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# Angiogenesis and Ovarian Function

N. C. Douglas, G. S. Nakhuda, M. V. Sauer, R. C. Zimmermann

*Ovarian follicle development and corpus luteum formation involves recurrent, regulated, and self-limited angiogenesis. In rodent and non-human primate models it was shown that the VEGF/VEGF receptor 2 signal transduction pathway is of critical importance for ovarian angiogenesis. Clinically manipulation of this pathway might be helpful in finding new treatment methods for ovarian cancer, ovarian hyperstimulation syndrome, polycystic ovarian disease, endometriosis, as well as new, non-hormonal contraceptive approaches.*

*Eine Voraussetzung für die physiologische Entwicklung von Follikeln und Corpora lutea im Eierstock ist eine zyklische, streng regulierte und zeitlich begrenzte Angiogenese. Es konnte im Nager- und Affen-Modell gezeigt werden, daß der VEGF/ VEGF Rezeptor 2 Signal-Transduktionsweg von herausragender Bedeutung für die Regulierung der Blutgefäßentwicklung im Ovar ist. Pharmakologische Beeinflussung dieses Signal-Transduktionsweges könnte zu neuen Behandlungsmethoden für das Ovarialkarzinom, das Überstimulierungssyndrom bei Gonadotropinbehandlung, das polycystische Ovarialsyndrom und die Endometriose führen, als auch zu neuen Wegen für die nicht-hormonelle Kontrazeption. J Fertil Reprod 2005; 13 (4): 7–15.*

Angiogenesis, or neovascularization, is the differentiation and growth of new blood vessels from preexisting microvasculature. Angiogenesis occurs as a series of steps involving (1) the breakdown of the basement membrane of existing blood vessels; (2) the migration of endothelial cells into the interstitial space towards an angiogenic stimulus; (3) the proliferation of endothelial cells; and (4) the formation of new capillary lumina and functional maturation [1–3]. The initial steps of endothelial cell invasion and migration are mediated by proteolytic enzymes and cell adhesion molecules. These enzymes include urokinase-type and tissue-type plasminogen activators (uPA and tPA) and matrix metalloproteinases (MMPs), while cell adhesion molecules include integrins and cadherins [4, 5]. "Angiogenic factors" including vascular endothelial growth factor (VEGF) [6], basic fibroblast growth factor (FGF-2) [7] and angiopoietins [8, 9] that promote endothelial cell survival, proliferation, and differentiation are attached to the extracellular matrix (ECM). Loss of integrity of the ECM results in an increased concentration of these factors. During the final phases of angiogenesis, local proteolysis is inhibited to allow for active synthesis of a new basement membrane and establishment of capillary lumina. Vessel maturation is complete once supportive pericytes and smooth muscle cells have been recruited to stabilize the new vessels. These vessels subsequently differentiate into arterioles and venules [1].

Angiogenesis is essential for organogenesis and cellular proliferation and differentiation during embryonic development [10, 11]. In adult animals and humans, angiogenesis can be classified as pathological or physiological. Pathological angiogenesis refers to the uncontrolled proliferation of capillary endothelium witnessed in atherosclerosis, hemangiomas, diabetic retinopathy and tumor growth and metastases. Physiological angiogenesis is a highly regulated process activated in wound healing [6] and involved in the female reproductive system during the non-pregnant estrous/menstrual cycle and implantation of embryos during pregnancy [12]. The ovary and endometrium in non pregnant adult animals and humans undergo cyclic changes [13, 14]. In the absence of conception, physiologic angiogenesis is followed by regression of

select ovarian and endometrial elements with re-initiation of the menstrual cycle.

The development of ovarian follicles, the functional units of the ovary, is a unique process that involves recurring, regulated, self-limited angiogenesis. Several experimental models have demonstrated that ovarian function is critically dependent on angiogenesis for follicular development, ovulation, and corpus luteum function. In the following review, we summarize data which demonstrate the critical role of angiogenesis in ovarian function.

