the major ovarian angiogenic factors belong to one of the families of heparin-binding angiogenic factors, namely the FGFs or the VEGFs. This hypothesis is consistent with the recent suggestion that the FGFs and VEGFs are probably key mediators of the angiogenic process in a variety of tissues *(3,46,47)*.

The angiogenic activity produced by bovine and ovine corpora lutea elutes from heparin-affinity columns at about the same NaCl concentrations as the major FGFs, namely acidic FGF (aFGF, or FGF-1) and basic FGF (bFGF, or FGF-2) *(48,49)*. In addition, we and others have shown in several studies that aFGF and bFGF proteins are present in bovine and ovine corpora lutea *(50–53)*. We also have shown that aFGF and bFGF stimulate proliferation of ovine luteal cells from several stages of the estrous cycle, but especially those from early in the cycle *(54)*. Moreover, Stirling et al. *(55)* have shown that the pattern of expression of bFGF mRNA by bovine corpora lutea closely follows that of angiogenic activity production *(43)*, and also is stimulated by luteinizing hormone (LH) as is secretion of angiogenic activity *(55,56)*.

When we attempted to immunoneutralize the endothelial mitogenic activity produced by the cow, pig, and sheep corpora lutea, antibodies against aFGF neutralized a relatively small amount (≈16%) of the angiogenic activity, whereas antibodies against bFGF neutralized a large amount (≈82%) of this activity *(48–50,57,58)*. We concluded, therefore, that bFGF and not aFGF was probably a major FGF involved in luteal angiogenesis *(6,7,48,49)*.

If bFGF is an important luteal angiogenic factor, however, it was not clear why similar amounts of its protein or mRNA were present across all stages of the estrous cycle *(48,50,51,55,58)*, since luteal angiogenesis occurs primarily early in the estrous cycle *(7,51,59)*. We therefore evaluated luteal expression of FGF receptors (FGFRs). Expression of both FGFR-1 (flg) and FGFR-2 (bek) proteins increased with the age of the corpus luteum; that is, their expression was least in the early cycle and greatest in the late cycle corpora lutea *(60)*. By contrast, immunohistochemical localization of FGFR-1 protein to the luteal parenchymal cells was greatest during mid cycle and was dramatically reduced during luteal regression. However, strong staining for FGFR-1 was present in luteal endothelial cells at all stages, and was especially abundant in those of the larger luteal microvessels late in the estrous cycle. FGFR-2 protein was present in parenchymal cells at all stages but was present in the vasculature, primarily the larger microvessels, only late in the estrous cycle *(60)*.

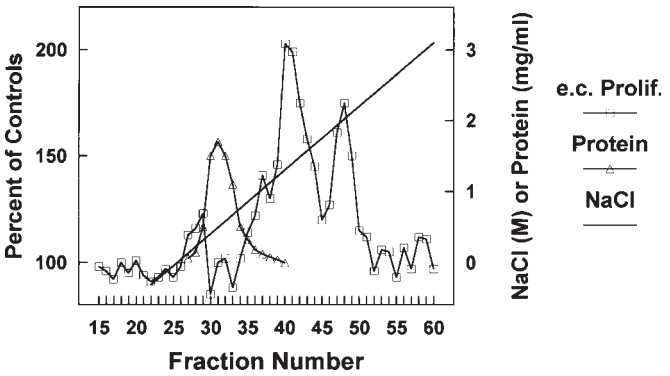

Fig. 2. Heparin-affinity chromatogram of a pool of luteal-conditioned media from sheep ($n = 16$ ewes). Each fraction was evaluated for its effects on endothelial cell proliferation (—h —) and its protein (– – n – –) and NaCl (- - - -) concentrations. The endothelial cell proliferation data are expressed as percentage of controls (unconditioned media). Before heparin-affinity chromatography, the endothelial cell mitogenic activity of this luteal-conditioned media pool was 294% of controls. (Adapted from ref. *49*.)

Based on these observations of FGFR expression, we concluded that the FGFs are probably involved not only in luteal angiogenesis, which occurs primarily early in the estrous cycle, but are playing additional roles in luteal function as well. For example, we and others have shown that the FGFs affect luteal progesterone production *(54,61,62)*. In addition, FGFs have been shown to inhibit cell death in several cell types *(63,64)*. Interestingly, many of the larger luteal microvessels are maintained during luteal regression, and we observed an increase in FGFR in these vessels, which could explain how they selectively avoid cell death while the rest of the luteal tissue is resorbed *(7,8,60)*. Therefore we have suggested that FGFs may affect not only luteal cell proliferation but also luteal cell function and turnover *(7,8)*.

