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Growth factors and steroidogenesis in the bovine placenta

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Abstract

The control of placental hormone biosynthesis is critical during gestation, since their coordinated action is essential for the normal progress of pregnancy. Hormonal synthesis regulation in placenta is still not elucidated and differs from that observed in other steroidogenic tissues since specific tropic hormones have not yet been identified. Cellular localization of growth factors in the placenta, including VEGF, EG-VEGF and bFGF, points that these factors have additional roles in the organ besides their well known modulation on cell proliferation and angiogenesis. *In vitro* experiments bring new evidence that growth factors play regulatory roles modulating processes related to steroid hormone secretion in the placenta. Importance of local estrogen function has been highlighted and a key enzyme for its synthesis is aromatase cytochrome P450. The objective of this review was to describe some aspects of placental steroidogenesis, mainly focusing on aromatase cytochrome P450 steroidogenic enzyme expression and growth factors as others potential modulators of hormonal synthesis in the organ.

Keywords: aromatase cytochrome P450, cattle, growth factors, placenta, steroidogenesis.

Introduction

An important aspect in raising dairy and beef cattle is the optimization of reproductive performance and productive efficiency. Several studies have pointed out that cows, mainly those with high milk production, present a gradual increase in reproductive problems, apparently from multifactorial causes (Lucy, 2001), including negative energy balance (Goff and Horst, 1997; Costa *et al*., 2007) and differential gene expression (Araújo *et al*., 2001). The placenta, beyond promoting maternal-fetal physiological exchange, synthesizes and secretes steroid hormones, such as progesterone and estrogens, which are responsible for the optimization of the environment for fetal growth (Hoffmann and Schuler, 2002). Therefore, its proper function is a determining factor for successful gestation. Regulatory mechanisms of placental function and activity, mainly those concerning the steroidogenic process, have not been totally explained yet. It is well described that estrogens are important regulators of caruncular growth and differentiation in the bovine placenta (Greven *et al*., 2007) and that sulfotransferase gene family, involved in estrogen biodisponibility, are markedly expressed during placentogenesis (Ushizawa *et al*., 2007). Several studies have emphasized the role of VEGF, EG-VEGF and bFGF as essential regulators of vasculogenesis and angiogenesis during gestation. However, the localization of these growth factors and their respective receptors in non-endothelial cells points to their participation in other physiological functions, such as stimulation of hormonal production in steroidogenic tissues (Jablonka-Shariff *et al*., 1997; Yamamoto *et al*., 1997; Winther *et al*., 1999; Winther and Dantzer, 2001). Thus, the aim of the present work was to review some aspects of placental steroidogenesis, mainly focusing on aromatase cytochrome P450 steroidogenic enzyme expression and growth factors as others potential modulators of hormonal synthesis in the organ.

Placental steroidogenesis

Steroid hormone synthesis starts when cholesterol, present in the cytoplasm, is transported to the inner mitochondrial membrane by steroidogenic acute regulatory protein - StAR (Herrmann *et al*., 2002; Sugawara and Fujimoto, 2004). Inside the mitochondrial inner membrane, the cholesterol side-chain cleavage cytochrome P450 enzymatic complex (P450scc) catalyzes the cholesterol conversion to pregnenolone (Hall, 1984). To form steroid hormones, the subsequent processing of pregnenolone requires enzymes related to smooth endoplasmic reticulum, such as 17α-hydroxylase/C17, 20 – lyase (P450c17α) and 3β-hydroxysteroid-dehydrogenase/isomerase (3β-HSD) (Carlone and Richards, 1997; Simpson and Davis, 2001), which belong to the Δ_5 and Δ_4 steroidogenic pathways (Fig. 1), respectively.