## Follicle morphogenesis

As early as four weeks post fertilization, primordial germ cells in developing human fetuses migrate from the yolk sac to the genital ridge where they play a critical role in the development of the gonad [15]. These germ cells, now referred to as oogonia in female fetuses, induce formation of pregranulosa cells from surrounding mesenchymal cells. In the human, oogonia undergo extensive proliferation such that there are approximately 600,000 oogonia at eight weeks post fertilization, reaching a peak number of 6 to 7 million at 20 weeks of gestation. Beginning at eight weeks post fertilization, oogonial development is influenced by

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concurrent processes of mitosis, meiosis, and oögonial atresia/apoptosis. As oögonia enter prophase of the first meiotic division, they become primary oocytes. Primary oocytes are enveloped by a single layer of flattened pre-granulosa cells to form primordial follicles. Most primordial follicles remain within a dormant, resting pool. Shortly after follicle formation, some primordial follicles undergo initial recruitment to enter the growing pool of primary follicles [16]. Regulation of the transition from a primordial to a secondary follicle is under active investigation. A single layer of cuboidal granulosa cells and early theca interna cells surround the oocyte in primary follicles. Secondary follicles are composed of an enlarged primary oocyte surrounded by the early zona pellucida, several layers of cuboidal granulosa cells and theca interna and externa layers. Granulosa cells in secondary follicles develop gap junction intercellular connections and acquire receptors for follicle-stimulating hormone (FSH), estrogens and androgens. Theca cells acquire luteinizing hormone (LH) receptors and the capacity to synthesize steroid hormones. The establishment of the theca layer is associated with the development of the follicular blood supply that is critical for continued follicle maturation. The transition from preantral to preovulatory follicle has been divided into eight classes based on granulosa cell number and follicle diameter (Figure 1) [17].

As follicular development progresses, the granulosa cell number, size of antral cavity and follicle diameter increase. Growing follicles can develop to the small antral stage in FSH- $\beta$  and FSH receptor deficient animals, however they do so more slowly and in fewer numbers than their normal counterparts [16]. Therefore, in the absence of gonadotrophin stimulation follicles can progress to the small antral stage but gonadotrophin stimulation accelerates cell division and differentiation. Hence, the development of follicles prior to the antral stage may not be gonadotrophin-dependent, but is gonadotrophin-responsive. Most antral follicles undergo atretic degeneration, primarily through apoptotic mechanisms. After the onset of puberty, gonadotrophin-dependent cyclic recruitment allows a small number of antral follicles to escape atretic (or apoptotic) demise and continue growth to the preovulatory stage [16]. With adequate FSH stimulation, granulosa cell proliferation and estrogen production in the leading preovulatory follicle exceed that of the cohort. The rise in estrogen levels from this dominant follicle initiates the LH surge that triggers the resumption of meiosis I and ovulation. After oocyte release, the ruptured follicle is reorganized into a highly vascularized corpus luteum. If pregnancy occurs, the corpus luteum produces and secretes hormones necessary for endometrial maturation and early embryonic development. In the absence of conception and gonadotrophin stimulation, structural and functional regression of the corpus luteum is achieved by a process known as luteolysis.

### Ovarian angiogenesis

Vascular development in the ovary is a cyclic process, spatially and temporally defined. Avascular primordial follicles depend on proximity to stromal vessels for nutrients. During the transition from an avascular, primary follicle to a vascular secondary follicle, angiogenesis occurs in the theca layer. How the secondary follicle becomes endowed with vasculature is unclear. This transition may be due to local transformation of mesenchymal cells into endothelial

cells or active migration of endothelial cell precursors from preexisting blood vessels. The mature vascular sheath, found in the theca layer of preovulatory follicles, consists of two concentric networks of vessels in the theca interna and externa layers. Arterioles and venules from the theca externa branch into the single-layer capillary plexus of the theca interna, but neither the basement membrane nor the granulosa layer of the follicle are traversed by capillaries. Hence, the granulosa layer receives nutrients and hormones by diffusion from the capillary network located in the peripheral theca layer.

Gonadotrophin dependent follicular growth is characterized by the expansion of the antrum through accumulation of fluid in the follicular cavity, cell division in the theca and granulosa layers, and expansion of the inner capillary plexus. The follicle selected for maturation and ovulation possesses a denser microvascular network than others in the same cohort [18]. Differential neovascularization is likely important in selection of the dominant follicle. Increased blood flow results in an increased supply of gonadotrophins as compared to less vascular secondary follicles in the cohort. High levels of estrogen produced by granulosa cells in the dominant follicle stimulate the LH surge which triggers ovulation. The collapse of the basement membrane that accompanies ovulation allows penetration of the luteinized granulosa layer by invading microvessels originating from the theca layer.

