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Angiogenic factors and ovarian follicle development

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Abstract

Ovarian follicles require an adequate blood supply for oxygen, nutrients and hormones, in addition to eliminating CO_2 and other metabolites. Acquisition of an adequate vascular supply is probably a limiting step in the selection and maturation of the dominant follicle. In this way, there is a progressive interest in the study of the growth factors involved in the angiogenic process. In addition, a better understanding about the mechanisms that regulate the expression and action of these factors could be a key point to increase the reproductive performance in females. Therefore, this review aims to summarize current data on the importance of the pro- and anti-angiogenic growth factors which regulate angiogenesis in ovarian follicle development.

Keywords: angiogenesis, follicle, growth factors, ovary.

Introduction

During embryo development, blood vessels differentiate from endothelial precursors by a process called vasculogenesis. Angiogenesis is the process of new blood vessel development from pre-existing vasculature that occurs in embryos and adults (Stouffer *et al*., 2001). In the last two decades, there was a progressive interest in the study of angiogenesis due to the association of this process with pathological conditions in adult tissues, such as tumoral growth and inflammation (Smith, 2001). In addition, several aspects of human and animal reproduction, such as clinical alterations that occur in the ovary and in the female reproductive tract depend on angiogenesis (Acosta *et al*., 2003). This process occurs throughout follicular development, allowing adequate nutritional and hormonal supply for ovarian follicle growth and oocyte development, as well as corpus luteum formation (Fraser and Lunn, 2000).

A wide range of growth factors have been identified that promote (pro-angiogenic) or inhibit (anti-angiogenic) angiogenesis (Stouffer *et al*., 2001). However, modulation of the expression and action of these factors can be a key point to increase female reproductive performance. In this review, aspects related to the importance of angiogenesis in ovarian follicle development, as well as the role of the regulatory pro- and anti-angiogenic growth factors, will be discussed.

Blood vessels formation

The vascular system is developed based on two distinct processes: vasculogenesis and angiogenesis. While blood vessels differentiate from endothelial precursors by a process called vasculogenesis during embryo development, in adults further vessel development from pre-existing vasculature occurs by intussusception or sprouting by a process called angiogenesis. Angiogenesis is characterized by a cascade of events that starts with capillary proliferation and culminates with the formation of a new microcirculation composed of arterioles, capillaries and venules (Redmer and Reynolds, 1996). During the development of new blood vessels, some features can be observed such as enzymatic degradation of the basal membrane of the pre-existing vessels, migration of endothelial cells marked by angiogenic stimulus and finally, endothelial cell proliferation (Redmer *et al*., 2001). This neovascularization is completed with the formation of a capillary network and differentiation of new capillaries into arterioles and veins (LeCouter *et al*., 2002).

Studies have demonstrated that circulating endothelial precursor cells, i.e., originated from bone marrow, may contribute to angiogenesis in adults (Carmeliet and Jair, 2000). In addition, recent studies indicated the presence of mitogenic endothelial cells, which are the primary components of capillaries, in specific organs, modulating angiogenic responses in a variety of organs (LeCouter *et al*., 2002).

It is important to note that in several adult tissues, capillary growth rarely occurs and the vascular endothelium represents a stable population of cells with low mitogenic rates (Klagsbrun and D`Amore, 1996). An exception is the rapid growth and regression that occurs in the female genital organs, associated with equivalent changes in its vascular network (Reynolds and Redmer, 1995). The mature ovary shows a highly developed vasculature, reflecting its high metabolic rate,

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which turns this organ into a unique model for studies of angiogenesis regulation during growth, differentiation and regression of normal tissues in adults (Redmer and Reynolds, 1996).

Follicular angiogenesis

The ovarian follicle is the structural and functional unit of the mammalian ovary, which supplies the necessary environment for oocyte growth and maturation (Telfer, 1996). The follicles are surrounded by somatic cells (granulosa and theca cells) and can be classified in preantral (primordial, primary and secondary) and antral (tertiary and preovulatory) follicles (Hulshof *et al*., 1994). It is known that vasculature is not equally distributed among the population of follicles of the adult ovary, since only theca cell layers, present in later follicular stages, have vessels. Quiescent primordial follicles and slow-growing preantral follicles do not have a vascular supply of their own, but instead rely on vessels in the surrounding stroma. Thus, Martelli *et al.* (2009) showed that an autonomous vascular supply starts to be evident in preantral follicles with diameter from 110 µm. However, as a follicular antrum develops, the thecal layer acquires a vascular sheath consisting of two capillary networks located in the theca interna and externa, respectively (Stouffer *et al*., 2001).