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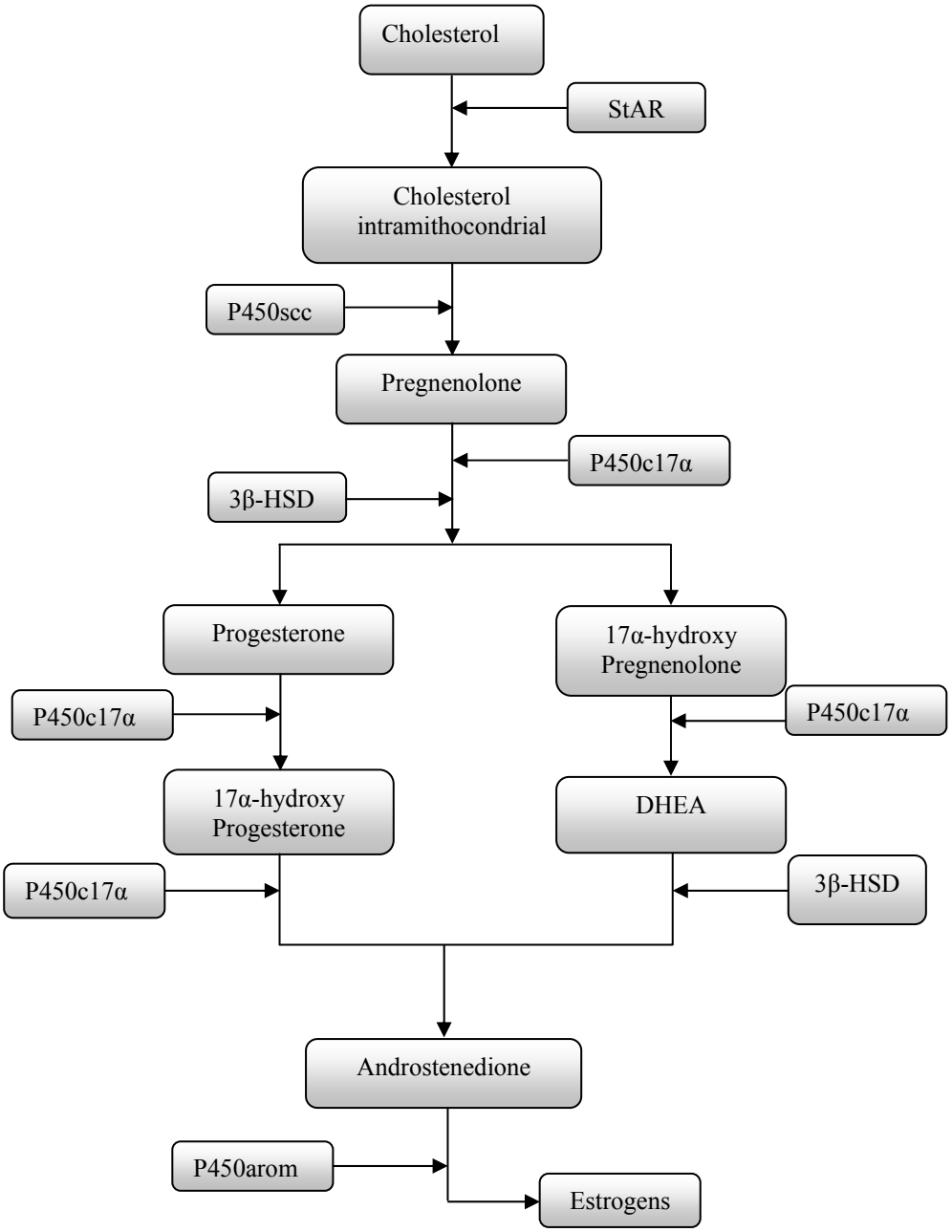

Figure 1. Principal steroidogenic pathways. Estrogen biosynthesis follows mainly the Δ_5 pathway (gray chart). P450scc: cholesterol side-chain cleavage cytochrome P450; 3β-HSD: 3β-hydroxysteroid-dehydrogenase/isomerase; P450c17α: 17α-hydroxylase/C17, 20-lyase; P450arom: aromatase cytochrome P450. Adapted from Schuler *et al*., 1994.

In Δ_4 pathway, pregnenolone is converted to progesterone by the enzyme 3β-HSD, which in turn, is converted to androstenedione by the enzyme 17α-hydroxylase/C17, 20 - lyase (P450c17α). On the other hand, in Δ_5 pathway, the enzyme P450c17α converts pregnenolone to dehydroepiandrosterone (DHEA), while 3β-HSD converts DHEA to androstenedione. Androstenedione is used as precursors for estrogen by trophoblastic cells (Albrecht and Pepe, 1990, 1998; Schuler *et al*., 1994). Estrogen biosynthesis is catalyzed by an enzymatic complex named aromatase cythocrome P450 (Conley and Hinshelwood, 2001). This complex binds the C19 steroid substrate (androstenedione and testosterone are the most common ones) and catalyzes specific reactions resulting in formation of phenolic A ring. NADPH cytochrome P450 reductase is an ubiquitous flavoprotein present in the endoplasmic reticulum of most cell types, which is responsible for transferring reducing equivalents to any microsomal form of cytochrome P450 (Simmons *et al*., 1985). P450arom is a member of a flourishing superfamily of genes, named the cytochrome P450 family, which

contains at the present time more than 220 characterized members belonging to 36 gene families (Nelson *et al*., 1993). In this context, P450arom is presently the sole member of gene family 19, designated Cyp19, based on the fact that the C19 angular methyl group is targeted by oxygen. The aromatase reaction apparently utilizes 3 mol oxygen and 3 mol NADPH for every metabolized C19 steroid mol (Thompson and Siiteri, 1974).

