

Review

## Angiogenesis in the corpus luteum

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### Abstract

The corpus luteum (CL) is a site of intense angiogenesis. Within a short period, this is followed either by controlled regression of the microvascular tree in the non-fertile cycle, or maintenance and stabilisation of the new vasculature a conceptual cycle. The molecular regulation of these diverse aspects is examined. The CL provides a unique model system in which to study the cellular and molecular regulation of angiogenesis. Vascular endothelial growth factor (VEGF) has been found to have a major role in the CL. By targeting its action at specific stages of the luteal phase *in vivo* by antagonists, profound inhibitory effects on luteal angiogenesis and function are observed.

### Introduction

The corpus luteum (CL) is a site of intense angiogenesis, the formation of a dense capillary network enabling the hormone-producing cells to obtain the oxygen, nutrients and hormone precursors necessary to synthesise and release large amounts of progesterone required for establishment and maintenance of early pregnancy. Endothelial cells, once differentiated, normally remain functional for periods of 2–3 years, but in the CL a non fertile cycle results in structural luteolysis and these newly formed blood vessels regress within a matter of weeks. [1]. However, in a fertile cycle, the life span of the CL, with its attendant vasculature, is prolonged. Therefore, within a short period, the CL must accommodate a period of angiogenesis, controlled regression of the vasculature in the non-fertile cycle, or maintenance of the vasculature. Investigations of the mechanisms of vascular growth and regression in this unique situation will provide insights into the angiogenic process and may lead to new strategies in treatment of luteal-based infertility and to a method of 'once a month' fertility control.

The molecular and cellular regulation of angiogenesis is complex with a growing list of potential regulators. In a previous review we illustrated the importance of inhibiting specific angiogenic factors *in vivo* to determine whether a specific factor, or its receptor(s), has a major role, or is of no physiological significance [2] using angiogenesis in the CL as an end point. The success of this approach has been dependent upon two developments. First, methods to accurately quantify proliferating endothelial cells on tissue sections have been made possible by immunocytochemical (ICC) staining for Ki67 or bromodeoxyuridine (BrdU) incorporation to identify proliferating cells together with dual staining with markers for endothelial cells. Secondly, the generation of specific inhibitors allows selective targeting of individual factors in the angiogenic pathway *in vivo*. However, such advances are dependent upon continued discoveries using molecular and *in vitro* techniques. Here, we introduce the cellular aspects of angiogenesis in the normal cycle, ask what has been learnt from examining cyclic changes in some of the potential regulatory factors involved, then examine the unresolved issues in the divergent processes

of regulation of luteolysis and luteal rescue. Finally, recent insights from *in vivo* manipulation are reviewed.

### Normal luteal phase

Angiogenesis in the CL has its origins in the vasculature of the developing follicle [3]. Blood vessels lying within the thecal layer are denied entry into the granulosa cell layer by the presence of the basement membrane. On collapse of the follicle inwards, the thecal layer, together with its attendant blood vessels, is situated within the folds of the granulosa compartment. There is loss of integrity of the basement membrane, accompanied by extensive tissue remodelling, with the commencement of invasion of the differentiating granulosa lutein cell-containing region by new blood vessel development from the pre-existing thecal vasculature. Variable amounts of blood and plasma extravasate into the follicular cavity where they form a fibrin-rich clot [4]. The next few days are associated with a period of intense angiogenesis with resultant newly formed microvessels extending throughout the tissue.

Comparison of the proliferation index through the early, mid- and late luteal phases shows that it is in the early luteal phase that the most intense angiogenesis is taking place in all mammals studied to date, e.g., rat [5] sheep [6] bovine [7], mare [8] marmoset monkey [9], rhesus monkey [10] and human [11,12]. Dual staining shows that at all stages of the luteal phase >80% of the dividing cells in the CL are endothelial [7-20]. In the mature gland, > 50% of cells are endothelial, all of the fully differentiated lutein cells are in contact with the endothelium and the CL has the highest blood flow of any tissue in the body.

