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—Review—

Angiogenic Factors (VEGF, FGF and IGF) in the Bovine Corpus Luteum

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Abstract. Angiogenesis, the development of the new capillaries by endothelial cell proliferation and outgrowth from pre-existing vessels, is one of the prominent features of early corpus luteum (CL). The process of angiogenesis is an important component of normal development and function of CL. Of the numerous promoters of angiogenesis and maintenance of new established capillaries that have been identified, the most important factors appear to be vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF). The biological effects of VEGF and FGF factor families are mediated by signaling through specific tyrosine kinase receptors. The VEGF, FGF and IGF family members in bovine CL are discussed with the literature available for large domestic animals. The highest expression for VEGF, VEGFR-2, FGF-2, FGFR, IGF-1, IGF-2 and IGFR1 were found during the early luteal phase (Ld 1–4) followed by a significant decrease afterwards. The VEGF and IGF-1 protein was localized predominantly in luteal cells. FGF-1 mRNA expression peaked ($P<0.05$) during mid-luteal stage and FGF-1 protein was localized in cytoplasm of luteal cells, but endothelial cells are always negative. FGF-2 protein during angiogenesis (d 1–5) was found in cytoplasm of endothelial cells and changed thereafter exclusively to the cytoplasm of luteal cells. In contrast, the IGF-2 protein is exclusively localized in pericytes and few endothelial cells. The results obtained (mainly in ruminant) suggest an important role of these growth factors for angiogenesis and furthermore for maintenance and function of the bovine corpus luteum.

Key words: Angiogenesis, Corpus luteum, Bovine, Vascular endothelial growth factor, Fibroblast growth factor, Insulin-like growth factor

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The ovarian cycle is characterized by repeating patterns of cellular proliferation, differentiation and transformation that accompany follicular development and the formation and regression of the corpus luteum (CL). Formation of the CL is initiated by the series of morphologic and biochemical changes in cells of the theca interna and granulosa of the preovulatory follicle. These changes, termed luteinization, occur with the preovulatory LH surge. In cattle the principal function of the CL is to secrete progesterone, during non-pregnant cycle as well as during pregnancy [1]. The CL is one of the few adult tissues that exhibits regular periods of growth, development and regression.

The CL is a heterogeneous tissue and besides endothelial cells, steroidogenic large luteal and small luteal cells it also consists of fibroblasts, smooth muscle cells and immune cells [2]. In complex tissue, the various cell types must interact to ensure normal growth and development. Tissue growth (follicle or CL) depends upon growth of new blood vessels and establishment of a functional blood supply. In the mature CL nearly every parenchymal cell is in contact with one or

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more capillaries [3]. An improved understanding of follicular growth and luteal function obviously has important implications for the regulation of fertility in mammals. In addition, because the CL is so dynamic, it provides an ideal model for studying the regulation of the process of angiogenesis and development.

Angiogenesis

Angiogenesis is the preferred term for processes leading to the generation of new blood vessels through sprouting from already existing blood vessels in a process involving the migration and proliferation of endothelial cells from pre-existing vessels. Blood vessel growth occurs in the embryo and rarely in the adult with exceptions such as the female reproductive system, wound healing, and pathological processes such as cancer [4]. In contrast, the term vasculogenesis is mainly used for the de novo generation of blood vessels occurring during embryogenesis [5]. In vasculogenesis, vessels form de novo through the assembly of endothelial precursors, called angioblasts. Vascular expansion is the enlargement of small or occluded vessels, which is observed frequently during the generation of collateral blood vessels. The process of angiogenesis includes degradation of the capillary vessel basement membrane, through which migrating endothelial cells form a sprout and proliferate to create a new lumen and further vessel maturation [6].