The early luteal phase is associated with intense angiogenesis as indicated by endothelial cell proliferation [19]. By mid-luteal phase, a mature vasculature characterized by maximal endothelial cell and pericyte area is present. This rich network of fenestrated capillaries is so rapidly formed in the corpus luteum, that by mid-luteal phase, the corpus luteum has the highest blood flow of any tissue in the body [20]. Analyses of corpus luteum morphology have shown that the majority of luteinized theca and granulosa cells are adjacent to two or four capillaries [21] and approximately 50% of the cells in the mature corpus luteum are endothelial cells [22–24].

The corpus luteum functions as a highly active endocrine organ synthesizing progesterone to create an adequate uterine environment to allow for implantation of the embryo and development of early pregnancy. In case a pregnancy does not occur, the corpus luteum ceases to function through a process which is called luteolysis. Luteolysis is characterized by degeneration of vasculature and steroidogenic cells with consequent decline in progesterone secretion. One possible mechanism involved in luteolysis is that capillary endothelial cells detach from the basement membrane, the density of blood vessels decreases, and all lutein cells disappear [11, 25]. A hyaline scar, the corpus albicans, remains. In contrast, during early pregnancy human chorionic gonadotrophin (hCG) synthesized by trophoblast cells induces a second wave of angiogenesis in the corpus luteum which results in an increase in endothelial cell and pericyte area. These changes “rescue” the corpus luteum and allow continued progesterone synthesis to occur [19].

These anatomical observations provide clear evidence that corpus luteum formation and function require the development of an extensive neovasculature, which once formed maintains function for a limited period of time in the absence of pregnancy. Hence, investigators hypothesized that mediators of angiogenesis were important for

corpus luteum formation as well as for folliculogenesis. Earlier studies found that the angiogenic activity produced in bovine ovaries bound strongly to heparin-affinity columns [24, 26]. Thus, members of the heparin binding family of angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (bFGF) were implicated in physiological ovarian angiogenesis [2, 3]. Although studies have not identified an essential role for bFGF in ovarian angiogenesis, VEGF is a critical regulator of follicular and luteal angiogenesis. Our understanding of the mediators of ovarian angiogenesis continues to be enhanced by ongoing research.

### **Regulation of ovarian angiogenesis**

VEGF and members of the VEGF gene family play a fundamental role in growth and differentiation of vascular and lymphatic endothelial cells [27, 31]. Human VEGF is a 45 kD basic, heparin-binding homo-dimeric glycoprotein that exists in four isoforms. Alternative exon splicing results in four different VEGF isoforms, having 121, 165, 189, and 206 amino acids, with VEGF165 being the predominant one. VEGF acts through two receptors: VEGF receptor-1 (VEGFR-1) and VEGFR-2 [32]. It is thought that VEGF dependent activation of VEGFR-2 is involved in mediating endothelial cell proliferation, survival, and vascular permeability, whereas VEGFR-1 might play an inhibitory role by sequestering VEGF and preventing interaction with VEGFR-2 [32]. VEGFR-3 is primarily expressed on lymphatic endothelial cells and functions as a receptor for VEGF family members VEGF-C and VEGF-D [29]. In the human ovary, the VEGF receptor is primarily detected in the theca interna of antral and developing follicles and the intensity of VEGF expression is higher in more mature follicles [33–35]. VEGF activity has not been detected in primordial, primary or atretic follicles. Inhibition of VEGF and VEGF receptor activity has helped to elucidate the role of this angiogenic molecule during folliculogenesis and corpus luteum formation and function.