In most tissues, the generation of new blood vessels is followed by recruitment of perivascular cells (pericytes), similar to vascular smooth muscle cells, to the outer wall of the vessel to afford stabilisation [13,15]. Recruitment of pericytes appears to take place rapidly in the CL as they are detectable in the early luteal phase and the percentage of vessels with associated pericytes increases as the luteal phase progresses in the bovine [13] and human CL [14]. Pericytes are also present in the pre-ovulatory follicle. In the sheep it has been proposed that perivascular cells, largely pericytes, in the ovulated follicle have an additional role as lead cells in guiding the blood vessels towards the former antrum [15]. In addition, in the sheep, capillary pericytes exhibit a high rate of pericyte proliferation in the early CL [15]. Further work is required to address the role of pericytes in early luteal angiogenesis and in guidance of blood vessels into the CL.

### Molecular regulation of luteal angiogenesis

In most tissues, hypoxia is the stimulus for the synthesis of angiogenic factors [16]. Hypoxia in luteal angiogenesis may be important at certain stages and more detailed

studies on this question are required. However, there is substantial evidence that luteal angiogenesis is hormonally regulated by luteinising hormone (LH) as most of the major potential regulatory factors are produced by the lutein cells, which respond to changing LH stimulation [9,17-21].

Vascular endothelial growth factor (VEGF, also known as vascular permeability factor, VPF) is the major specific stimulator of endothelial cell proliferation, acting through two tyrosine kinase receptors, VEGFR-1 (flt-1) and VEGFR-2 (KDR) [16]. The first VEGF to be discovered, VEGF-A, is the most important in the CL, but some of the other members of this family [16] may also have a specialised role [17]. VEGF production increases in luteinizing granulosa cells of the ovulatory follicle [18] and is stimulated by addition of human chorionic gonadotrophin (hCG) to luteinized granulosa cells in culture [19]. In most species, VEGF mRNA is detected in the new CL in the granulosa-derived lutein cells, e.g., mouse [20] and human [21,22]. Several studies have examined whether changes in expression of angiogenic factors and their receptors may help explain the diverse changes in luteal vasculature. In the human, *in situ* hybridization showed that VEGF-A mRNA continues to be expressed at a high level throughout the luteal lifespan [22]. This may serve as a survival factor for endothelial cells and as a permeability factor. Other studies provide evidence for a fall in VEGF mRNA during luteal regression. Using northern blot analysis, a fall in VEGF expression from the mid- to late luteal phase was found in human CL [24,25]. In rhesus monkey CL, RT-PCR studies showed a 2 fold increase in VEGF expression between the early and mid-luteal phase followed by a 2 fold decline from mid- to late [23]. It is possible that the demonstration of a decline in expression in the late luteal phase are the result of more regressed specimens being studied [23,24] than the *in situ* investigation [22].

In primates, VEGF protein is localised in the hormone-producing cells of the CL, being highest in the granulosa-derived cells [4,14,21,23-25]. Similar results were reported in the equine CL [8]. VEGF is present throughout the luteal phase becoming less widespread during luteolysis. In apparent contrast, in sheep CL a highly specific antibody localised VEGF in vascular pericytes but it was absent from the hormone-producing cells [15].

With respect to VEGF receptors, *in situ* data for the CL is limited, probably because of low levels of expression, although endothelial cells are likely to be the site of expression [16]. Northern blot analysis and semi quantitative PCR in macaque or human CL [23-25] demonstrated expression of VEGFR-1 and VEGFR-2 mRNA at all stages of the luteal phase with a 2-3 fold decline in the

late luteal phase. ICC localises VEGFR-1 and VEGFR-2 to luteal endothelial cells, but also to hormone-producing cells [21,24,25].

Co-ordination of blood vessel formation and regression also involves other factors. These include the angiopoietins Ang-1 and Ang-2, which are of particular interest with respect to regulation of the rapidly-changing luteal vasculature because Ang-1 stimulates stabilisation of blood vessels while Ang-2 causes destabilisation [26]. Ang-2, acting through its tyrosine kinase receptor Tie-2, aids the action of VEGF by reducing endothelial contact with the extracellular matrix, and hence with adjacent endothelial cell interactions. In contrast, Ang-1 acts via the same receptor as a competitive antagonist and enhances the stability of the newly formed blood vessels by recruiting pericytes [26]. A potential role for the angiopoietins in regulating both luteal angiogenesis and vascular regression at luteolysis was proposed from *in situ* hybridisation observations in the rat ovary [26]. On formation of the CL, high levels of VEGF were expressed uniformly; expression of Ang-2 was more restricted, suggesting an action in serving to enhance the angiogenic effects of VEGF at certain loci, while localised expression of Ang-1 may serve to stabilise the new blood vessels [26]. At luteolysis, VEGF mRNA decreased and the Ang-2:Ang-1 ratio increased. It was proposed that this change would destabilise vessels in the CL and lead to vascular regression via apoptosis [26]. Supporting evidence for this attractive scenario has been tentative. *In situ* hybridization in human CL demonstrated intense expression of Ang-2 in a small number of endothelial and a few hormone producing cells while Ang-1 expression was low and diffuse [22]. A rise in Ang-2 mRNA expression has been reported using PCR in the regressing bovine CL [13]. In the rhesus monkey, both Ang-1 and Ang-2 mRNA rose at the late luteal phase [23].