In cattle, estrone (E_1) is the principal estrogen secreted throughout gestation, predominantly in its sulfoconjugated form - E1SO4 (Hoffmann *et al.,* 1997; Zhang *et al*., 1999). Sites of estrogen production are the fetal cotyledons, which together with the maternal caruncular tissue, form multiple sites of placentation, the placentomes (Hoffmann *et al*., 1979; Robertson and King, 1979; Schuler *et al*., 1994). Placental estrogens can be detected in bovine fetal fluids as early as day 33 of gestation, while in maternal plasma estrone sulfate concentrations start rising around day 70 (Eley *et al*., 1979).

Even though placental estrogen production starts in early gestation, focus on its biological role in cattle has been limited to preparation of genital tract for parturition and mammary glands for lactation. Detection of a high estrogen receptor α (ERα) expression in caruncular stromal and epithelial cells (Schuler *et al*., 2002) shed new insights on the role of placental estrogens in cattle, classifying them primarily as local regulators of caruncular growth, differentiation and other functions throughout gestation (Greven *et al*., 2007). Moreover, the subsequent plasma estrone sulfate profile is used as an indicator of feto-placental developmental status and fetal viability (Zhang *et al*., 1999).

Bovine placental steroidogenesis differs from that observed in other tissues, such as the ovaries, once it is not modulated by cyclic nucleotides as intracellular messengers (Ullmann and Reimers, 1989). It has been demonstrated that calcium (Ca^{++}) acts as second messenger in steroidogenic stimuli signaling (Ullmann and Reimers, 1989) modulated by protein kinase C (Shalem *et al*., 1988; Shemesh *et al*., 1989) and calmodulin (Ullmann and Reimers, 1989). Moreover, the regulation of bovine placental steroidogenesis is still not elucidated, since specific tropic hormones have not yet been identified. In this case, the control of hormonal production is attributed to relative levels and tissue-specific arrangement of steroidogenic enzymes (Izhar *et al*., 1992; Schuler *et al*., 2006).

Control mechanisms of steroidogenic enzyme expression

As previously mentioned, estrogen biosynthesis is catalyzed by aromatase cytochrome P450, which is encoded by the Cyp19 gene (Conley and Hinshelwood, 2001). In cattle, this gene has been mapped to band q2.6 of chromosome 10 (Goldammer *et al*., 1994). Although the length of the bovine Cyp19 gene locus cannot be determined precisely, it may exceed 56 kb (Fürbass *et al*., 1997). The main sites of Cyp19 expression are the gonads (Masters *et al*., 2003; Mendelson *et al*., 2005) and, in humans and cattle, the placenta (Simpson *et al*., 1994; Corbin *et al*., 1995; Hinshelwood *et al*., 1995; Fürbass *et al*., 1997; Delarue *et al*., 1998; Vanseslow *et al*., 1999; Schuler *et al*., 2006). However, during development, differentiation, and to a minor extent throughout adult life, Cyp19 transcripts have been found in many organs, including skin, adipose tissue (Lueprasitsakul and Longcope, 1991), brain (Harada *et al*., 1992; Lephard *et al*., 1992) and adrenal glands (Conley *et al*., 1996).

In humans, tissue-specific expression of Cyp19 is regulated by the use of various spatially separated promoter regions, resulting in transcripts variants with different 5' untranslated regions (5'-UTRs), which are regulated by distinct hormones and second messengers (Clyne *et al*., 2004). On the contrary, sequences that encode P450arom protein remain identical (Mendelson *et al*., 2005). Thus, in humans, aromatase expression in the ovary is regulated by action of FSH through cAMP in the promoter II (Michael *et al*., 1995; Simpson *et al*., 2000), whereas in the placenta, promoter I.1 is stimulated in response to retinoids (Sun *et al*., 1998). Conversely, in adipose tissue, promoter I.4 drives Cyp19 expression under the control of glucocorticoids, class 1 cytokines or TNF-α (Zhao *et al*., 1995a,b, 1996). Although the alternative first exons are untranslated, their presence in the mature mRNA allows identification of the specific promoter used to derive a given transcript.