## **Folliculogenesis**

Using the rhesus monkey as a model, we demonstrated that VEGF, acting through VEGFR-2, is a crucial physiologic component in follicular growth in the non-human primate. Administration of anti-VEGFR-2 antibody starting on days 2–4 of the menstrual cycle, the early follicular phase, temporarily interrupts follicle development. Specifically we noted (1) levels of inhibin B, a marker of small antral follicles, initially decrease, (2) there is a delay in the rise of estradiol levels and in attainment of the preovulatory estradiol peak, and (3) the length of the follicular phase increases from 10–12 days in controls to 20–42 days in anti-VEGFR-2 treated rhesus monkeys [36]. Although all treated animals develop an ovulatory cycle, disruption of VEGF activity in the early follicular phase delays selection of the dominant follicle. These results demonstrate that VEGF, acting through VEGFR-2, is necessary for the selection of recruited antral follicles. Administration of anti-VEGF antibody to rhesus monkeys in the late follicular phase delays maturation of the dominant follicle [37]. Inhibition of VEGF action and VEGFR-2 [unpublished observations] interrupts the late follicular phase rise in estradiol, increases mean FSH levels, and lengthens the follicular phase. After a variable delay, estradiol concentrations increase to reach a preovulatory peak. Ovulation, normal luteal function, and a normal post-treatment cycle ensue. As in the early follicular phase disruption, the effects of inhibiting VEGF

action are completely reversible. The reversible nature of VEGF inhibition was recently confirmed [38]. A single intravenous (i.v.) injection of VEGF TrapR1R2, a receptor based VEGF antagonist, during the follicular phase of the macaque ovarian cycle inhibits follicular maturation and ovulation. However, normal ovarian activity resumes when VEGF TrapR1R2 levels fall below threshold concentrations and subsequent follicular and luteal phases are of normal length [38].

VEGF activity correlates with vascular endothelial cell proliferation, maintenance and permeability [39, 40]. Thus, it is likely that short-term inhibition of angiogenesis by antibodies blocking VEGF activity and VEGFR-2, results in the observed derangements in folliculogenesis. However, the specific mechanism by which inhibition of VEGF activity blocks follicular development remains unclear.

These dynamic observations were confirmed by morphological characterization of the inhibition of follicular angiogenesis in the marmoset monkey [41, 42]. Treatment of marmosets with VEGF TrapA40 for 3 days starting at the time of ovulation results in a significant reduction in proliferation in the theca of secondary and tertiary follicles and a reduction of endothelial cell area [41]. VEGF TrapA40 is a soluble truncated form of human VEGFR-1 (Flt-1) that has high affinity for VEGF, neutralizes the actions of VEGF and prevents receptor binding. In a second series of experiments, Fraser's group utilized VEGF TrapR1R2, a novel soluble receptor based antagonist containing portions of the extracellular ligand binding domains of human VEGFR-1 (Flt-1) and VEGFR-2 (KDR). Inhibition of VEGF action by administration of VEGF TrapR1R2 for 10 days during the follicular phase of the marmoset cycle confirmed and extended the previous observations [42]. TrapR1R2 treatment results in a significant reduction in; (1) the endothelial cell area, (2) proliferation in the theca, and (3) the mean thickness of the thecal layer in secondary and tertiary follicles. Medium and large antral follicles and corpora lutea were not detected in ovaries of TrapR1R2 treated animals. This study demonstrates that suppressing follicular angiogenesis by VEGF inhibition disrupts the development of antral follicles and subsequently prevents ovulation. Inhibition of ovulation in this study may be due to the increased duration of VEGF TrapR1R2 treatment and/or mechanism of action as compared to the anti-VEGFR-2 treatment [36].

Fraser et al. [38] recently demonstrated that a single i.v. injection of TrapR1R2 at the mid-follicular or late follicular phase in the macaque produces a dose-related suppression of ovarian function. Administration of TrapR1R2 at the mid-follicular phase, the time of selection of the follicle that will ovulate, causes a dose-dependent decline in serum inhibin B and estradiol levels and a sustained increase in LH and FSH concentrations. Administration of TrapR1R2 at the late follicular phase results in a preferential increase in FSH and serum progesterone is maintained at follicular phase levels. In all treated macaques, ovulation and corpus luteum function failed to occur at the anticipated time. As mentioned above, TrapR1R2 inhibition of ovarian function in the macaque is completely reversible. Thus, after a dose-dependent delay, normal ovarian activity resumed in all treated animals. Taken together, these five studies support the hypothesis that VEGF mediated angiogenesis is necessary for folliculogenesis in non-human primates. The use of antiangiogenic therapies to manipulate follicular maturation in a single ovarian cycle without deleterious effects on subsequent cycles and ovarian function has the

potential to make vast contributions to reproductive medicine.