Acquisition of an adequate vascular supply is probably a limiting step in the selection and maturation of the dominant follicle destined to ovulate (Stouffer *et al*., 2001). Some studies have shown that angiogenesis is followed by a vasodilatation, a functional adaptation for the occurrence of ovulation, as well as by the development of theca endocrine function (Jiang *et al*., 2003). Thus, there are evidences that theca cells angiogenesis have a primary role in follicular development (Tamanini and De Ambrogi, 2004).

The development and growth of the theca vascular network are probably controlled by paracrine and angiogenic factors produced by granulosa cells. In addition to those factors, Vascular Endothelial Growth Factor (VEGF), whose levels increase according to follicular growth, can induce the formation of a primitive capillary network during the early phases of antral follicle development. Moreover, the regulation of angiogenesis seems to be dependent on the interaction among other growth factors that can act in different moments, some of them stimulating growth, while others, mediating endothelial cell reorganization in more complexes vascular structures (Grasselli *et al*., 2003).

The degeneration of the capillary network is a relevant phenomenon that causes follicular atresia through the interruption of the metabolic supply for follicular cells. In addition, an increase in vascular density around antral follicles contributes to the inhibition of atresia. However, some studies have suggested that microvascular changes of atretic follicles are a consequence and not the cause of atresia (Macchiarelli *et al*., 1993).

As the corpus luteum begins its formation, thecal capillary sprouts begin to migrate towards and grow into the folds of the stratum granulosum. The growth of new capillaries during luteal angiogenesis follows a cascade of events including changes in the basement membrane, migration and proliferation of endothelial cells and development of capillary lumina (Plendl, 2000).

Pro-angiogenic growth factors

A variety of parameters, including oxygen tension, aging and endocrine or local factors can modulate the expression of angiogenic factors. It is generally believed that a decline in local oxygen concentrations (hypoxia) is a primary initiator of angiogenesis in normal and pathologic tissues (Hazzard and Stouffer, 2000). In the ovary, pro-angiogenic factors promote vascular permeability, supporting antrum formation and the events that induce follicular rupture (Tamanini and De Ambrogi, 2004). Several pro-angiogenic factors are well-known, such as fibroblast growth factor-2 (FGF-2), VEGF, angiotensin II (ANG II), insulin like growth factor-1 (IGF-1), epidermal growth factor (EGF), angiopoietin (ANPT) and endothelin-1 (ET-1). However, those that seem to be most important in angiogenesis are FGF-2, VEGF and ANG II (Redmer *et al*., 2001). Table 1 and Fig. 1 summarize the effects of pro-angiogenic factors on ovarian follicular development.

Table 1. Summary of the effects of angiogenic factors on ovarian follicular development.

Angiogenic factors	Effects on ovarian follicular development
FGF-2	Oocyte and granulosa cell survival Primordial follicle activation Granulosa and theca cell proliferation
VEGF	Primordial follicle survival Mitogenic effect in granulosa cells Transition from primary to secondary follicles
ANG II	Regulates oocyte maturation, ovulation and steroidogenesis
IGF-1	Follicular growth and survival Increases steroidogenesis