Ruminants, horses and swine, which express P450arom in their placentas, also use placenta-specific promoter regions to regulate Cyp19 gene expression through alternative splicing (Hinshelwood *et al*., 1995). In the bovine placenta, two major Cyp19 transcript variants have been detected: one variant comprises exon 1.1, and the other harbors exon 1.3 (Fürbass *et al*., 1997). Upstream sequence to exon 1.1, namely promoter 1.1 (P1.1), has been identified as the promoter region responsible for Cyp19 gene expression in placenta (Hinshelwood *et al*., 1995; Fürbass *et al*., 1997; Vanseslow *et al*., 1999; Kalbe *et al*., 2000). The bovine P1.1 shows only 39% of sequence homology with the placenta-specific promoter of the human aromatase gene (Hinshelwood *et al*., 1995), but does not show any similarity with placenta-specific promoter 1.5 of ovine gene (Vanseslow *et al*., 1999). Furthermore, locations of these placenta-specific promoters are very different: whereas the bovine and human promoters are located far distal (Brunner *et al*., 1998; Kamat *et al*., 1999), the ovine promoter is located proximal to the translation start site (Vanseslow *et al*., 1999) as shown in Fig. 2. Thus, placenta-specific Cyp19 promoters are not homologous among species, suggesting that this gene was achieved independently several times during evolution, even in closely related species such as cattle and sheep (Fürbass *et al*., 2001).

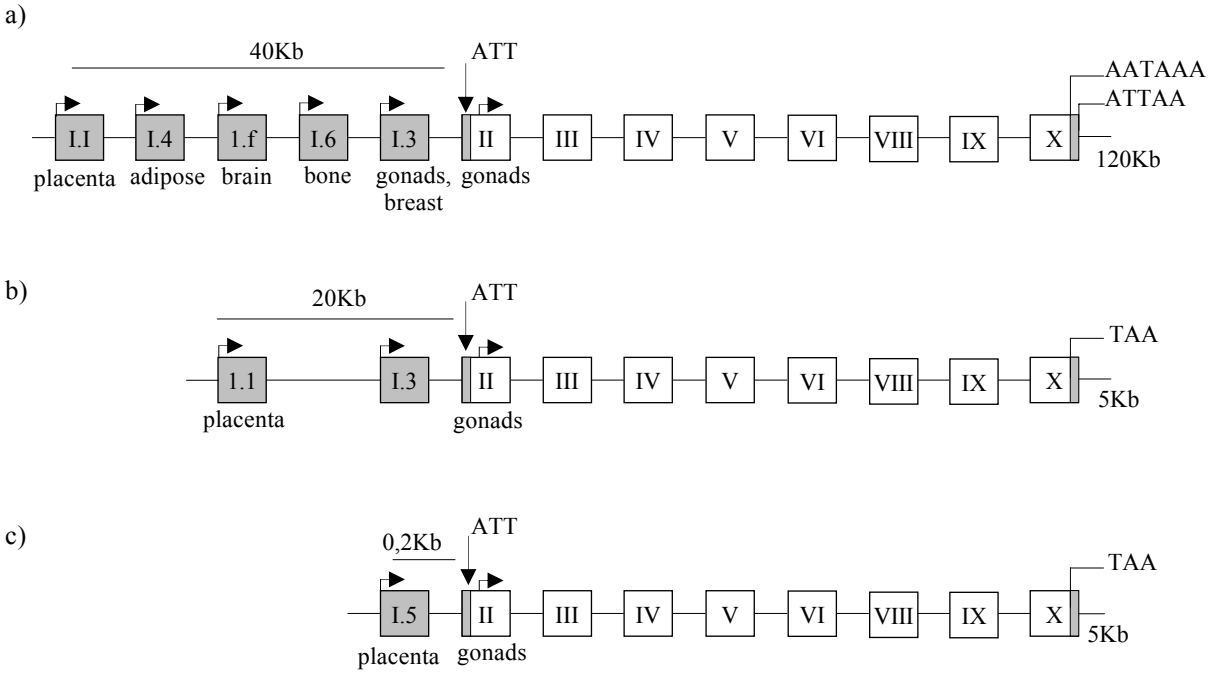

Figure 2. Schematic representation of the human (a), bovine (b) and ovine (c) *CYP19* gene. The coding region comprises exons II-X. Untranslated exons are shaded. The major placental promoter is the most distally located, and the ovarian promoter II is the most proximal. The main sites of expression are indicated under the first exons associated with *CYP19* transcripts in each tissue. Since only exons II to X are translated, the protein products in each tissue are identical.

The beginning of the transcription process is mediated by three types of RNA polymerases (I, II and III), specific for a genes' single group transcription (Hib, 2003). Gene expression regulation is critically dependent on the chromatin structure (Felsenfeld *et al*., 1996; Kadonaga, 1998), which is affected by DNA methylation patterns (Nan *et al*., 1998; Bhattacharya *et al*., 1999). DNA methylation is associated with more condensed chromatin and hence with transcriptional repression. However, during gene repression, the number of methylations has less influence than their location, since DNA methylation at promoter sequence can inhibit the activity of the gene, whereas several methylations at encoded regions do not modify them. In addition, one gene can be methylated in a cellular type, but not in another (Hib, 2003).