Of the numerous promoters of angiogenesis that have been identified, the most important factors appear to be vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF). Numerous other possible inducers of angiogenesis have been identified, including members of transforming growth factors (TGF) family, angiopoietins, epidermal growth factor (EGF), insulin like growth factors (IGF), tumor necrosis factors (TNF), angiotensin-2, endothelins, and proteins of the extracellular matrix (ECM). These factors and compounds differ in cell specificity and also in the mechanisms by which they induce the growth of new blood vessels. Not all compounds that are active *in vivo* show the same spectrum of activities for endothelial cells *in vitro*. Many of these factors are pleiotropic and, among other things, may induce the migration and proliferation of endothelial cells and the production of collagenase and plasminogen activator. Some of these factors are chemotactic. They elicit their effects probably by attracting other cells to the site of growth, by activating these cells and by inducing them to secrete angiogenic factors. As many angiogenic factors are mitogenic and chemotactic for endothelial cells their biological activities can be determined *in vitro* by measuring the induced migration of endothelial cells or the effect of these factor on endothelial cell proliferation [7].

Physiological angiogenesis is mainly restricted to the female reproductive system where it occurs cyclically in the ovary (folliculogenesis and corpus luteum formation) and the uterus as well as during pregnancy [8, 4]. Angiogenesis is a complex process in which a delicate balance between promoters and inhibitors is maintained. Disturbance of this balance may result in a disrupted physiologic state or various pathologic conditions [6]. Therefore, the precise control of angiogenesis in the ovary is critical for normal ovarian function [9].

Ovarian angiogenesis

Vascular development or angiogenesis is a critical component of normal growth and function of ovarian follicles and corpora lutea. Recent studies have confirmed, for example, that dominant follicles not only are more vascular but also take up more serum gonadotropins than nondominant antral follicles, leading to the suggestion that increased vascularity may be a primary determinant of follicular dominance [10]. Although it is well established that the ovarian function is regulated primarily by the pituitary gonadotropins FSH, LH and their receptors (FSHR, LHR); it is also evident that locally produced factors such as steroid hormones, peptides and growth factors have modulatory roles in follicular development [11]. Angiogenesis, the formation of new capillaries to a dense network, may play an important role in the selection process suggested by studies in monkeys that demonstrate that the selected follicle possesses a more elaborate microvascular use than other follicles [10].

Mature CL are so vascular, in fact, that the majority of parenchymal (steroidogenic) cells are adjacent to one or more capillaries, which is not surprising because up to 85% of the cells that proliferate during luteal growth are endothelial cells [1, 12]. Conversely, inadequate luteal function has been associated with decreased luteal

vascularity and several investigators have suggested that reduced ovarian blood flow plays a critical role in luteal regression [13].

Ovarian angiogenic factors

Relatively few studies have evaluated the effects of growth factors and cytokines on proliferation of follicular or luteal cells. Because the ovary tissues are so dynamic, they provide a unique model for studying the regulation of angiogenesis during growth, differentiation and regression of normal adult tissues. The ovary was among the first organs in which growth factors with angiogenic activity were detected [14].

Vascular endothelial growth factors (VEGFs)

Vascular endothelial growth factor (VEGF) is a homodimeric heparin binding glycoprotein of 46–48 kDa (24 kDa subunits). The VEGF family currently comprise six members: VEGF-A (which denotes the originally identified VEGF), placenta growth factor (PlGF), VEGF-B, VEGF-C, VEGF-D and VEGF-E [5, 15]. The human VEGF gene has a length of approximately 12 kb and is composed of eight exons, separated by seven introns. It is now well established that alternative exon splicing of a single VEGF gene results in the generation of five different molecular species, having respectively 121, 145, 165, 189 and 206 amino acids after signal sequence cleavage ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{189}$, $VEGF_{206}$). Bovine VEGF is one amino acid shorter than the human factor, and the bovine and human sequences show a homology of 95 percent. It was found that $VEGF_{121}$ is soluble secreted form; $VEGF_{145}$ and $VEGF_{165}$ are also secreted, although a significant fraction remains bound to the cell surface and the extracellular matrix, whereas $VEGF_{189}$ and $VEGF_{206}$ are mostly cell associated. $VEGF_{165}$ is the most common molecular form produced by a variety of normal and transformed cells. In contrast, $VEGF_{206}$ is very rare form [16]. The different isoforms of VEGF have different properties *in vitro* and this may apply also to their *in vivo* functions. VEGF is a highly specific mitogen for vascular endothelial cells. *In vitro* the shorter forms of VEGF stimulate the proliferation of macrovascular endothelial cells. VEGF does not appear to enhance the proliferation of other cell types. The VEGF protein is produced also by tumor cells, and normal cell types, including macrophages, lung epithelial cells, kidney epithelial cells, follicular cells in the pituitary, corpus luteum cells, ovarian follicle cells, aortic smooth muscle cells. VEGF expression is influenced by numerous factors including hypoxia, hormones, growth factors and cytokines [17].