Perhaps the mechanism by which expression of VEGF and the angiopoietins change to induce endothelial cell apoptosis differs between species. In those such as the cow in which luteolysis is brought about by endogenous luteolysin, e.g. prostaglandin F<sub>2</sub> alpha, a rapid sequence of molecular and cellular changes may be demonstrated associated with endothelial cell apoptosis. In primate species in which luteolysis is a more protracted process, and endothelial cell apoptosis is observed for a short time frame [1], these changes may be obscured.

Many other factors have been suggested to influence luteal angiogenesis. Estrogens may induce angiogenesis by acting directly on endothelial cells or by inducing angiogenic factors in other cell types; possible actions of progesterone, activin A, follistatin and other factors have been discussed elsewhere [27]. Recently, connective tissue growth factor (CTGF) has been proposed as a promoter of migra-

tion of endothelial cells in the CL [28]. Insulin like growth factor binding protein-3 is highly expressed in luteal endothelial cells and may be involved in determining accessibility of IGF [29].

Of particular current interest is a novel regulator of ovarian angiogenesis, endocrine gland VEGF (EG-VEGF) identified from the human ovary and which has been proposed as a steroidogenic endocrine gland specific angiogenic regulator [30]. Although highest levels are found in the ovary, testis and adrenal, EG-VEGF is also present at lower levels in other tissues such as the small intestine, where its action appears to be in regulating contraction of gastrointestinal smooth muscle [31]. The sequence was discovered independently from the small intestine and named prokineticin-1 [31]. Functionally, EG-VEGF/prokineticin-1 is similar to VEGF: it induces endothelial cell proliferation, migration and fenestration of endothelial cells but the activity seems selective to endocrine glands [30]. It also induced extensive angiogenesis when delivered by adenovirus to the mouse ovary, but not in other tissues [30,32]. EG-VEGF/prokineticin-1 consists of 86 amino acids. It is structurally distinct from VEGF but displays an 80% homology to a protein in the venom of the black mamba snake [30]. EG-VEGF/prokineticin-1 acts via G protein-coupled receptors, prokineticin-R1 and prokineticin-R2 [33].

EG-VEGF mRNA has been reported in rhesus monkey CL, and RT PCR studies showed an increase as the CL matures [34], and by *in situ* hybridization in the human CL [35]. A factor related to EG-VEGF/prokineticin-1 has also been discovered and termed Bv8 or prokineticin-2 and is highly expressed in the testis, but is apparently absent from the CL [35].

Fenestration of the endothelial cell membrane plays a role in the high permeability of the ovarian endothelial cells. The fenestrae are highly permeable to fluid and small solutes and are thought to facilitate the large exchange of materials between interstitial fluid and plasma associated with the CL. Since EG-VEGF and VEGF have an additive response *in vitro* it is suggested that they may also cooperate *in vivo* to induce the fenestrated phenotype and to promote angiogenesis [32], especially as both molecules are produced in the CL. Studies on the localisation of EG-VEGF and its receptors in the CL, how it is regulated by LH and/or hypoxia and *in vivo* studies to determine role of EG-VEGF are now required. The idea that there are angiogenic factors exhibiting tissue specificity means that more specific targeting should be possible than could be achieved by inhibition of VEGF.

In summary, the approaches described above have provided strong evidence for the role of various angiogenic

factors in the regulation of luteal angiogenesis. Comparing changes in expression ratios at different stages of the luteal phase has provided an indication of how the divergent changes in the vasculature may be controlled. However, in most studies in primates, especially in the human CL, major changes in gene expression of angiogenic factors or their receptors do not appear to precede vascular regression. Thus, the molecular regulation of vascular regression in the CL needs further investigation. Given the divergent nature of the various stages of the CL lifespan, it is surprising that more work has not been done on gene analysis by differential display or micro arrays. Since the regressive process are likely to involve localised changes in gene expression by specific cell types, further studies using *in situ* hybridization, ICC and specific vascular markers are required. Such investigations may also benefit from laser capture techniques.