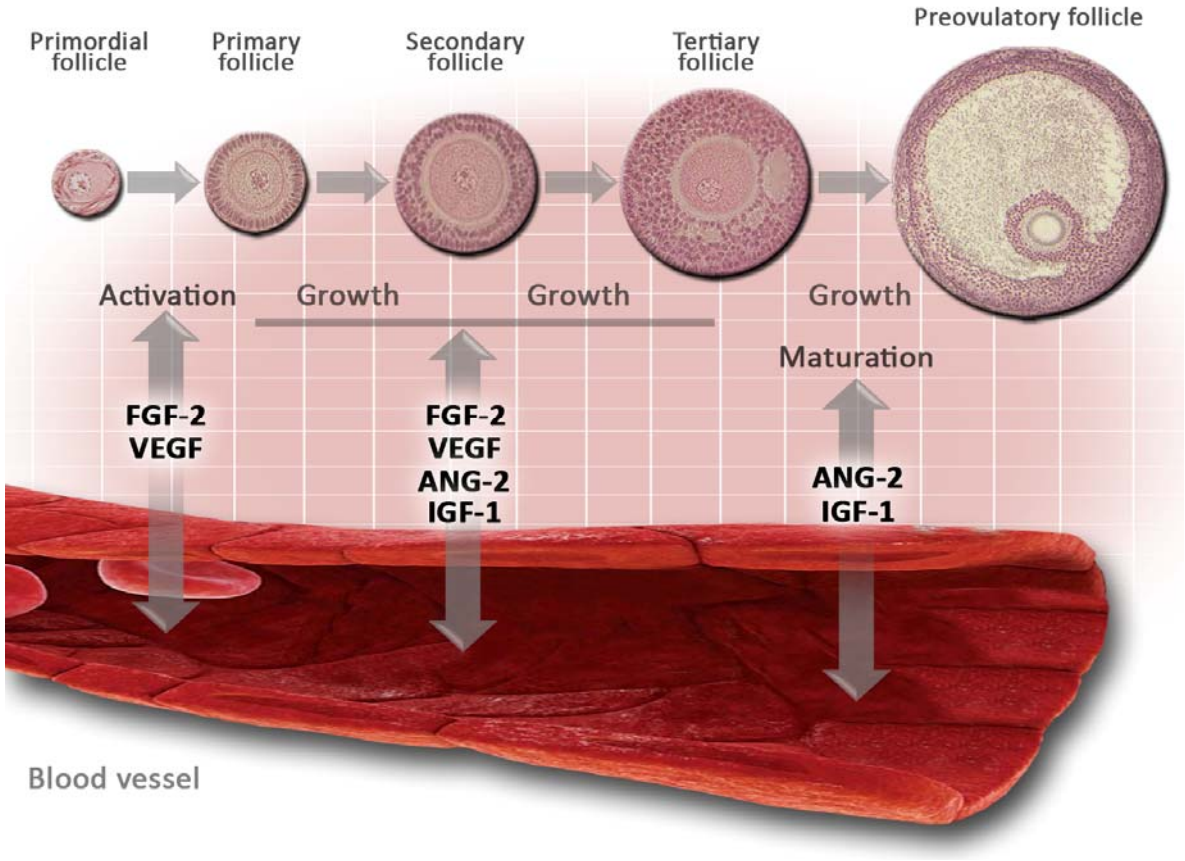

Figure 1. Angiogenic growth factors act in different stages of follicular development.

Fibroblast growth factor-2 (FGF-2)

FGF-2, also known as basic FGF (bFGF), was the first angiogenic factor indentified in the ovary (Gospodarowicz *et al*., 1985). The localization of FGF-2 in endothelial cells suggests that it is an important factor for endothelial growth (Gospodarowicz *et al*., 1985).

FGF-2 is also found in ovarian follicles (rat: Nilsson *et al*., 2001; human: Ben-haroush *et al*., 2005) and corpus luteum (rat: Asakai *et al*., 1993; bovine: Schams *et al*., 1994), while its receptors are expressed in growing follicles (Wandji *et al*., 1995). In medium and large swine follicles, mRNA for FGF-2 and its receptor FGFR-2 was detected in granulosa and theca cells, respectively. In the bovine ovary, the expression of the mRNA for FGF-2 in inner theca significantly increases during final follicular growth; however this expression was weak in granulosa cells (Shimizu *et al*., 2002). This factor exerts an antiapoptotic effect in granulosa cells, favoring the production of other angiogenic factors (Grasselli *et al*., 2002).

Some in vitro studies have shown that addition of FGF-2 to the culture medium promoted primordial and primary follicles growth (Nilsson *et al*., 2001), granulosa and theca cell proliferation (Wandji *et al*., 1996), as well as oocyte survival (Zhou and Zhang, 2005). Recently, Matos *et al*. (2007) demonstrated that FGF-2 at 50 ng/mL, stimulated goat primordial follicle activation after 5 days of in vitro culture.