The biological activities of VEGF are mediated through two high affinity receptor tyrosine kinases. The Flt-1 (fms-like-tyrosine kinase) or VEGFR-1 and the Flk-1 (fetal liver kinase-1) or VEGR-2 possess seven immunoglobulin-like domains in the extracellular domain, a single transmembrane region and a consensus tyrosine kinase sequence that is interrupted by a kinase insert domain. VEGF binds to VEGFR-1 with a Kd of approximately 10–20 pM [18]. VEGFR-2 has a somewhat lower affinity for VEGF: the Kd has been estimated to be approximately 75-125 pM [19]. The expression of VEGFR-1 and VEGFR-2 genes is largely restricted to the vascular endothelium [20].

VEGF has been shown to regulate most steps of the angiogenic process, including endothelial cell degradation of extracellular matrix, migration, proliferation, and tube formation.

Fibroblast growth factors (FGFs)

Fibroblast growth factors (FGF) constitutes a family of related 16–18 kDa proteins controlling normal growth and differentiation of mesenchymal, epithelial, and neuroectodermal cell types. Acidic fibroblast growth factor (FGF-1) and basic fibroblast growth factor (FGF-2) are prototypic members of a large family of 20 structurally related proteins [21, 22]. All factors are products of different genes, some of which are Oncogene products (FGF-3 to FGF-5). The FGF-1 gene has a length of approximately 19 kb and is composed of three exons, separated by two very large introns. FGF-1 is one of the angiogenesis factors and has been shown to promote wound healing. The FGF-2 gene has a length of more than 36 kb; it is composed of three exons separated by two 16 kb introns [23]. The sequences of bovine and human FGF-2 differ in only 2 amino acids.

FGF-2 is heparin binding growth factor, which occurs in several isoforms resulting from alternative initiations of translation: an 18 kDa cytoplasmic isoform and four larger molecular weight nuclear isoforms (22, 22.5, 24, and 34 kDa). Most of the reported functions are for 18 kDa isoform which is widely distributed in many tissues. The smallest FGF-2 isoform (18 kDa)

occurs predominantly in the cytosol and functions in an autocrine manner, where the higher molecular weight forms (22, 22.5, 24 kDa) are associated with the nucleus and exert activities through an intracrine pathway [24, 25]. The mechanism by which the factor is released by the cells is not known.

The biological activities of FGFs are mediated through high affinity tyrosine kinase receptors (FGFR-1 to FGFR-4) and heparan sulfate proteoglycan low affinity receptors. FGF-2 activation of FGFR induces FGFR dimerization and subsequent transphosphorilation, which initiates signaling cascades. These FGFRs are characterized by the presence of two or three immunoglobulin-like domains in the extracellular region and a tyrosine kinase domain in the intracellular region of the receptor. Although all the different splice variants of the four FGFRs can be activated by FGF-1, most of the FGFR variants have narrower specificity for the different FGF ligands. Alternative splicing in the extracellular region of FGFR-1 to FGFR-3 generates receptor variants (IIIb and IIIc) with different ligand binding affinities. In particular FGF-2 activates the splice variant FGFR2-IIIc but not FGFR2-IIIb [26]. A multifunctional role of FGF-2 is suggested by the many different receptor phenotypes expressed in various cell types [22]. Evidence of various experimental systems suggests that FGF-2 is a potential regulator of a variety of genes. FGF-2 stimulates the growth of fibroblasts, myoblasts, osteoblasts, neuronal cells, endothelial cells, keratinocytes, chondrocytes, and many other cell types. FGF-2 has pleiotropic roles in many cell types and tissues; it is a mitogenic, angiogenic and survival factor, which is involved in cell migration, cell differentiation and in a variety of development processes.