All of the previous studies were performed in animals with an intact hypothalamus-pituitary-ovarian axis in which active feedback is present. To further characterize the role of VEGF in follicle development, we chose to alter VEGF function in a model in which the pituitary, the endogenous source of gonadotrophins and a key component of the feedback loop, is absent. Thus, we chose the prepubertally hypophysectomized (HX) mouse model to characterize the role of VEGFR-2 activity in follicular development [43]. Hypophysectomy prevents advanced follicle growth and maturation. However, administration of exogenous gonadotrophins can induce follicular maturation and ovulation [44–46]. We demonstrated that administration of PMSG (pregnant mare serum gonadotrophin) to HX mice stimulates theca layer angiogenesis and follicular development. Blocking VEGFR-2 activity inhibits gonadotrophin-dependent follicular angiogenesis, antral cavity formation, and granulosa cell proliferation. In the absence of endothelial cell proliferation, exogenously administered gonadotrophins are unable to drive follicle development to the preovulatory stage. Most of the advanced follicles are early antral follicles, with few if any preovulatory follicles (Figure 1). The fundamental role of angiogenesis in follicular development is further supported by our recent demonstration that vascular-endothelial cell cadherin (VE-C), an angiogenesis related adhesion molecule, is required for neovascularization in the follicular phase [47]. In this study, administration of an antibody to VE-C to HX mice during gonadotrophin stimulation inhibits angiogenesis and preovulatory follicle formation.

The targeted overexpression of VEGF and endocrine-gland-derived vascular endothelial growth factor (EG-VEGF) in the rodent ovary has expanded our knowledge of the role of angiogenic factors in ovarian physiology [48]. EG-VEGF is an angiogenic mitogen selective for endocrine gland endothelium. Endogenous expression of EG-VEGF is highest in ovary and testis, followed by adrenal gland and placenta. Using an adenovirus vector, targeted overexpression of both VEGF and EG-VEGF in the rat ovary results in increased ovarian weight, increased angiogenesis and disruption of ovarian stroma by large cystic spaces. These cysts contain proteinaceous material and hemorrhage likely due to leakage from the new vasculature [48, 49]. Thus, enhanced VEGF expression in the rat ovary supports the role of VEGF in angiogenesis and vascular permeability. Furthermore, EG-VEGF may contribute to VEGF independent ovarian angiogenesis.