Vascular Endothelial Growth Factor (VEGF)

VEGF, also known as vascular permeability factor (VPF), is a potent mitogenic factor that stimulates endothelial cell migration. It has also a role in the structural maintenance, increase of capillary permeability (Redmer *et al*., 2001) and a survival factor for endothelial cells of microvessels (Stouffer *et al*., 2001). The VEGF family is composed of at least six members (VEGF A, B, C, D, E and F), and the human VEGF-A gene is organized in eight exons, separated by seven introns. Alternative exon splicing results in the generation of four different isoforms, having 121, 165, 189, and 206 amino acids, respectively, after signal sequence cleavage (VEGF121, VEGF165, VEGF189, VEGF206; Stouffer *et al*., 2001; Ferrara, 2004). VEGF A expression was demonstrated in preantral follicles. The protein VEGF A has been identified in oocytes of human primordial follicles (Otani *et al*., 1999; Harata *et al*., 2006) and human and rat primary follicles (Celik-Ozenci *et al*., 2003). In swine and bovine follicles, VEGF A is weakly expressed during early development

and this expression becomes higher in granulosa and theca cells of dominant follicles (Barboni *et al*., 2000; Greenaway *et al*., 2005). Expression of mRNA for VEGF in the granulosa and theca cells, as well as the protein for VEGF in all follicle compartments and follicular fluid significantly increase according to the stage of follicular development (Yamamoto *et al*., 1997; Greenaway *et al*., 2004; Taylor *et al*., 2004). Recently, VEGF A expression in rats was occasionally observed in early preantral follicles and was always detected in preantral follicles during the late stages of development (Abramovich *et al*., 2009; Martelli *et al*., 2009). Furthermore, there are two known VEGF receptors (VEGFR-1 and VEGFR-2) that bind to VEGF-A. VEGFR-1 is expressed in quiescent and proliferative endothelial cells (Berisha *et al*., 2000) and induced the formation of vessels by VEGF (Boonyaprakob *et al*., 2003). VEGFR-2 is expressed specially in angiogenic endothelial cells and regulates the effects of VEGF on the proliferation and migration of these cells (Celik-Ozenci *et al*., 2003).

High VEGF concentrations cause a destabilization of the blood vessels, resulting in a new vascular network development, while VEGF deficiency results in blood vessel regression (Hanahan, 1997). Furthermore, Hazzard *et al*. (1999) demonstrated that gonadotropins stimulated VEGF secretion in primate preovulatory follicles and can act as regulatory factors of VEGF production. In this way, modulation of the hormones that influence VEGF expression, such as human (hCG) and equine (eCG) chorionic gonadotrophin, luteinizing hormone (LH) and follicle stimulating hormone (FSH), as well as their levels in the follicle, are possibly one of the keys to control ovarian follicular angiogenesis. There are also in vitro (Pepper *et al*., 1992) and in vivo (Asahara *et al*., 1995) indications of synergistic effects between angiogenic growth factors. In bovine, association between VEGF and FGF-2 induced an in vitro angiogenic response, which was stronger and faster than the effect produced by these two factors individually.

Recent in vitro studies suggested that VEGF has a mitogenic effect in granulosa cells and can stimulate follicular growth in rats (Otani *et al*., 1999). In this species, Kezele *et al*. (2005) identified that the gene encoding for VEGF-A is an important regulator of primordial follicle development. In addition, Danforth *et al*. (2003) showed that VEGF increases the number of primary and secondary follicles in rat ovaries. Recently, a study verified that VEGF promoted the transition from primary to secondary follicles in bovine (Yang and Fortune, 2007). Furthermore, a study associated VEGF production and the increase of blood vessel content to follicular activation, i.e., the transition from the primordial to primary follicle stage (Mattioli *et al*., 2001). Another study showed that endogenous VEGF is essential to rodent primordial follicle survival (Roberts *et al*., 2007). Moreover, the inhibition of VEGF activity produced an increase in ovarian apoptosis through an unbalance in the pro and antiapoptotic protein rate, leading to a great number of atretic follicles (Abramovich *et al*., 2006). Other authors observed that the direct injection of VEGF into the ovary increases vasculature (Shimizu, 2006), the number of antral follicles and inhibits apoptosis (Quintana *et al*., 2004).