Insulin-like growth factors (IGFs)

There are clear evidences for a functional role of IGFs on luteal tissue. IGF-1 and IGF-2 have stimulatory effects on progesterone secretion of sheep [27], pigs [28] and cattle [29, 30]. However, the IGFs may have additional actions to include angiogenesis and apoptosis. IGF-1 and IGF-2 have also been implicated in neovascularization occurring in response to injury [31], in the rabbit cornea model [32] and localization in the bovine CL [33]. The interaction of the IGF receptor with IGF-1 or IGF-2 protects different types of cells from apoptosis including ovarian cells [34]. The biological actions of IGF-1 and IGF-2, which include proliferation and cellular differentiation, are regulated by six binding proteins (IGFBP1-6).

VEGF in bovine corpus luteum

Because of the importance of vascular development in the growth, development, and function of ovarian tissues, we have therefore focused in our studies on follicular and luteal angiogenic growth factors and their receptors, such as expression of mRNA, concentration and localization of their protein during CL development (angiogenesis), maintenance and regression.

All components of the VEGF system were found in the bovine corpus luteum during the estrous cycle [35, 36]. Analysis of VEGF transcript by RT-PCR shows that CL tissue express predominantly the smallest isoforms ($VEGF_{121}$ and $VEGF_{165}$). The highest mRNA expression for VEGF and VEGFR-2 mRNA was detected during the early luteal phase followed by a significant decrease of expression during the mid and late luteal phase and a further decrease of VEGF mRNA after regression [37]. In contrast, no significant change in VEGFR-1 mRNA expression during the estrous cycle was found. VEGF protein concentration in CL tissue was significantly higher (20.9–23.4 ng/g wet weight) during the early luteal phase (d 1–7) followed by a decrease at the late luteal phase (14.3–18.7 ng/g) and especially after CL regression (2.8 ng/g). As achieved by immunohistochemistry, VEGF protein was localized predominantly in luteal cells. High VEGF protein and transcripts concentrations and increased VEGFR-2 expression during the early luteal phase coincide with luteal vascularization [38]. These results suggest an important role of VEGF in angiogenesis of the newly formed CL. High VEGF mRNA expression and protein levels during matured vasculature in the mid stage CL suggests also a survival function for endothelial cells and luteal cells.

The mRNA expression for VEGF in the bovine CL agrees with observations by in situ hybridization in rat luteal cells [20] or in primate CL during the early and late luteal phase by Northern analysis or in situ hybridization [39]. Highest mRNA expression levels were observed as evaluated by RPA in sheep CL on days 2–4 and on day 8 or days 14–15 stages of the estrous cycle [40]