P1.1 DNA is hypomethylated in the placental cotyledons and methylated in caruncles, testis and corpus luteum (Fürbass *et al*., 2001), suggesting that the fetal compartment shows high aromatase activity during gestation and parturition (Tsumagari *et al*., 1993). Furthermore, a survey of Cyp19 transcript variants presented a tissue specific distribution in cattle, in which abundant amounts of exon 1.1 transcripts were present in post-parturition placenta, containing the cotyledons, in contrast to low concentrations found in testis and corpus luteum (Fürbass *et al*., 1997).

The bovine placental P1.1 region is an example of a promoter in which there is no consensus TATA motif, resulting in several transcription start sites within a stretch of 2023 bp. However, a putative transcriptional initiator region (INR) matching the consensus structure spans the major transcriptional start site 2 and is essential to position RNA polymerase II in the absence of consensus TATA motif (Fürbass *et al*., 1997). These elements are recognized by binding proteins that interact with cell general transcription machinery providing a proper environment for the formation of a competent transcriptional complex (Weis and Reinberg, 1992). Thus, in this case the placental P1.1, consensus binding motifs for the ubiquitous upstream stimulating factor (USF) mediate the binding of a transcriptional factor IID with a deficient promoter TATA motif (Bungert *et al*., 1992). USF 1 and 2 act as transcriptional amplifiers (Gao *et al*., 2003; Nowak *et al*., 2005) and inhibitors of several target genes, including human placental Cyp19 gene (Jiang and Mendelson, 2005).

In addition, although main human and bovine placenta-specific promoters (PI.1 and P1.1) are not related, both have binding sites to nuclear factor interleukin-6 (NF-IL6), which is a member of CEBP (CCAAT/enhancer binding protein) transcriptional factor family, characterized by containing a basic-

leucine zipper domain required to form dimers and bind DNA (Akira *et al*., 1990). A hexameric sequence motif, AGGTCA (i.e. half of an estrogen receptor-binding site) occurs twice within P1.1. This motif has been reported as a steroidogenic factor – 1 (SF-1), also known as adrenal 4-binding protein – Ad4BP, binding site (Lala *et al*., 1992; Morohashi *et al*., 1992).

SF-1 has emerged as a key regulator of endocrine function within the hypothalamic-pituitary-gonadal axis and adrenal cortex and as an essential factor in sex differentiation. SF-1 was first identified as a transcription factor with limited tissue distribution that recognized a conserved regulatory motif in the proximal promoter regions of genes encoding the cytochrome P450 steroid hydroxylases. These studies established that SF-1 was responsible, at least in part, for tissue-specific expression of genes involved in steroid hormone biosynthesis (Lynch *et al*., 1993; Morohashi *et al*., 1993; Parker and Schimmer, 1997). Subsequently, it has been demonstrated that SF-1 belongs to nuclear hormone receptor family (Honda *et al*., 1993), which represents a group of transcriptional factors mediating the actions of diverse ligands, including steroid hormones, thyroid hormone, vitamin D and retinoids (Mangelsdorf *et al*., 1995).

Within SF-1 sequence, two transactivator domains have been identified: AF-2, responsible for binding of co-activators providing an essential function for the constitutive activity of SF-1 (Lund *et al*., 2002), and AF-1, which is regulated by mitogen-activated kinase (MAPK) phosphorylation and is involved in interactions with various cofactors and in stabilization of the AF-2 activating function (Crawford *et al*., 1997; Hammer *et al*., 1999; Desclozeaux *et al*., 2002).

In the complex light of transcription regulating mechanisms, the multifaceted effects of hormones and growth factors on the development and function of tissues are described briefly. For example, ovarian follicular cells are under cyclic control by the differentiation-inducing gonadotropins FSH and LH, in combination with essential growth-promoting effects by hormones and growth factors like insulin and/or insulin-like growth factor- I (McArdle *et al*., 1991). Synergistic activation by LH and IGF-1 appears to provide the full stimulus for ovarian follicular cell differentiation into luteal cells (Richards *et al*., 2002). Many of the genes induced or up-regulated during the luteinization process, for example genes coding for steroidogenic P450 enzymes, steroid acute regulatory protein (StAR) and insulin-like factor-3 (INSL-3), appear to be controlled by SF-1 binding to specific binding sites in their promoter regions (Lala *et al*., 1992; Ivell *et al*., 1999). However, according to Walther *et al*. (2006), SF-1 knockout indicates that it plays an important role in theca cells. Probably, the established activating functions of SF-1 only play a minor role in the control of reproductive functions during theca cell luteinization, and an interconnection exists between MAP kinase and cyclic AMP-protein kinase-A pathways, constituting a non-classical pathway of growth factor and hormone action.