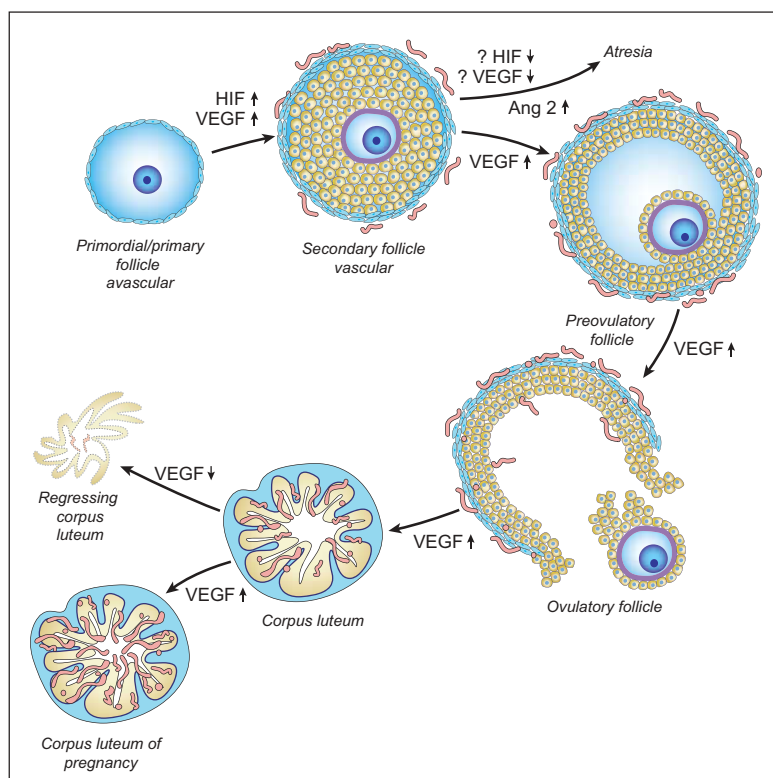
## Corpus luteum formation and function

Angiogenesis provides hormonal precursors to steroid producing lutein cells of corpora lutea and transports newly synthesized progesterone from corpora lutea into systemic circulation. A lack of progesterone production by the corpus luteum results in early pregnancy failure. In the human and non-human primate, endothelial cell proliferation is highest in the early luteal phase and decreases during

the midluteal phase [50, 51]. To investigate the consequences of inhibition of angiogenesis on primate corpus luteum development and function, Fraser et al. [52] administered a mouse monoclonal antibody against VEGF to marmoset monkeys at the time of ovulation. Although corpora lutea formed in treated animals, the degree of vascularization and extent of endothelial cell proliferation in the early luteal phase were reduced. Plasma progesterone levels were reduced by 60%. Fraser et al. also demonstrated that inhibition of VEGF in mid luteal phase interrupts ongoing angiogenesis [53]. Administration of anti-VEGF monoclonal antibody to marmoset monkeys starting day 7 after ovulation resulted in a 50% reduction in progesterone production, decreased endothelial cell proliferation, and increased endothelial cell apoptosis. Treated animals also had increased numbers of pericyte associated mature endothelial cells that are believed to be VEGF independent [54]. Taken together, these studies demonstrate the importance of VEGF in initiation and maintenance of luteal phase angiogenesis and corpus luteum function in non-human primates.

Fraser confirmed and extended the previously described observations by inhibiting VEGF with VEGF TrapA40 [55]. Administration of VEGF TrapA40 to marmoset monkeys at the time of ovulation or on luteal day 3 after ovulation decreases endothelial cell proliferation, reduces the microvasculature tree and leads to a decline in plasma progesterone. Since rapid actions of VEGF are attributed to VEGF mediated effects on vascular permeability [6], the rapid decline in plasma progesterone after a single injection of VEGF TrapA40 on luteal day 3 might be due to suppression of ovarian vascular permeability.

Studies in rodents have yielded similar results. Ferrara et al. [56] used a rat model of gonadotrophin-induced ovulation to determine the role of VEGF in corpus luteum



**Figure 2.** Some of the principle factors involved in the regulation of angiogenesis in the ovary (mod. from [80])

angiogenesis. In this model, induction of ovulation results in increased ovarian vascularity and a 10-fold increase in ovarian weight. Administration of truncated soluble murine Flt-1 receptor, a construct similar to VEGF TrapA40, prior to initiating ovulation induction results in avascular corpora lutea with central ischemic necrosis. As expected, suppression of corpus luteum angiogenesis results in inhibition of corpus luteum development and progesterone release. Of note, preexisting ovarian vasculature was not affected by administration of soluble Flt-1 in these studies.

VEGFR-2 is expressed in the corpora lutea of rodents [56] and signaling through this receptor mediates the angiogenic action of VEGF [57]. To establish the role of VEGF/VEGFR-2 signaling in corpora lutea survival, proliferation and function in non-pregnant and pregnant rodents, we disrupted receptor function using a monoclonal antibody against VEGFR-2 [58, 59]. Hormonal induction in immature mice results in neovascularization of corpora lutea, increased numbers of corpora lutea and a three-fold increase in ovarian weight [58]. Administration of anti-VEGFR-2 antibody prior to and during ovulation induction results in suppression of ovarian weight, reduced numbers of corpora lutea, and decreased progesterone secretion. Corpora lutea that were present were smaller in size with decreased vascularity and areas of necrosis. In contrast, permanent vasculature such as that found in the kidney was not affected by treatment with anti-VEGFR-2 antibody. Targeted inhibition of VEGFR-2 immediately prior to ovulation confirmed previous works that show that VEGF activity is needed for corpus luteum function as well as folliculogenesis.