A portion of the angiogenic activity produced by bovine and ovine corpora lutea also elutes from heparin-affinity columns at about the same NaCl concentrations as would be expected for VEGF, which is now thought to be the major factor involved in the initial stages of angiogenesis *(8,47–49,65,66)*. To evaluate the expression of VEGF in ovine corpora lutea, we cloned and sequenced ovine VEGF cDNA and developed a sensitive ribonuclease protection assay to quantify VEGF mRNA *(67)*. Although VEGF mRNA was present in ovine corpora lutea throughout the estrous cycle, its levels were greatest early in the cycle, when luteal vascularization also is occurring *(7,8)*.

When we attempted to immunoneutralize the endothelial chemotactic activity produced by ovine corpora lutea, only about 25% of the activity was neutralized with anti-bFGF, whereas 65% was neutralized with anti-VEGF *(68)*. By contrast, only a small amount (10–25%) of the endothelial chemotactic activity produced by mid cycle corpora lutea was neutralized by treatment with anti-VEGF or anti-bFGF. These observations are consistent with those indicating that the greatest levels of VEGF mRNA are present in the early cycle corpus luteum, and that VEGF is a potent stimulator of endothelial cell migration as opposed to endothelial cell proliferation *(7,8,47,67)*. Based on these studies, therefore, it seems that VEGF is probably a major angiogenic factor involved in the tremendously rapid luteal vascularization that occurs after ovulation.

Additional observations provide further support for the hypothesis that VEGF is an important regulator of luteal vascular development. For example, in monkey corpus luteum, VEGF mRNA also is present in greatest amounts in the early luteal compared with the late luteal phase and, in addition, is reduced by treatment with a gonadotropin-releasing hormone antagonist *(69)*. Others have found VEGF mRNA or protein in luteal tissues or luteinized granulosa cells *(44,70–72)*. These observations provide further support for the hypothesis that VEGF is an important regulator of luteal vascular development.

Recent Work

Recently, we have reported that VEGF mRNA expression in cultured ovine luteal cells is increased by about 30% with LH treatment, and by 300% under low O_2 conditions *(73)*. These studies were based partly on the observation that human chorionic gonadotropin or LH can induce VEGF mRNA expression in preovulatory rat follicles and in cultured bovine luteal cells *(74,75)*. If VEGF is the major luteal angiogenic factor, its regulation by LH would make sense because LH is an important luteotropic factor and is critical for normal luteal development and function *(31)*.

Additionally, O_2 is a potent stimulator of VEGF expression across a number of cell and tissue types, which is consistent with the concept that metabolic demand is the primary factor regulating vascular development in all tissues *(18,46)*. Because the developing corpus luteum resembles a healing wound, and thus would be expected to be hypoxic until the luteal parenchymal lobules become well-vascularized *(76)*, it is also logical to expect that O_2 levels would be major regulators of luteal VEGF expression, and our data support this contention *(73)*.

To determine the cell types expressing VEGF during luteal vascularization, we utilized an affinity-purified polyclonal antibody against a 15 amino acid peptide from the N-terminal region of ovine VEGF *(67)*. Consistent with the mRNA and immunoneutralization studies, localization of VEGF protein appeared to be greatest during early luteal development and least late in the estrous cycle, during luteal regression *(8,77)*. In addition, VEGF protein was present in luteal connective tissue cells, luteal vascular smooth muscle, and most interestingly, luteal capillary pericytes (Fig. 3) *(8,77)*.

Thus, at least in the ovine corpus luteum VEGF protein is localized to thecal-derived connective tissue tracts, arterioles, and capillary pericytes. Neeman et al. *(78)* found that follicular development is accompanied by elevated levels of VEGF mRNA. Consistent with these observa-