Growth factors

Vascular endothelial growth factor (VEGF) and its receptors

The VEGF family is constituted by various proteins identified by letters: VEGF-A to F (Paavonen *et al*., 1996; Achen *et al*., 1998; Meyer *et al*., 1999; Suto *et al*., 2005; Yamazaki *et al*., 2005), and placental growth factor - PLGF (Park *et al*., 1994). Native VEGF or VEGF-A is a heparin-binding homodimeric glycoprotein of 45 kDa, encoded by a single gene, and able to promote growth of vascular endothelial cells derived from arteries, veins and lymphatics (Ferrara, 2004).

Although endothelial cells are the primary targets of VEGF, several studies have reported mitogenic effects on certain nonendothelial cell types, including retinal pigment epithelial cells (Guerrin *et al*., 1995), pancreatic duct cells (Oberg-Welsh *et al*., 1997), Schwann cells (Sondell *et al*., 1999), and possible regulatory actions on placental cell functions (Pfarrer *et al*., 2006a).

Alternative exon splicing results in the generation of five different VEGF isoforms, which contain 120, 145, 165, 188 and 205 amino acids. The three smallest isoforms ($VEGF_{120}$, $VEGF_{145}$ and $VEGF_{165}$) are diffusible and act in a paracrine manner, while the others ($VEGF_{188}$ and $VEGF_{205}$) are associated with the cellular membrane and act in an autocrine manner (Ferrara *et al*., 1992; Ng *et al*., 2006). VEGF primary transcript derives from a single gene organized in eight exons separated by seven introns. Alternative splicing involves exons 6 and 7, while the amino acids encoded by exons 1, 5, and 8 are conserved in all VEGF isoforms (Wellmann *et al*., 2001).

Two tyrosine kinase receptors mediate VEGF biological activities: VEGFR-1/Flt-1 - Fms-like tyrosine kinase 1 (De Vries *et al*., 1992), and VEGFR-2/KDR - Kinase insert domain containing region (Terman *et al*., 1992). Both VEGFR-1 and VEGFR-2 show seven Ig-like structures in the extracellular domain, a single-transmembrane region, and a tyrosine kinase sequence that is interrupted by a kinase-insert domain (Shibuya *et al*., 1990). VEGFR-3 or Flt-4 (Kaipainen *et al*., 1995) is another member of the same RTKs family, showing binding affinities for VEGF-C and VEGF-D (Karkhainen *et al*., 2002). In addition to RTKs, VEGF interacts with a family of coreceptors, the neurophilins (Ferrara, 2004).

The interaction of VEGF with its receptors, Flt-1 and KDR, occurs through two distinct domains present in VEGF molecule, which are located in the opposite terminal of VEGF monomer (Ferrara, 2004). $VEGF_{165}$ and $VEGF_{188}$ activate Flt-1 (Plouet *et al*., 1997; Poltorak

et al., 1997) while 120, 145, 165, and 188 isoforms activate KDR (Poltorak *et al*., 1997). In contrast, $VEGF_{205}$ is a rare isoform, which is detected only in fetal liver and placenta (Burchardt *et al*., 1999), and is almost totally sequestered by extracellular matrix (Ferrara and Davis-Smith, 1997).

In placentomes, VEGF system was located in uterine epithelium, trophoblast, as well as in vascular tissue and uterine glands. The presence of VEGF system in the maternal-fetal interface, and in vasculature indicates that bovine VEGF may have: (1) classical functions in angiogenesis and vascular permeability, (2) chemotactic activity in endothelial capillaries, (3) autocrine influence in giant trophoblastic cells migration, which facilitates maternal-fetal interchange (Pfarrer *et al*., 2006a), and (4) modulatory action in trophoblastic function (Winther and Dantzer, 2001), specifically in steroidogenesis.

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

Endocrine gland derived endothelial growth factor is a recently identified angiogenic factor, which expression of mRNA and protein is restricted to endocrine glands (Le Couter *et al*., 2001). Other organs, like the small intestine, may contain lower levels of this factor (Li *et al*., 2001). Also known as prokinectin 1 (PK-1), EG-VEGF binds to two closely homologous G-protein coupled receptors (PK-R1 and PK-R2), inducing the enhancement of cell proliferation and survival (Kisliouk *et al*., 2005). It has been demonstrated that EG-VEGF allows the development of endothelial fenestrations, increasing vascular permeability (Le Coulter *et al*., 2001). This effect promotes the transport of LDLs (low density lipoproteins) into the steroidogenic cells, which secrete progesterone and other products into the bloodstream (Fraser *et al*., 2005) and highlights its role as a pro-survival factor.