### Corpora lutea and pregnancy

Studies of corpora lutea of pregnancy have further elucidated the role of angiogenic factors in corpus luteum function. Administration of anti-VEGFR-2 antibodies to pregnant mice has provided data which suggest that endothelial cell survival, proliferation and vascular permeability depends on the functioning of an intact VEGF/VEGFR-2 pathway to maintain corpora lutea function [59]. By 24 hours after a single post- or pre-implantation injection of the anti-VEGFR-2 antibody to CD-1 mice, luteal size, blood vessel density, and ovarian progesterone secretion start to decrease. Epithelial cell elimination through apoptosis and endothelial cell detachment from vascular basement membranes were noted after anti-VEGFR-2 antibody administration. By pregnancy day 13.5, there was a complete absence of embryonic structures. However, progesterone replacement was able to completely reverse the effects of the VEGFR-2 blocking antibody and pregnancies continued to develop normally. By contrast, administration of the anti-VE-C antibody caused no discernable effect on the pregnancy corpora lutea [47]. Although administration of an anti-VE-C antibody blocks follicular and luteal angiogenesis, anti-VE-C does not disrupt mature non-dividing, non-proliferating vessels in established corpora lutea of pregnant mice. Taken together, these results suggest that VEGF stabilizes mature vessels in pregnancy corpora lutea and mediates neovascularization and vascular permeability, while VE cadherin is specifically required for formation of new blood vessels.

In humans, rescue of the corpus luteum using hCG treatment, as occurs in early pregnancy, results in increased VEGF expression, endothelial cell proliferation and vessel stabilization [19]. This burst of angiogenesis has been ob-

served in rats but is absent in early pregnancy in the marmoset and rhesus monkeys [60]. Endothelial cell proliferation, pericyte area, and VEGF levels in corpora lutea of early pregnancy in the marmoset were similar to that of the late luteal phase of nonpregnant cycles [60]. Immunoneutralization of VEGF decreases plasma progesterone levels in mated animals but pregnancy rates in the marmoset are not significantly reduced. These data are in contrast to those obtained using the murine model. It is likely that mice are similar to rats in that increased angiogenesis accompanies early pregnancy.

### Regulation of angiogenic pathways

The molecular regulation of angiogenesis has been most extensively studied in models of tumor angiogenesis. In a variety of conditions, such as wound healing and malignant tumors, hypoxia is a major stimulus for synthesis and upregulation of angiogenic factors [1, 10]. However, in the ovary, the primary mediators of angiogenesis are likely regulated by gonadotrophin hormones, LH and FSH. Angiogenic factors include hypoxia-inducible factor (HIF), VEGF, and angiopoietins (Ang-1 and Ang-2). Other factors that are of lesser importance include endocrine gland vascular endothelial growth factor (EG-VEGF) [48], nitric oxide [61], and leptin [62].

HIF-1 mediates transcriptional activation of VEGF [63]. Given that HIF regulates VEGF and angiogenesis in cancers, it is likely that HIF has a role in ovarian angiogenesis. The role of HIF in the ovary is under active investigation. Angiopoietins bind primarily to Tie-2, a member of the Tie family of endothelium-specific tyrosine kinase receptors. Binding of Ang-1 to Tie-2 enhances stability of newly formed blood vessels. In the presence of VEGF, Ang-2 makes vasculature more responsive to angiogenic stimuli by destabilizing preexisting vasculature. However, in the absence of VEGF, Ang-2 causes endothelial cell apoptosis and vascular regression [63, 64].

Our understanding of the molecular regulation of ovarian angiogenesis is far from complete. Figure 2 contains a schematic of some principle factors involved in the regulation of ovarian angiogenesis.

### Clinical applications

Several clinical processes involving the ovary may be amenable to manipulation by modulating angiogenesis. These processes include ovarian neoplasms [57], ovarian hyperstimulation syndrome (OHSS) [65, 66], and polycystic ovarian syndrome (PCOS) [67, 68]. Conditions of the uterine corpus, such as endometriosis, fibroids and menorrhagia, are regulated by ovarian steroid hormones and may be affected by antiangiogenic therapies.