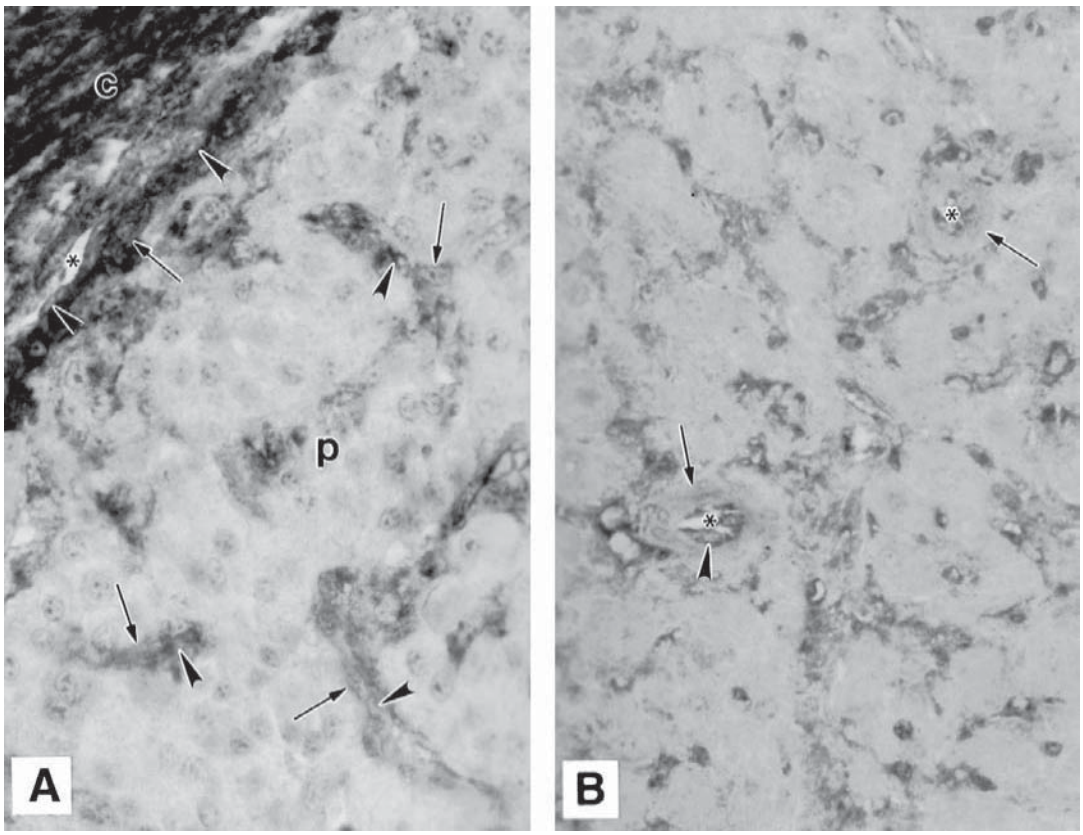

Fig. 3. Dual staining for VEGF (brownish staining) and BS-1 lectin (a specific endothelial cell marker; bluish staining) in histological sections of **(A)** early cycle, developing and **(B)** mid cycle, mature ovine corpora lutea. In (A), note that the cells staining for VEGF protein (arrows) are located in the thecal-derived luteal capsule (c) and also in association with the microvessels of the developing parenchymal lobule (p). In the mature corpus luteum (B), note that VEGF protein is confined primarily to the vascular smooth muscle (arrows) of the luteal arterioles (*). In both (A) and (B), note that the cells containing VEGF protein (arrows) are distinct from but closely associated with the luteal endothelial cells (arrowheads). Recently, by using dual immunofluorescence, we have confirmed that these VEGF-containing cells colocalize with smooth muscle α-actin, a specific marker for vascular smooth muscle and capillary pericytes *(80)*. Data are taken from previously unpublished observations (V. Doraiswamy, D. A. Redmer, and L. P. Reynolds). Original magnification: ×400.