Moreover, EG-VEGF is able to increase the VEGF mRNA expression affecting angiogenesis directly and indirectly (Kisliouk *et al*., 2005). In the first trimester human placenta, it has been demonstrated that EG-VEGF mRNA peaks in the ninth gestational week, whereas VEGF mRNA peaks in the seventh and remains elevated (Hoffmann *et al*., 2006). Levels of EG-VEGF receptors mRNA are not correlated to each other during human pregnancy: PK-R1 mRNA shows the same expression profile as EG-VEGF, while PK-R2 increases in the end of the first gestational trimester (Hoffmann *et al*., 2006). Protein expression of EG-VEGF reflects mRNA findings: immunohistochemistry tests localized EG-VEGF mainly in the syncytiotrophoblast layer at the sixth and also in the cytotrophoblast at the eigth and ninth gestational weeks (Hoffmann *et al*., 2006). Cellular localization of VEGF was adjacent but distinct from that for EG-VEGF, suggesting both factors may play complementary biological roles in the human and mouse placenta (Hoffmann *et al*., 2006, 2007) and also corpus luteum (Chung *et al*., 2004).

A functional hypoxia-response element (TACGTGCGGC) was localized to the promoter region of human EG-VEGF gene (Le Couter *et al*., 2001). In fact, EG-VEGF transcription and translation are regulated by hypoxic conditions. Other potential regulators of the EG-VEGF gene are luteinizing hormone, human chorionic gonadotropin (Fraser *et al*., 2005) and progesterone, specifically for the endometrium (Battersby *et al*., 2004; Fraser *et al*., 2005). However, other factors, presenting expression peaks in the first gestational trimester may also act as regulators of EG-VEGF gene and protein expression (Hoffmann *et al*., 2006).

PK-R1 (EG-VEGFR1) and PK-R2 (EG-VEGFR2) are G-Protein coupled receptors showing 85% amino acid sequence homology. In the human placenta both receptors are found; trophoblast cells express large amounts of PK-R1 between the eight and tenth weeks of gestation, exactly during the time in which the placenta undergoes developmental hypoxia. Like EG-VEGF, but not PK-R2, PK-R1 seems to be modulated by hypoxia (Hoffmann *et al*., 2006). The exact localization of PK-R1 and PK-R2 could be elucidated using mouse placenta and house produced antibodies against these proteins. Labyrinth was the major expression site for both PK-R1 and PK-R2 during the whole gestation, although PK-R1 could not be detected after day 14 post-coitus (Hoffmann *et al*., 2006, 2007). Both receptors have already been isolated from different body compartments like gut, nervous system and endocrine glands, pointing towards functional flexibility. PK-R1 and PK-R2 are activated by nanomolecular concentrations of recombinant EG-VEGF, leading to calcium, IP3 and MAPK mobilization (Lin *et al*., 2002a,b; Masuda *et al*., 2002).

Fibroblast growth factors (FGFs) and their receptors

Fibroblast growth factors (FGFs) are small polypeptide growth factors, sharing common structural features. To date, twenty-five distinct FGFs have been identified, numbered consecutively from 1 to 25 (Katoh and Katoh, 2005). FGFs induce mitogenic, chemotactic, and angiogenic activity in cells of mesodermal and neuroectodermal origin (Basilico and Moscatelli, 1992). FGF family has a strong affinity for heparin and HLGAGs (Burgess and Maciag, 1989), as well as a central core of 140 amino acids that is highly conserved among different family members. Despite nomenclature, the use of FGF initials does not mean that all factors stimulate fibroblast activities (indeed, FGF-7 does not stimulate fibroblasts) but rather that they belong to the same family due their structural similarities (Zhu *et al*., 1991).

Alternative exon splicing of bFGF (FGF-2) gene results in the generation of five distinct isoforms with 18, 22, 22.5, 24 and 34-kDa (Arnaud *et al*., 1999; Okada-Ban *et al*., 2000). This alternative splicing regulates the localization of the isoforms in cells, and can modulate their function (Bugler *et al*., 1991). The 18-kDa form is located in the cytoplasm (Renko *et al*., 1990; Bugler *et al*., 1991; Yu *et al*., 1993; Davis *et al*., 1997) acting in a paracrine/autocrine manner (Bikfalvi *et al*., 1995; Davis *et al*., 1997; Arese *et al*., 1999). In contrast, the high molecular weight forms are located in the nucleus (Renko *et al*., 1990; Bugler *et al*., 1991; Yu *et al*., 1993; Davis *et al*., 1997) and exert their effects in an intacrine manner, independent of receptor activation (Bikfalvi *et al*., 1995; Davis *et al*., 1997; Arese *et al*., 1999).