Endocrine gland-derived vascular endothelial growth factor (EG-VEGF)

Endocrine gland-derived vascular endothelial growth factor (EG-VEGF) was identified as a novel human endothelial cell mitogen, through a bioassay assessing the ability of a library of purified human secreted proteins to promote the growth of primary adrenal cortex capillary endothelial cells (LeCouter *et al*., 2001). EG-VEGF does not belong to the VEGF family or other known families of endothelial mitogens but instead is a member of a structurally related class of peptides including the digestive enzyme colipase, the Xenopus headorganizer, Dickkopf (Glinka *et al*., 1998), venom protein A (VPRA; Joubert and Strydom, 1980) or mamba intestinal toxin-1 ‘‘MIT-1’’ (Schweitz *et al*., 1999), a non-toxic component of Dendroaspis polylepis polylepis venom, and the secreted proteins from Bombina variegata designated Bv8 (Mollay *et al*., 1999). Its receptors were designated EG-VEGFR-1 and EG-VEGFR-2 (Masuda *et al*., 2002). EG-VEGF selectively promoted proliferation, survival and chemotaxis of endothelial cells isolated from steroidogenic tissues (Lin *et al*., 2002). Indeed, exogenous EG-VEGF in the ovary (LeCouter *et al*., 2001) or testis (LeCouter *et al*., 2003) can dramatically affect vascular leakage. Northern blot analysis of a panel of RNAs from a variety of human tissues revealed EG-VEGF expression in ovary, testis, adrenal and placenta (LeCouter *et al*., 2001). In situ hybridization analysis demonstrated that steroidogenic cells within these glands are the source of EG-VEGF (LeCouter *et al*., 2001). Granulosa cells in primordial and primary follicles express EG-VEGF strongly. As the secondary follicle matures, EG-VEGF expression in granulosa cells declines (Ferrara *et al*., 2003). At approximately 5 days post-ovulation, both VEGF-A and EG-VEGF are strongly expressed in a portion of granulosa lutein cells, whereas 8 days post-ovulation EG-VEGF expression is intense in the theca lutein cells, while VEGF expression has diminished to the point where only weak signal remains in the peripheral thecal cells (Corner, 1956).

Angiotensin II (ANG II)

ANG II is a potent vasoactive peptide, which is converted from ANG I by angiotensin conversion enzyme, and induces neovascularization in rabbit retina (Fernandez *et al*., 1985), mouse and human endometrium (Hu *et al*., 1996; Li and Ahmed, 1996;

Walsh *et al*., 1997), as well as in the bovine corpus luteum (Walsh *et al*., 1997). ANG II acts by its binding to a group of receptors, classified as ANG receptor type 1 (AT-1) and 2 (AT-2). Both receptors are expressed in the ovary, and their presence and distribution differ significantly among the species and follicular development stages (Pucell *et al*., 1991; Obermüller *et al*., 1998). In rabbit ovaries, AT-2 receptor is expressed predominantly in granulosa cells of preovulatory follicles, while AT-1 is located in theca and stroma cells (Yoshimura *et al*., 1996). In mouse, AT-2 is expressed exclusively in granulosa cells of large atretic follicles, while AT-1 is expressed in all structures of ovarian follicles (Pucell *et al*., 1991). The presence of ANG II receptors in the ovary suggests a possible role of this peptide on this organ. Mitsube *et al*. (2003) reported that only the blockage of AT-2 increases blood flow in the mouse ovary, suggesting that vasoconstriction occurs specifically through this receptor. In addition, another study observed high ANG II levels during proestrous in the mouse, and this fact may be associated with the high vascular proliferation that occurs in this phase of the estrous cycle (Costa *et al*., 2003). Moreover, ANG II improves angiogenic activity of VEGF in bovine microvascular cells (Otani *et al*., 2000).