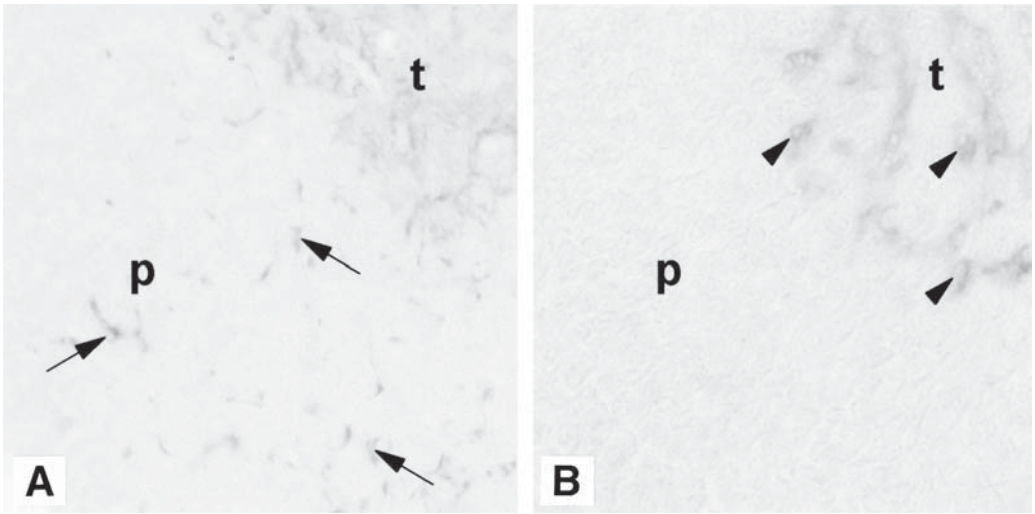

Fig. 4. Staining for **(A)** VEGF and **(B)** BS-1 lectin (a specific endothelial cell marker) in serial histological sections of a developing sheep corpus luteum within about 6 h after ovulation. In (A), note that the VEGF-expressing cells (arrows), which are probably derived from the thecal (t) region, have already invaded deep into the granulosa-derived parenchymal lobule (p). In the serial section (B) note that the thecal-derived endothelial cells (arrowheads) have begun to invade the developing granulosa-derived region but lag behind the VEGF-expressing cells shown in (A). (Reproduced from ref. *77*.) Original magnification: ×200.

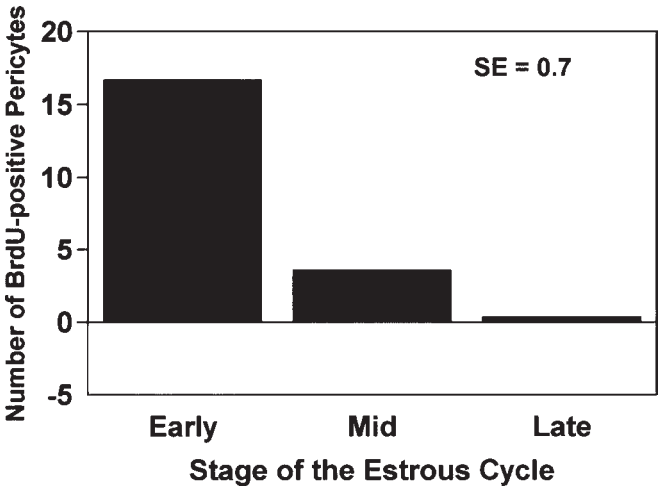

Fig. 5. Proliferation of luteal pericytes at several stages of the estrous cycle in sheep. Data are expressed as the number of BrdU-positive pericytes (BrdU is a thymidine analog that is incorporated into nuclear DNA during the S phase of the cell cycle and thus is a marker for cell proliferation) *(11)* per 0.56 mm^2. Pericytes were identified by their histological location and by immunohistochemical staining for smooth muscle α-actin, a specific marker for vascular smooth muscle and capillary pericytes. Data are from previously unpublished observations (K. Fischer, K. C. Kraft, D. A. Redmer, and L. P. Reynolds). Using 6 μm as the thickness of the histological sections, and estimating the number of luteal pericytes at each stage of the estrous cycle from the data of Farin et al. *(14)*, the labeling index (percentage of pericytes labeled with BrdU) was ≈5.2% for early, ≈0.8% for mid, and ≈0.05% for late cycle corpora lutea.

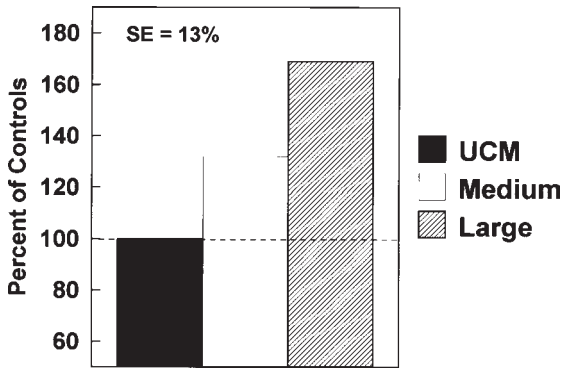

Fig. 6. Effects of media conditioned by bovine granulosa cells (n = 20 medium [h][5–10 mm diameter] and 13 large [▨] [>10 mm diameter] follicles) on migration of bovine retinal perictyes. Data are expressed as percentage of unconditioned media (j) controls. Granulosa-conditioned media from all follicles stimulated ($p < 0.01$) migration of perictyes, with that from large follicles having a greater effect ($p < 0.10$) than that from medium follicles. Granulosa cell cultures and the pericyte migration assay were conducted as described previously *(57)*. (Reproduced from ref. *90*.)