and agrees with our VEGF mRNA expression data and protein tissue concentrations in the bovine [41]. A higher mRNA expression was also found during the early luteal phase in cow CL by RT-PCR [42]. The localization of VEGF in luteal cells was also observed in bovine and human CL [36, 42, 43]. But in contrast to the bovine CL, the immunostaining in human granulosa and theca lutein cells became weak in the mid and late luteal phase [43]. We could show in the regressed CL that mRNA expression and tissue levels decreased significantly [37, 44]. No hybridization signals were observed from regressing CL in primates [39]. Immunostaining of luteal cells from regressing CL disappeared in our study and this agrees with other observations in the bovine CL [42]. But in contrast, we could observe some positive staining for VEGF in smooth muscle cells of arteriols. Our finding that VEGF is predominantly localized in luteal cells underline these assumptions suggesting that VEGF may act as a chemoatractant for sprouting endothelial cells. This is supported by the dominant expression of the secretory forms of $VEGF_{121}$ and $VEGF_{165}$. Like in our study [37], $rhVEGF_{120}$, $rhVEGF_{164}$ and $rhVEGF_{188}$ isoforms were found in sheep CL [40]. Evaluation from the RPA showed that $VEGF_{120}$ represented approximately one third of the total mRNA encoding VEGF in the CL and that this proportion did not vary with stage of the estrous cycle [40]. The targets for VEGF localized in luteal cells are endothelial cells where both receptors (VEGFR-1 and VEGFR-2) are found. Probably the growth stimulatory signals are transducted to a major extent via VEGFR-2. This type of receptor is clearly regulated in the bovine CL, despite our results are presenting a high expression of VEGFR-1 [36]. Since both receptors bind VEGF with high affinity [18, 19], it is possible that co-expression of VEGFR-1 and VEGFR-2 in endothelial cells leads to formation of heterodimers in response to VEGF. Porcine aortic endothelial cells, individually expressing VEGFR-1 or VEGFR-2 after transfection, differed in their abilities to migrate towards VEGF [45]. The VEGFR-1 expressing cells failed to migrate, whereas the VEGFR-2 expressing cells migrated efficiently with a maximal response at 10 ng/ml VEGF. Thus it is likely that VEGFR-2 expressed in endothelial cells could mediate chemotaxis. In our study [37] we demonstrate that VEGF and its receptors are expressed clearly in the bovine corpus luteum during estrous cycle and pregnancy. The highest mRNA expression for VEGF and VEGFR-2 was detected during the early luteal phase, correlating with highest VEGF protein tissue concentrations and localization in luteal cells, and coincide with luteal vascularization [37].

Concerning regulation of VEGF expression, oxygen tension plays a major role, both *in vitro* and *in vivo*. LH or eCG is a potenst stimulator of VEGF mRNA expression in bovine and porcine granulosa cells [35, 46, 47]. IGF-1 stimulate dose dependently and significantly VEGF secretion [47]. VEGF was further stimulated by TNFα and forskolin. The same signaling pathway by stimulation of cyclic adenosine monophosphate production and proteinkinase A activation for luteinization and neo-vascularization demonstrates a close temporal and spatial relationship of these normal physiological processes.

FGF in bovine corpus luteum

The mRNA expression of FGF-1 in bovine CL increased significantly during mid luteal state [41]. In contrast, FGF-2 expression is highest during very early luteal phase (days 1–2), and for FGFR during days 1–4 and decrease thereafter to a lower plateau. The mean concentrations for FGF-2 in tissue [25] are high during the early luteal phase, decrease significantly on days 5–7 and increase again during the late luteal phase, and after luteolysis. Immunohistochemical analysis showed specific labeling of endothelial and luteal cells [44]. During the early stages (days 1–4) FGF-2 was detected strongly in cytoplasm of capillary endothelial cells and in smooth muscle cells of arteries. During cycle days 4–7 staining pattern for FGF-2 changes dramatically. Beginning with the mid luteal phase (days 8–12), most of the capillary endothelial cells are no longer positive for FGF-2. The reaction product is now localized exclusively in the cytoplasm of luteal cells and consist for the late luteal phase and after regression. With the change of localization there is also a change in molecular size from 18 KDa to 16 KDa, latter representing the truncated form having 15 amino acids less than the regular form [25]. In contrast, FGF-1 immunostaining is very weak in the cytoplasm of luteal cells during early luteal phase, followed by a stronger staining during mid luteal stage and regression, endothelial cells are always negative. The different localization of FGF-1 and FGF-2