FGFs produce their mitogenic and angiogenic effects in target cells by signaling through cell-surface, tyrosine kinase receptors. Five distinct genes encode the high affinity receptors: FGFR-1 to 5 (Sleeman *et al*., 2001). All FGFRs have one extracellular domain, a single-transmembrane region, and a tyrosine kinase domain. The extracellular portion comprises three Ig-like domains, D1, D2, and D3 (Stauber *et al*., 1999).

Alternative splicing in exon III results in the generation of three isoforms of FGFR-1, FGFR-2 and FGFR-3 (IIIa, IIIb and IIIc), which present different degrees of affinity with FGFs. For example, FGF-1 activates all variants, while bFGF activates mainly IIIc isoforms (Coleman, 2003). Isoform IIIa is a truncated protein unable to translate extracellular signaling (Ornitz *et al*. 1996; Igarashi *et al*. 1998). The fifth FGF receptor differs from the others because it has one membrane protein, and an extracellular domain composed of 16 cysteines (Stauber *et al*., 1999).

Both maternal and fetal tissues express bFGF throughout gestation (Rider and Piva, 1998; Zheng *et al*., 1998), indicating that it is an essential factor for differentiation of vascular and nonvascular mesoderm-derived tissues (Yamaguchi and Harpal, 1994; Reynolds *et al*., 2000). In bovine placenta, bFGF was localized in epithelial cell cytoplasm, maternal and fetal stroma, as well as in endothelial cells. Both FGFR-1 and FGFR-2 were observed in cytoplasm and nucleus of epithelial cells, maternal and fetal stroma, and endothelial cells, while FGFR-3 presented predominantly nuclear localization in all cell types throughout gestation. According to Pfarrer *et al*. (2006b), in cattle, members of FGF family are located specifically in giant trophoblast cells, suggesting that these cells exert classical hormonal production function, beyond playing important roles in the regulation of placental growth, differentiation, and angiogenesis.

An intimate cross-talk exists among bFGF and the different members of the VEGF family during angiogenesis, vasculogenesis, and lymphangiogenesis. Experimental evidence points to the possibility that bFGF induces neovascularization indirectly by activation of the VEGF/ VEGFR system (Sheghezzi *et al*., 1998; Auguste *et al*., 2001; Tille *et al*., 2001; Gabler *et al*., 2004). Concerning hormone production modulation, a cross-talk mechanism among bFGF and VEGF could not be excluded.

Steroidogenesis modulated by growth factors

Functional studies related to role of growth factors on steroidogenesis modulation are described for VEGF and bFGF, but not EG-VEGF. *In vitro* studies demonstrate that VEGF and bFGF exert modulatory effects in cell steroidogenesis from distinct species. Both VEGF and bFGF influence estradiol-17β and progesterone production in swine granulosa cells (Grasselli *et al*., 2002), and increase progesterone production in early bovine corpus luteum (Kobayashi *et al*., 2001). Modulation of FSH-induced steroidogenesis by bFGF, inhibits estradiol-17β and progesterone synthesis in bovine (Vernon and Spicer, 1994) and mouse granulosa cells (Yamoto *et al*., 1993), as well as androstenedione production in chicken granulosa cells (Li and Johnson, 1993). Furthermore, both VEGF and bFGF act in bovine placenta in a paracrine and/or autocrine manner, increasing progesterone synthesis depending on gestational stage.

Until now, evidence about the possible influence of VEGF, EG-VEGF and bFGF in placental estrogen synthesis does not exist. Although experimental data demonstrate effects of these growth factors in ovarian and corpus luteum steroidogenesis, they do not explain totally if their action is mediated though direct effect on steroidogenic enzymes or not. For example, inhibition of cytochrome P450 17α-hydroxylase expression mediates the inhibitory effect of bFGF on androstenedione production from chicken granulosa cells (Li and Johnson, 1993), but VEGF and bFGF progesterone stimulation in early bovine corpus luteum is possibly mediated by the influence of these factors on the luteal secretion of prostaglandin F2α and angiotensin II (Kobayashi *et al*., 2001).

The regulation of bovine placental steroidogenesis can be characterized as a multifactorial process, where locally produced hormones, cytokines and growth factors, including VEGF and bFGF, participate in an autocrine and/or paracrine manner, to play decisive roles in fetal development and successful reproduction.

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