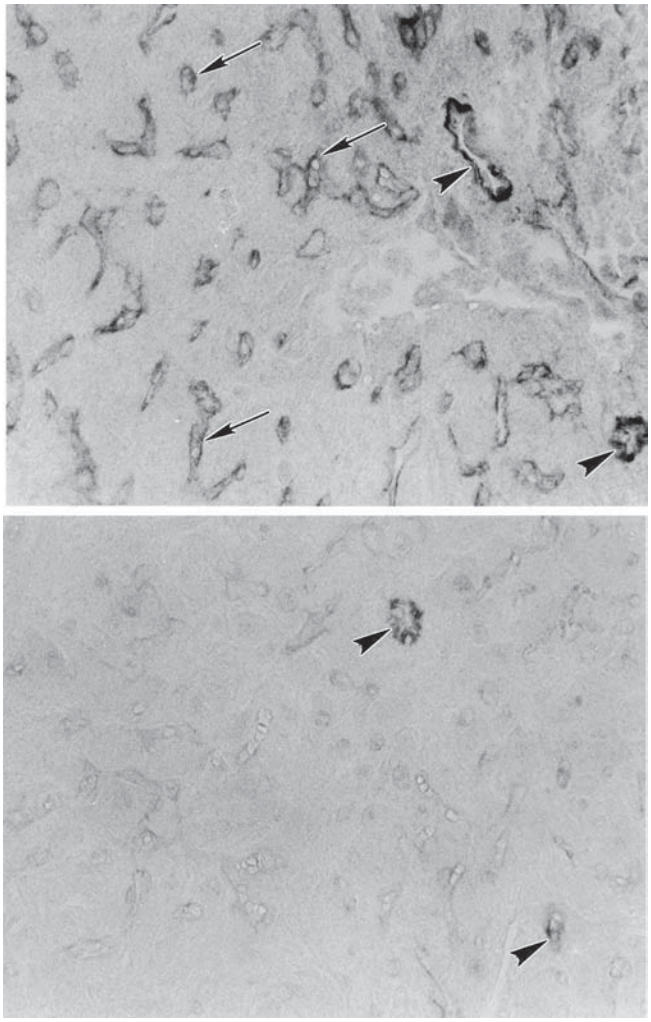

Fig. 7. Immunohistochemical localization of endothelial NOS (eNOS) in **(A)** early cycle, developing and **(B)** midcycle, mature ovine corpora lutea. In the developing corpus luteum (A), note the strong staining for eNOS in endothelial cells of the arterioles (arrowheads) and capillaries (arrows). In the mature corpus luteum (B), note that eNOS is greatly reduced and is confined to arteriolar endothelium (arrowheads). Data are from previously unpublished observations (D. A. Arnold, K. C. Kraft, D. A. Redmer, and L. P. Reynolds). Original magnification: ×400.

tions, we recently have shown that VEGF is expressed exclusively in the theca and not the granulosa of preovulatory bovine and ovine follicles *(77)*. We also found that the VEGF-expressing thecal pericytes invade the granulosa layer within hours after ovulation, before or coincident with the thecal endothelial cells (Fig. 4) *(77)*. In addition, we recently have shown a high rate of proliferation of pericytes in the early corpus luteum (Fig. 5). Interestingly, under hypoxic conditions, VEGF itself has been shown to be mitogenic for pericytes *(79)*.

Previous studies have shown that the thecal-derived microvasculature is responsible for vascularizing the developing corpus luteum, and that pericytes are involved in this process *(7,8,44,51)*. Within the last decade, various observations have indicated that the primary role of perivascular cells, including vascular smooth muscle cells and capillary pericytes, is to regulate endothelial cell function and angiogenesis during tissue growth and development *(80–83)*. In addition, vascular smooth muscle cells and

