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Expression of VEGF-receptor system in conceptus during peri-implantation period and endometrial and luteal expression of soluble VEGFR-1 in the pig

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Abstract

In view of the importance of vascular events observed during gestation, it was hypothesized that the VEGF-receptor system plays a critical role during early pregnancy and maternal recognition of pregnancy in pigs. This hypothesis was tested by examining the expression of the VEGF-receptor system in the porcine conceptus. Additionally, the endometrium, corpus luteum (CL) and embryos were studied for the expression of soluble VEGF receptor 1 (sVEGFR-1), the strong endogenous antagonist of VEGF. The expression patterns show that VEGF164 mRNA levels increase gradually in line with conceptus development, whereas VEGF120 and VEGFR-2 remain unchanged during the peri-implantation period. Interestingly, elevated VEGFR-1 expression was observed in conceptuses on days 15–16 of gestation ($P < 0.05$). Comparison of the endometrial sVEGFR-1 mRNA expression revealed up-regulation on days 12 and 15–16 of pregnancy ($P < 0.01$ and $P < 0.05$, respectively). Furthermore, increased sVEGFR-1 levels were observed on day 12 of the estrous cycle in the CL ($P < 0.05$). Concluding, it seems that conceptus-derived VEGF164 plays crucial role in peri-implantation vascular events in pigs. These results support a potential role of VEGFR-1 in the proper growth and development of porcine conceptus during pregnancy. Moreover, expression patterns of sVEGFR-1 in the endometrium of pregnant pigs suggest that it may participate in vascular remodeling important for successful implantation. Finally, luteal sVEGFR-1 may be involved in the maintenance of CL function whenever pregnancy occurs in pigs.

Keywords: Angiogenesis; VEGF-receptor system; Soluble VEGFR-1; Reproductive tract; Conceptus

1. Introduction

The embryonic signal that initiates luteal maintenance occurs by about day 12 in the pig [1] and is necessary not only for the maternal recognition of pregnancy but also to prepare the uterine environment for implantation. One of the first effects of the embryo-derived estrogenic signal is increased uterine blood supply that is thought to support, e.g. prostaglandin production in the endometrium and maintenance of functional corpora lutea (CL) [2,3]. Early stages of embryo implantation are characterized by elevated endometrial vascular permeability in many species, including primates [4], rodents [5], sheep [6] and pigs [7,8]. Increased vessel permeability seems to be a prerequisite for the induction and direction of tissue growth and remodeling, and facilitates the angiogenesis associated with the maternal component of placenta formation [9].

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The most potent activator of vascular permeability and angiogenesis is vascular endothelial growth factor (VEGF). The VEGF family has several members, including VEGFA, VEGFB, VEGFC, VEGFD, placental growth factor (PlGF) and some homologs with VEGF-like activity (for a review see [10]). In addition, the various forms of VEGFA (also referred to as VEGF) that differ in their total number of amino acids are generated by alternative splicing of a single gene. Four different isoforms of VEGF were initially reported in humans VEGF121, VEGF165, VEGF189, VEGF206 [11,12], however, to date several less frequent and tissue specific splice variants have also been reported. VEGF121 is an acidic polypeptide that does not bind heparin and is a freely diffusible protein. VEGF165, the predominant isoform, is a secreted protein, but a significant fraction binds to the cell surface and extracellular matrix (ECM). Highly basic VEGF189 and VEGF206 bind with a high affinity towards heparin and are almost completely sequestered in the ECM. Interestingly, the ECM-bound isoforms can be released in diffusible form by plasmin cleavage, which generates a bioactive fragment. Therefore, it is tempting to believe that VEGF165 has optimal characteristics regarding bioavailability and biological potency (for a review see [10]).

The effects of VEGFs are almost exclusively mediated via two related receptors (R) VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1 or KDR). The VEGFR-1 mRNA can be spliced to generate forms encoding either the full-length membrane-spanning receptor or a soluble form, sVEGFR-1 that is truncated on the C-terminus. The sVEGFR-1 produced by endothelial cells acts as a negative modulator of the bioavailability of VEGF [13]. The VEGFR-1 was the first identified VEGF receptor, although its precise function is still under debate. It was initially suggested that VEGFR-1 is rather a ''decoy'' receptor, able to reduce the number of unbound, circulating VEGF molecules available to bind to VEGFR-2. However, recent studies suggest that VEGF binding to VEGFR-1 may result, at least, in induction of a mitogenic signal, recruitment of endothelial progenitor cells or release of tissue specific growth factors. Although VEGF binds to VEGFR-2 with a lower affinity than to VEGFR-1, this receptor is considered as a major mediator of the mitogenic, angiogenic, and vascular permeability-enhancing effects of VEGF (for a review see [10]).

In rodents, VEGF is the key mediator of estrogen-induced uterine permeability and is essential for successful implantation [14,15]. Interestingly, vessel permeability appeared in conjunction with blastocyst elongation at day 12 in pigs [7]. Moreover, we have recently shown that VEGF is expressed in endometrial and luteal tissues during the estrous cycle and early pregnancy suggesting possible participation of this protein in pregnancy-associated vascular events [16,17]. We have also shown that VEGF-receptor system expression in the porcine endometrium is controlled by factors that facilitate the creation of a unique micro-environment for successful embryo implantation in pigs. Both insulin-like growth factor I and relaxin seem to be the most potent and pregnancy status-dependent inducers of VEGF secretion and mRNA expression in the porcine uterus; probably acting together with estrogens of embryonic origin [18,19].

Elucidating the mechanisms controlling vascular permeability and angiogenesis in the porcine uterus is important for understanding the physiological role of this process in the development of a receptive endometrium and the implantation process in the pig. Identification of specific anti-angiogenic agents in porcine reproductive tract such as sVEGFR-1 is of considerable importance for understanding pathophysiology of significant early embryo loss that occurs in pigs during the first 5 weeks of early pregnancy. The overall aim of the present study was to address the hypothesis that the VEGF-receptor system plays a critical role during early pregnancy and in the maternal recognition of pregnancy. Since there are no data about VEGF-receptor system expression in the porcine conceptus during peri-implantation period and presence of sVEGFR-1 in the pig reproductive tissues, the gene expression of *VEGF120*, *VEGF164*, *VEGFR-1*, *sVEGFR-1, VEGFR-2* was investigated.

2. Materials and methods

2.1. Animals and tissue collection

Thirty prepubertal crossbred gilts from one commercial herd of similar age (approximately 5 months) and weight (95–105 kg) were checked daily for estrous behavior. After at least two consecutive estrous cycles of normal length, gilts presenting a third estrus were randomly assigned into two experimental groups: cyclic/non-pregnant ($n = 15$) and pregnant ($n = 15$). The second group of 15 gilts was artificially inseminated at 12 and 24 h after onset of estrus. Pigs were slaughtered at a commercial abattoir on day 9 ($n = 5$ per status), 12 ($n = 5$ per status) and 15–16 ($n = 5$ per status) of the estrous cycle or pregnancy (day 0 = first day of estrus). Immediately after slaughter the concepti were flushed from both uterine horns with 20 ml of phosphate-buffered saline (PBS; pH 7.4). Pregnancy was confirmed