### Pregnancy

For the maintenance of pregnancy, the lifespan of the CL must be extended. The question of whether this involves further angiogenesis and increased vessel stabilization requires to be investigated since failure of the CL due to a malfunction of the vasculature could be a factor in miscarriage or infertility.

In the rodent, prolactin has a pivotal role. Prolactin secretion induced by mating leads to increased endothelial cell proliferation in the CL of pregnancy [36]. In prolactin receptor deficient mice, CL angiogenesis is inhibited [37].

In the primate, the CL of pregnancy avoids luteolysis as a result of 'rescue' by chorionic gonadotropin (CG) which provides a major stimulus to prolong the function of the hormone-producing cells. To investigate the question of cellular and molecular changes in angiogenesis in primate CL stimulated by CG, three sources have been employed: 1) ovaries from experimental animals in which timing of conception has been determined, 2) CL from patients with ectopic pregnancy 3) CLs from rhesus monkeys or women following treatment *in vivo* with increasing doses of hCG starting at the mid-luteal phase, to mimic the endogenous stimulation of early pregnancy.

Following hCG treatment in rhesus monkeys there was no associated burst of angiogenesis [10]. Similar conclusions were reached from an early investigation after hCG treatment in the human [11]. In marmosets, no increase in CL angiogenesis was observed during early pregnancy [38]. This suggests that the vascular tree required for the CL of pregnancy is already established during the normal luteal phase.

A complication that may arise when analysing changes in angiogenesis on luteal sections is that vascular density

may be influenced not only by angiogenesis but also by changing volume of non-endothelial cells [1]. For example, in the human CL there is an increase in luteal cell volume from early to mid-luteal phase and a decrease during luteal regression which influences vascular density [1]. Expansion of granulosa lutein cell volume during luteal rescue spreads proliferating endothelial cells further apart. When changes in a particular cell content are quantified per unit area of tissue, change in luteal cell volume (translated to area in tissue sections) has to be addressed, or results will reflect the effects of expanding or contracting tissue. When this was taken into account, hCG induced luteal rescue was found to be associated with a significant increase in endothelial cell content of the human CL [14]. This, taken with the fact that endothelial cell proliferation increased at this stage, suggests that rescue of the human corpus luteum is associated with an increased angiogenic activity.

Early pregnancy may be associated with an increase in vessel stability of the newly formed vasculature achieved by recruitment of periendothelial support cells such as pericytes. Rescue of the human corpus luteum is associated with high pericyte coverage. Furthermore, it was shown that the percentage of non-endothelial cell proliferation (presumably pericytes) was highest during luteal rescue [14].

Examination of whether similar changes occurred in early pregnancy in the marmoset, showed that neither luteal cell area, endothelial cell area, pericyte area or endothelial cell proliferation differed from the mature CL of the non-pregnant cycle [38]. It would appear that the mature luteal vasculature of the non-pregnant marmoset is equipped with the cellular components to perform the functions of the vasculature of the CL of early pregnancy.

With respect to molecular changes in the rescued CL, it appears that the production of those factors associated with maintenance of the vasculature is continued under the influence of hCG with Ang-1 acting to stabilise the vessels, possibly by recruiting pericytes. In CL obtained at 6–8 weeks of pregnancy in women, VEGF mRNA was higher than at mid-luteal phase [21]. VEGF mRNA quantified by *in situ* hybridization [22] and VEGF immunostaining [14] increased after hCG-induced rescue. However, no rise in Ang-1 mRNA was detected to explain increase in pericytes [22]. Again, in the marmoset model, none of these changes were observed [38].

Clearly, further investigation, especially in the human, is required to determine the molecular regulation of the CL of pregnancy.