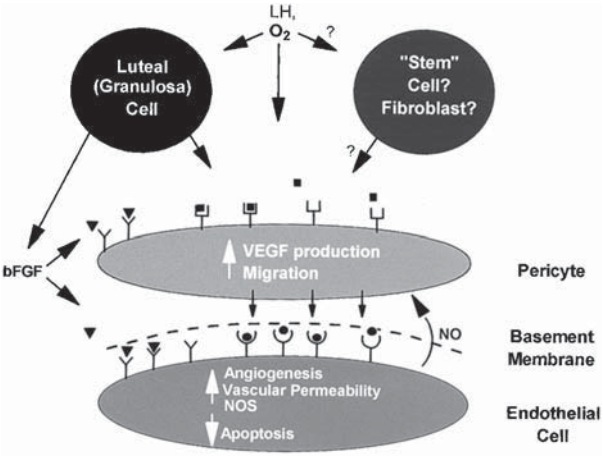

Fig. 8. Our current working model for luteal vascularization. bFGF and VEGFs seem to be the major luteal angiogenic growth factors. Granulosa cells and the luteal cells derived from them are capable of producing bFGF, which may regulate not only migration of pericytes but also endothelial cell survival (via inhibition of cell death). Similarly, VEGF derived from the perivascular cells may stimulate not only luteal angiogenesis but also luteal vascular permeability and blood flow (via increased NOS production). Likewise, NO produced by the luteal endothelial cells can act in a paracrine loop to stimulate relaxation of arteriolar smooth muscle, and thus vasodilation, as well as increase VEGF production by capillary pericytes, and thus angiogenesis. Although LH stimulates production of angiogenic factors by luteal tissues, whether this effect is direct or mediated by luteal parenchymal or other cells is unknown. Oxygen tension seems to be a major regulator of luteal VEGF production; again, whether this effect is direct or indirect is not known. (Adapted from ref. *8.*)

pericytes have been shown not only to produce VEGF but to increase its expression under hypoxic conditions *(84–87)*. Thus, these observations, taken together with studies showing that VEGF is critical for normal vascular development, are consistent with the hypothesis that thecal-derived perivascular cells may direct vascularization of the developing corpus luteum via production of VEGF.

If the thecal-derived pericytes regulate luteal vascularization, we wondered what would stimulate them to invade the developing luteal lobules soon after ovulation. Because bFGF is a potent stimulator of pericyte migration and VEGF expression ([*87*] H. E. MacDonald, D. A. Redmer, and L. P. Reynolds, unpublished observations), and may be produced by granulosa cells *(88,89)*, we hypothesized that bFGF secreted by the granulosa cells stimulates invasion of thecal pericytes. In support of this hypothesis, we recently have shown that granulosa cells of preovulatory ovine follicles produce a factor that stimulates migration of pericytes (Fig. 6) *(90)*.

Recent work also has shown that nitric oxide (NO), which is primarily an endothelial product and a potent vasodilator, can stimulate VEGF production and angiogenesis *(91,92)*. Similarly, VEGF, which we have shown to be resident in luteal perivascular cells, can stimulate endothelial NO synthase (eNOS) expression and thus NO production *(93–96)*. We have therefore proposed the existence of a paracrine loop, whereby luteal endothelial cells release NO, which stimulates perivascular VEGF production, which in turn stimulates endothelial expression of eNOS. This paracrine loop would thereby serve as a feed-forward system to maximize vasodilation and angiogenesis during luteal development. In support of this proposal, we recently found that eNOS is expressed in endothelial cells of luteal arterioles and capillaries early in the estrous cycle but that its expression is greatly reduced by mid cycle (Fig. 7). In addition, expression of NOS has been shown in the human corpus luteum *(97)*. These recent observations have led us to propose a new model for luteal vascularization (Fig. 8).

Implications

As mentioned previously, luteal dysfunctions, which may result from inadequate luteal angiogenesis, include reduced progesterone production, premature luteolysis, or persistent corpus luteum *(32,34–38)*. Insufficient progesterone secretion is unable to maintain the appropriate length of the luteal phase during the estrous cycle or pregnancy *(32,34–36)*. In cows, inadequate progesterone production has been associated with infertility and early embryonic death especially early in gestation *(98,99)*. In humans, luteal phase defect is a factor in as high as 20% of infertility cases and 60% of repeated first-trimester abortions *(40,100,101)*.

Whether reduced luteal vascularity is secondary to luteal insufficiency or, conversely, whether inadequate luteal vascularization is a cause of luteal dysfunction is not known at present. Although cause and effect are difficult to establish, two recent studies have shown that inhibition of VEGF action in the developing corpus luteum can lead to both reduced luteal vascularity and luteal progesterone production *(66,102)*. Ferrara et al. *(66)* and Dickson and Fraser *(102)* therefore concluded that VEGF expression plays a critical role in luteal vascularization and function. Furthermore, it seems reasonable to suggest that inappropriate follicular or luteal VEGF expression may contribute to luteal dysfunction and thereby be an important cause of infertility. This possibility, along with the recent spate of clinical work on regulators of angiogenesis *(103)*, leads us to believe that regulation of luteal and follicular angiogenesis could become a novel and powerful method for regulating fertility.

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