ANG II regulates oocyte maturation (Kuo *et al*., 1991), ovulation (Yoshimura *et al*., 1996) and steroidogenesis (Yoshimura *et al*., 1993) through the modulation of ovarian blood flow (Mitsube *et al*., 2003). ANG II is well known for its vasoconstrictor action; however this effect was not observed in the ovary after treatment with ANG II (Costa *et al*., 2003). The vasodilatation was observed in rabbit ovaries perfused in vitro with ANG II and this effect can be due to the release of gonadotropins stimulated by ANG II (Kuo *et al*., 1991). In addition, Shuttleworth *et al*. (2002) suggested a possible role of ANG II in swine early folliculogenesis and steroidogenesis. In bovine, ANG II restored the inhibitory effect of follicular cells on oocyte maturation (Giometti *et al*., 2005; Stefanello *et al*., 2006) and stimulated nuclear and cytoplasmic maturation of swine oocytes (Li *et al*., 2004). In addition, ANG II appears to regulate the induction of several autocrine growth factors, such as platelet derived growth factor, transforming growth factor-β, FGF-2 and IGF-1, and induces the angiogenic activity through the paracrine function of VEGF in microvascular cells (Otani *et al*., 2000).

Insulin like growth factor-1 (IGF-1)

The IGF system appears to have indirect effects on angiogenesis through stimulatory action for VEGF production in luteal cells, as well as through the stimulus of endothelial cell proliferation and differentiation (Schams *et al*., 2001). In bovine, a high expression of IGF-1 in theca interna was observed before the phase of follicular selection, while the expression increased in granulosa cells after this phase. In addition, mRNA for IGF-1 receptor (IGFR-1) was present in theca interna and granulosa cells with increased levels during final follicular development (Schams *et al*., 2002).

In association with FSH, addition of IGF-1 to the in vitro culture medium of preantral follicles stimulated follicular growth in several species (human: Louhio *et al*., 2000; bovine: Gutierrez *et al*., 2000; rats: Zhao *et al*., 2001; mouse: Liu *et al*., 1998). Experiments performed by Zhou and Zhang (2005) showed that IGF-1 promoted the growth and maintained the viability of oocytes from caprine preantral follicles. In swine, 50 ng/mL of IGF-1 promoted follicular growth, stimulated granulosa cell proliferation and prevented apoptosis of preantral follicles cultured for 4 days in the presence of serum (Mao *et al*., 2004). Furthermore, different concentrations of IGF-1 (10, 50 and 100 ng/mL) increased follicular diameter and steroidogenesis of mouse preantral follicles cultured in vitro for 6, 10 and 12 days (Demeestere *et al*., 2004). In a recent study, Thomas *et al*. (2007) showed that follicular diameter was increased over control levels by addition of 50 ng/ml of IGF-I during 6 days of culture.

Anti-angiogenic growth factors

Angiogenesis is also modulated by inhibitory factors, such as thrombospodin, angiostatin, endostatin, 2-metoxiestradiol, hyaluronic acid, platelet factor-4, tumoral necrose factor α and interferon γ. These substances blocked endothelial cell proliferation and migration, as well as in vitro capillary formation (Espinosa-Cervantes and Rosado-Garcia, 2002).

Regarding thrombospodin 1 and 2, they bind to their receptor CD36 and inhibit angiogenesis, inducing endothelial cell apoptosis. In rat ovaries, the expression of thrombospondin 1 and CD36 mRNA is high in granulosa cells of preantral and antral follicles and is limited in theca cells. Nevertheless, there is no expression of mRNA for thrombospodin 2 in ovarian follicles. Furthermore, thrombospodin 1 is strongly expressed in small follicles where the vascularization is absent, showing that expression of thrombospodins decreases during follicular maturation together with the increase in VEGF levels (Petrik *et al*., 2002).

Platelet factor-4 inhibits angiogenesis both in vivo and in vitro and the inhibitory effects are due to the formation of complexes with FGF-2, inhibiting FGF-2 binding to its receptors (Perollet *et al*., 1998).

Final considerations

Increasing evidence suggests that physiological angiogenesis in ovarian follicles and corpus luteum are fundamental features of mammalian reproduction.

Failures in vascular development in these structures may be the reason for several ovarian dysfunctions observed during the estrous cycle and pregnancy. Therefore, it is necessary to evaluate both in vivo and in vitro influences of the angiogenic factors, alone or in association, on the survival (anti-apoptotic effects) of ovarian cells in different species. This information will provide novel opportunities for therapeutic interventions and improvement of the efficiency of assisted reproduction in humans and animals in the future.

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