## Regression

Because the CL, including its vascular system, disappear during structural luteolysis, it has been tempting to believe that the programmed cell death associated with apoptosis is the principal mechanism involved. A fundamental question is whether regression of the vasculature plays a role in the functional and structural regression of the surrounding hormone-producing cells. The most compelling evidence for the involvement of luteal endothelial cell apoptosis come from observations on the regressing CL in the sheep [39] and guinea pig [40] in which blood vessels become occluded with cellular debris, presumably from endothelial cells. It was proposed that this cascade would result in ischemia and hypoxia leading to apoptosis of endothelial cells followed by apoptosis of hormone-producing cells. A major difficulty in determining the role of endothelial cell apoptosis in luteolysis may be the fact that the timing of luteal regression and the mechanisms which regulate it vary between species. For example, the period the CL functions by producing progesterone ranges from less than a day in the rodent to 21 days in the marmoset [41-43]. Also, the degenerative period can be very rapid (one cycle in the hamster) to gradual (several cycles in rat and human) [41,42]. In the hamster, the destruction of the CL between days 2 and 3 of the 4 day cycle is associated with massive apoptosis both of hormone-producing and endothelial cells [41]. However, the initiation of apoptosis was not apparent until several hours after the onset of the decline in plasma progesterone (functional luteolysis). In the human, a peak of apoptosis is found in regressing CL observed in both the theca-lutein and granulosa-lutein areas during the peri-menstrual period, from a few days before to a few days after menstruation [42]. Similarly, apoptosis peaked in CL of the early follicular phase in the marmoset [43]. These, and other observations [44] suggest that apoptosis is rarely observed in the primate CL and is confined to a short time span. Apoptosis represents an initial step in structural luteolysis, after which most steroidogenic cells survive to undergo a process of gradual involution [42]. The studies in the sheep showing a rapid degeneration of blood vessels during luteolysis [39] may be related to species such as the sheep, cow and horse in which luteolysis is induced by prostaglandin which may induce rapid changes in blood flow resulting in a luteolytic cascade [45]. In primates, there is little evidence to suggest that luteal vascular degeneration is a trigger for luteolysis [46], but the onset of the former would undoubtedly lead to a reduction in the supply of oxygen and nutrients, and perhaps contribute to lutein cell death. The availability of inhibitors of angiogenesis for *in vivo* studies should provide a new approach to help elucidate the relationship between regression of the vasculature and functional and structural integrity of the hormone-producing cells.

## Manipulation of angiogenesis

Studies of angiogenic factors in the CL at various stages of the cycle as described above provide useful pointers to their physiological role in regulation of luteal angiogenesis. The next stage of investigation should involve specifically inhibiting angiogenic factors *in vivo* using effects on luteal angiogenesis as a indicator of effectiveness. A large number of anti-angiogenic agents are being generated. Antagonists developed specifically to target individual angiogenic factors are the most useful, since specific factors may be targeted, their role established, and overall controlling pathways unravelled. Striking results have been obtained by inhibiting VEGF, either by specific VEGF antibody [47], anti-VEGF receptor antibody [48], soluble decoy receptors created by fusing the first three domains of VEGFR-1 to the Fc portion of human IgG [49] or higher affinity VEGF 'traps' also based upon the VEGF receptors [50,51].

When soluble VEGFR-1 fusion protein was injected directly into the pre-ovulatory follicle of rhesus monkeys the day before or the day of the mid-cycle LH surge decreased subsequent luteal progesterone in the serum, while the length of the luteal phase was of normal duration [52]. However, treatment at this time did not effect luteal angiogenesis or development of the microvascular tree.

In marmosets, the intense angiogenesis in the early CL has been targeted. In agreement with results which show that LH/hCG stimulates VEGF production, suppressing endogenous LH by GnRH antagonist treatment commencing 1 day post-ovulation inhibits early luteal angiogenesis [9]. However, withdrawal of LH *in vivo* is likely to disrupt not only VEGF but also the action a variety of luteal factors and cell-cell interactions likely to be necessary for angiogenesis and survival of hormone-producing cells.

Using treatment schedules at specific stages in the luteal phase, the role of VEGF in luteal angiogenesis has been examined in the marmoset [50,53-55]. VEGF inhibitors have been administered in the following schedules: 1) from day 0-3 or, 2) day 0-10 of the luteal phase, 3) luteal day 3-4 or, 4) luteal days 7-10. Ovaries were studied at the end of these treatment periods. Treatments 1 and 2, commencing at or shortly after ovulation, targeted VEGF during the period of intense luteal angiogenesis. The first schedule was over a three-day period, so that the CL was examined at a time of normal maximal proliferative activity. The second continued this treatment to the mid-luteal phase, by which time the microvascular tree is established in normal animals. The initial short-term treatment markedly reduced the number of proliferating cells, while the more prolonged treatment resulted in a change in the vascular tree: the larger vessels were still present, but there

was a dearth of the numerous small capillaries found in the normal CL [53]. CL function was inhibited as demonstrated by a reduction in the concentrations of plasma progesterone [53].