suggests different functions in the CL. The dominant localization of FGF-2 in endothelial cells and pericytes at the early stage suggests that FGF-2 is the dominating factor for endothelial growth [48]. FGF-2 was also demonstrated by Northern analysis in bovine CL with higher expression late in the luteal phase [49]. Our results for localization of FGF-1 and FGF-2 primarily in the cytoplasm of large and small luteal cells during mid and late luteal phase in bovine CL agrees with earlier observations [50]. In contrast to our findings, no staining was observed in endothelial cells especially during the early stage (days 1–4). In sheep CL from days 5, 10 and 15 after estrus FGF-1 and FGF-2 were immunolocalized in the cytoplasm of large and small luteal cells with no clear changes [51]. FGF-2 act directly on the secretory function of bovine luteal cells [52] and may be a potential luteotropic factor in this tissue during the estrous cycle and pregnancy. In sheep FGF-1, FGF-2 and LH stimulated proliferation and progesterone production of luteal cells especially from the early luteal phase [53]. In sheep CL presence of FGFR-1 and FGFR-2 were evaluated by using Western analysis, immunohistochemistry and topical autoradiography. Western analysis demonstrated that the levels of FGF-1 and FGF-2 were similar in the early and mid-cycle CL but increased in the late stage of the estrous cycle. Immunohistochemistry and topical autoradiography demonstrate that both parenchymal and nonparenchymal (endothelial, fibroblastic) cells express FGF-1 and FGF-2. The distribution suggest that FGF is involved in the regulation of luteal parenchymal and vascular function [54]. Another member of FGF family, keratinocyte growth factor (FGF-7) mRNA expression was recently demonstrated in bovine CL and the protein is primarily localized in small luteal cells. It is assumed that FGF-7 may participate in paracrine communication within the bovine CL [55].

IGF in bovine corpus luteum

The highest mRNA expression by RT-PCR for IGF-1, IGF-2 and IGFR-1 was observed during the early luteal phase (days 1–4), followed by a decrease to a lower plateau of the cyclic CL. Levels of mRNA expression of the pregnant CL were comparable to the lower cyclic plateau. These trends were confirmed by RPA (ribunoclease protection assay) and localization of the proteins [56]. The bovine corpus luteum in general has been shown to express IGF-1 mRNA [57–61]. In most cases CL were collected from day 5 onwards. Therefore, the highest expression of IGF-1, IGF-2 and IGFR-1 during days 1-4 was missed.

The pronounced mRNA expression for IGFR-1 and localization in large luteal cells agrees with results in pig CL [62].

From our data it is assumed that the IGF system plays an important role especially for the development of the early CL by actions on luteinization of granulosa-lutein cells and stimulation of oxytocin and progesterone production [29, 58, 63]. *In vitro* data by a novel microdialysis system (intact luteal tissue) demonstrate distinct and stage-specific effects of IGF-1 and IGF-2 on progesterone and oxytocin secretion with long lasting effects even after stop of the perfusion, especially during the late luteal phase and early pregnancy [30, 64]. Since the proteins for IGF-1 [33] and IGFR-1 are mainly localized in large luteal cells, the regulation seems at least in part autocrine. A positive immune reaction was seen only for IGF-1 to a moderate extent in the stroma. However, it was confined to a subset of endothelial cells. Therefore, some paracrine actions are likely. The IGF system may have rather indirect effects on angiogenesis in the early CL by stimulatory actions for vascular endothelial growth factor production in luteal cells [47] as well as by proliferation and differentiation of endothelial cells. In contrast to IGF-1, IGF-2 positive immunoreactivity was restricted to the perivascular fibroblasts of large blood vessels and to the pericytes of capillaries [33]. This supports our assumption that IGF-2 acts as an autocrine/paracrine growth factor, affecting the proliferation and differentiation of these cells. The specific cellular localization of IGF-1 and IGF-2 is similar to that observed in pigs [56]. It is well established that the number and localization of pericytes play an important role in the modulation of endothelial migration and proliferation [66–68].