The role of VEGF in the formation of ovarian neoplasms has been suggested [35]. Analysis of cyst fluid shows that VEGF is present in functional, benign, borderline and malignant ovarian tumors, but is most markedly elevated in cancer [69]. Indeed, anti-angiogenic therapy in the form of a monoclonal antibody against human VEGF (bevacizumab [Avastin]) has demonstrated activity against an ovarian epithelial carcinoma that was refractory to other modalities [70]. Dopamine can inhibit VEGF induced angiogenesis in a well-characterized mouse ovarian carcinoma

model [71]. Intraperitoneal injection of dopamine at non-toxic doses reduces ascites and peritoneal angiogenesis. The prospect of more effective therapies against advanced ovarian cancer is encouraging.

OHSS is a potentially life threatening complication resulting from use of gonadotrophins, antiestrogens, and GnRH agonists to achieve controlled ovarian hyperstimulation. The pathophysiology of OHSS is still unknown, however the clinical manifestations of extravascular fluid accumulation with consequent intravascular volume depletion and hemoconcentration are related to increased permeability of peripheral vasculature. While the administration of hCG to hyperstimulated ovaries may be the inciting event, VEGF appears to play an important role in mediating the increased vascular permeability that ensues [65, 66, 72]. The ascitic and follicular fluids of women with OHSS contain high concentrations of VEGF [65, 73]. It has been shown that the concentration of VEGF contained in the follicular fluid of women undergoing IVF is related to the number of oocytes produced [73]. VEGF induces endothelial cell permeability by disrupting tight junctions and rearranging the actin cytoskeleton. Furthermore, these investigators demonstrated the permeability effects were 98% reversible with the administration of anti-VEGF antibody [73]. Unfortunately, measurements of circulating VEGF have failed to predict the onset of OHSS in women undergoing superovulation [74]. Nevertheless, inhibition of VEGF by administration of sFlt-1, a soluble truncated form of VEGFR-1 that neutralizes VEGF action [75], or SU5416, a compound that inhibits VEGFR-2 phosphorylation [66] in rodent models of OHSS ameliorates symptoms and suggests that anti-angiogenic agents may play a role in the management of this condition.

Serum levels of VEGF are significantly elevated in women with PCOS [67, 76]. In polycystic ovaries, EG-VEGF is strongly expressed in the stroma and spatially related to new blood vessels [77]. Studies are needed to clarify the precise role of angiogenesis in the clinical manifestations of PCOS, however one might speculate that predisposition of these patients to OHSS may be related to a relative hypervascularity of polycystic ovaries.

It is well recognized that development and maintenance of endometriosis is associated with angiogenesis. VEGF-A and -C mRNA are significantly upregulated in large endometriomas (>6 cm) and VEGF-A gene expression in peritoneal endometriotic lesions is statistically higher than that in normal peritoneum [78]. Nap et al [79] recently tested the ability of several anti-angiogenic agents to interfere with the maintenance and growth of endometriotic lesions. These investigators noted significantly decreased microvessel densities and a decreased number of established endometriosis lesions, except in areas in which vessels were protected with pericyte [79]. Angiogenic inhibition may be a viable option in the treatment of endometriosis.

Physiologic ovarian function can also be targeted by angiogenic modulation. Fraser et al [38] demonstrated that single injections of VEGF TrapR1R2 blocked ovulation in macaques, and showed dose related suppression of ovarian function. Furthermore, the effects were reversible, with normal ovarian function resuming when levels of VEGF TrapR1R2 fell below a threshold level. Such findings suggest a potentially significant clinical application for VEGF TrapR1R2 in situations where temporary suppression of

ovulation is desirable, such as required for contraception or treatment of endometriosis [38]. The role of angiogenesis in implantation and pregnancy also suggests that inhibition of VEGF may be useful for emergency contraception, treatment of ectopic pregnancy, or therapeutic abortion [59].

## Conclusion

Ovarian function is critically dependent on angiogenesis for follicle development, ovulation and corpus luteal function. Experiments in rodents and non human primates demonstrate that VEGF is the primary modulator of angiogenesis in the ovary. Inhibition of VEGF action disrupts folliculogenesis and corpus luteum formation and function while overexpression of VEGF is noted in pathological conditions such as ovarian cancer, endometriosis and OHSS. The ability to manipulate the ovarian cycle by modulating angiogenesis and the potential to treat ovarian pathology by reducing VEGF expression have promising clinical implications.

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