The third and fourth schedules addressed the issue of whether inhibition of VEGF could intervene with ongoing angiogenesis. Treatment on day 3–4 [50] or 7–10 [54] resulted in a marked decrease in angiogenesis. Interestingly, treatment at these times was associated with a fall in plasma progesterone. Recent studies in which the more potent VEGF trap R1R2 has been administered on days 7, 14 or 19 post ovulation show that functional luteolysis results [55]. Such an effect would not be expected if the treatment is acting by inhibiting angiogenesis alone. Three possibilities arise. One is that treatment is suppressing serum LH. Second, inhibition of VEGF is destroying existing luteal blood vessels, or third, that ovarian permeability is being compromised. It is possible to discount the possibility that LH is decreased as studies of inhibition of VEGF in macaques show LH to rise [48], presumably as a result of withdrawal of negative feedback. Also, in contrast to the situation observed for treatment with the GnRH antagonist, the morphological features of the lutein cells in the anti-VEGF animals remain essentially intact [53–55]. There is an increase in endothelial cell death after inhibition of VEGF at the mid-luteal phase, but it is not extensive [54,55]. Thus, it would appear that ovarian permeability is being compromised such that either LDL-cholesterol required for progesterone synthesis does not gain entry into the cell or release of progesterone from the hormone producing cells is restricted. This possibility remains to be investigated.

Overall, these data demonstrate that inhibition of VEGF during early luteal development prevents the intense angiogenesis normally occurring at this time, leading to a failure of development of the microvascular tree. This in turn has an adverse effect on the CL whose ability to secrete progesterone is severely restricted. An additional factor contributing to the fall in progesterone secretion after inhibition of VEGF may be a suppression of ovarian microvascular permeability.

With respect to the regulation of luteal activity in early pregnancy, as reviewed above, the increase in angiogenesis in the human CL after hCG does not appear to occur in the non-human primate model [38]. In view of the fact that the period of intense luteal angiogenesis in the fertile cycle of the marmoset is restricted to the early luteal phase in the non-human primate, the effects of inhibiting VEGF during this period were examined in marmosets in family groups, in which pregnancy rate is over 80% [38]. We used the same treatment schedule which inhibited angiogenesis, prevented formation of the microvascular tree

and suppressed plasma progesterone in the normal cycle. The results confirmed that the VEGF antibody markedly suppressed plasma progesterone in the mated females. However, after cessation of treatment 10 days post-ovulation, a degree of recovery of progesterone secretion was observed so that in 5 of the 10 treated marmosets, the CL was subsequently successfully rescued. These animals went on to deliver normal offspring at the same time as the controls. The fact that pregnancy was successfully established in the face of a marked reduction in plasma progesterone concentrations suggests that in the marmoset, the normal quota of luteal progesterone is in excess of requirement. Furthermore, the rise in plasma progesterone post-treatment in half of the animals, implies that angiogenesis is capable of re-initiation after cessation of VEGF inhibition. Further studies are required to determine the outcome in other species and to determine effects of the more potent VEGF traps.

## Conclusions

Considerable progress has been made on describing the cellular and molecular events associated with angiogenesis in the CL. Further work is needed to elucidate the divergent changes seen in the vasculature during luteolysis and rescue. The lack of clarity as to whether changes in the vasculature precede luteolysis and to the cellular events associated with early pregnancy may in part be the result of species differences. The use of luteal angiogenesis as a target for investigation of the physiological role of individual angiogenic factors has fulfilled its promise. Inhibition of VEGF has a profound inhibitory effect upon luteal function. The mechanism appears to involve suppression of other functions, probably permeability, in addition to inhibition of angiogenesis. These results may help shed light on the phenomenon of luteal insufficiency and have clinical relevance in our ability to manipulate luteal function. Finally, the observations made on inhibition of VEGF should form a platform for deciphering the role of other putative angiogenic regulators.

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