As described by ourselves [56], there is clear evidence for mRNA expression for all six IGFBPs in CL, with distinct differences between the BPs. The message for IGFBP-1, -2 and -6 is weak and shows no clear regulation during the cycle. In contrast, IGFBP-3, -4 and -5 are highly expressed with clear changes for IGFBP-3 and -4. Highest expression for IGFBP-3 correlates with the highest expression for

IGF-1, IGF-2 and IGFR-1 during the early luteal phase (days 1–4) followed by a clear decrease at the mid-luteal stage. IGFBP-3 is postulated to serve as the principal carrier and storage reservoir for IGFs within the intravascular compartment. Possibly this is also the case during the very early luteal phase in tissue, when the up-regulation of IGF-1 and -2 expression is relatively high.

General Discussion and Conclusions

The results described for VEGF, FGF and IGF family members in bovine CL are schematically presented and summarized in Fig. 1, and suggests an important role of these systems in angiogenesis of the newly forming CL. Growth factor activation enables quiescent, resting endothelial cells to proteolytically degrade their underlying extra cellular matrix, to invade and directionally migrate towards the angiogenic stimulus, and to proliferate and organize into new three-dimensional capillaries [69]. VEGF has been shown to induce plasminogen activators [70]. The localization of VEGF in luteal cells suggests that VEGF may act as chemoatractant for sprouting endothelial cells. This is supported by the dominant expression of the secretory forms of $VEGF_{121}$ and $VEGF_{165}$ whose receptors are found exclusively on endothelial cells. FGF-2 is observed exclusively in the early bovine CL in vascular cells and may act as an amplification system for VEGF. When added simultaneously, VEGF and FGF-2 induced an *in vitro* angiogenic response that was far greater than additive and that occurred with greater rapidity than the response to either cytokine alone [71]. This synergism was confirmed recently under *in vivo* conditions [72]. A similar synergistic system of VEGF/FGF-2 seems to exist in dominant bovine follicles [73]. Recently *in vivo* evidence was given in the monkey for the importance of VEGF and angiogenesis for luteal function [74]. The relative high expression and tissue levels of VEGF and FGF-2 and its receptors after established angiogenesis suggests a maintenance function of VEGF and FGF-2 for the endothelial cells of the surrounding capillaries or luteal cells themselves. Alon *et al.* [75] showed that a certain threshold concentration of VEGF is required to inhibit apoptosis of the endothelial cells and is essential for the stabilization of the newly formed blood vessels. The IGF system may have rather indirect effects on angiogenesis in the early CL by proliferation and differentiation of endothelial cells by IGF-1, and by affecting the proliferation and differentiation of pericytes of capillaries. In conclusion, the results suggest an important role of the VEGF/FGF and IGF family members in angiogenesis of the newly forming corpora lutea, and the maintenance of capillary function surrounding the luteal cells.

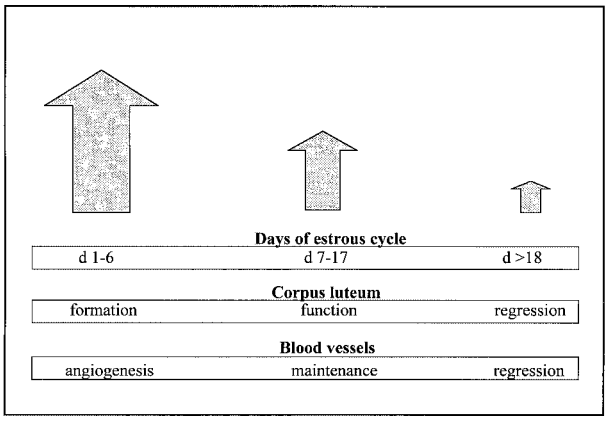

Fig. 1. Schematic presentation of mRNA expression for the angiogenic factors VEGF, FGF and IGFs in bovine corpus luteum during estrous cycle.

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