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Expression of the vascular endothelial growth factor receptor system in porcine oviducts after induction of ovulation and superovulation

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ABSTRACT

This study was performed to determine the influence of insemination as well as treatment with human chorionic gonadotropin (hCG) and equine chorionic gonadotropin (eCG) on expression of the vascular endothelial growth factor (VEGF) system in porcine oviducts. In the first experiment, 10 gilts were assigned to 2 groups: cyclic (treated with phosphate-buffered saline; n = 5) and inseminated (n = 5). In experiment II, 15 gilts were assigned to 3 groups: inseminated (control; n = 5), induced ovulation and inseminated (750 IU eCG, 500 IU hCG; n = 5), and superovulated and inseminated (1500 IU eCG, 1000 IU hCG; n = 5). Oviducts (isthmus and ampulla) were collected 3 days after phosphate-buffered saline treatment (experiment I) or insemination. Blood samples were collected during slaughter for E_2 (estradiol) and P_4 (progesterone) analysis. Levels of messenger RNA (mRNA) of the VEGF system were analyzed by real-time polymerase chain reaction and protein by Western blot and E_2 and P_4 using radioimmunoassays. Insemination by itself decreased $VEGF_{120}$ mRNA expression and VEGF-A protein level in the oviductal isthmus ($P < 0.05$) but did not alter $VEGF_{164}$ mRNA. Expression of *Flt-1* (c-fms-like tyrosine kinase VEGFR-1) mRNA increased in the isthmus of inseminated relative to cyclic gilts ($P < 0.05$), whereas *KDR* (fetal liver kinase-1 VEGFR-2) mRNA levels decreased in both the oviductal isthmus ($P < 0.05$) and ampulla ($P < 0.001$). Superovulation decreased $VEGF_{120}$ and $VEGF_{164}$ mRNA expression in the isthmus compared with the inseminated group ($P < 0.05$), and lowered protein levels of VEGF-A in the isthmus of both stimulated groups ($P < 0.001$). Expression of *Flt-1* mRNA was affected by hCG and eCG treatment in both gonadotropin-stimulated groups in the isthmus as well as in the ampulla ($P < 0.001$) and protein levels in the ampulla of superovulated gilts ($P < 0.05$). Protein levels of KDR were reduced in the oviductal ampulla of gilts in both the induced ovulation and superovulated groups ($P < 0.05$). The concentrations of both E_2 and P_4 increased significantly in superovulated group of gilts ($P < 0.01$ and $P < 0.05$ for E_2 and P_4, respectively). Our study showed that insemination alone as well as ovarian stimulation affected the mRNA and protein profiles of the VEGF system in the porcine oviduct. Disrupted VEGF system expression may be crucial to many events occurring during the periovulatory period and consequently could lead to deprivation of VEGF-dependent factors that are necessary for proper fertilization, gamete transport, and embryo development.

1. Introduction

The oviduct is a venue for many crucial events within the female reproductive tract. This dynamic organ provides

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a favorable environment for final maturation of spermatozoa and oocytes and fertilization and early embryo development. Beside its basic function, which is transport of gametes and embryos, the oviduct secretes fluid, which is a complex mixture of plasma-derived products and proteins actively synthesized by oviductal tissue [1]. Transudation of blood plasma is one of the most important mechanisms for oviductal fluid (ODF) formation [2]. Therefore, proper oviductal vascular permeability must be maintained.

Vascular endothelial growth factor (VEGF) is a well known, potent angiogenic, and permeability-inducing factor [3]. VEGF can exist as an active glycosylated homodimer (34–46 kDa) or 2 monomers (17–23 kDa) lacking biological activity [4]. As a result of alternative splicing, 8 different VEGF-A isoforms can be expressed in humans [5]. In pigs, 7 isoforms have been confirmed, which differ from those of humans by lacking 1 amino acid [6]. The predominant VEGF-A isoforms are $VEGF_{121}$ and $VEGF_{165}$, produced by various normal and transgenic cells.

VEGF transmits its signal via several receptors. The most physiologically relevant ones are 2 tyrosine kinase family receptors: c-fms-like tyrosine kinase VEGFR-1 (Flt-1) and fetal liver kinase-1 VEGFR-2 (Flk-1/KDR) [5]. Despite the fact that Flt-1 (180 kDa) has a high affinity for VEGF-A (K_d ~10–20 pM), its activation results in only a weak mitogenic effect [7]. In contrast, although KDR (230 kDa) has a weaker affinity for VEGF-A binding (K_d ~75–125 pM) in comparison with Flt-1, the response after binding to this receptor is definitely stronger. Therefore, the KDR receptor is considered as the major receptor in the VEGF system.

Expression of the VEGF system in the oviduct was confirmed in several species including human [2], cattle [8], and pig [9]. However, many factors may influence VEGF system expression, for example, steroid hormones or exogenous gonadotropins, which are commonly used for improving animal breeding [10,11]. The most popular protocol for induction of ovulation and superovulation is a combination of equine chorionic gonadotropin (eCG) for follicular growth stimulation and human chorionic gonadotropin (hCG) to induce ovulation [12,13]. It is well known that stimulation with gonadotropins is an excellent tool for estrous cycle regulation and induction of ovulation in the pig [14,15]. On the other hand, administration of hCG and eCG may influence expression of the VEGF system. In the human uterus, gonadotropin stimulation increased *VEGF-A* messenger RNA (mRNA) expression and VEGF concentration during the peri-implantation period [11]. In rat ovaries, treatment with hCG and eCG changed expression of VEGF-A as well as Flt-1 [16]. Finally, hCG and eCG reduced VEGF levels in porcine follicular fluid and granulosa cells [17]. Disturbances in VEGF system expression may result in altered permeability, migration, and survival of oviductal endothelial cells, which in turn changes the local environment in the oviduct. Therefore, the present study was undertaken to determine, for the first time, whether administration of exogenous gonadotropins (hCG and eCG) used to induce ovulation and superovulation affects VEGF/VEGFR system expression in the porcine oviduct.

2. Materials and methods

2.1. Experimental scheme

The experimental procedures were approved by the Animal Ethics Committee, University of Warmia and Mazury in Olsztyn (No. 69/2008/N). Twenty-five crossbreed gilts of similar age (5–5.5 mo), weight (100–110 kg), and genetic background were observed daily for the onset of estrus. After exhibiting 1 natural cycle, gilts were allocated to 2 experiments.

2.1.1. Experiment I

Ten gilts were assigned to 2 groups: cyclic (n = 5) and inseminated (n = 5). The onset of estrus (day 0) was determined as the day of occurrence of a standing reflex in the presence of a boar. Gilts were considered to be in estrus when standing in response to the backpressure test during boar exposure. Gilts from the cyclic group received 100 mL intrauterine infusions of phosphate-buffered saline (PBS; pH 7.4), whereas the others received 100 mL of diluted semen, via a transcervical catheter, 12 and 24 h after detection of their third estrus.

Diluted semen contained 2.5×10^9 spermatozoa in a Safe Cell Plus (IMV Technologies, Szczecinek, Poland) commercial extender. The ratio of neat semen to semen extender was determined according to the concentration and motility of the spermatozoa.

2.1.2. Experiment II

Fifteen gilts were divided into 3 groups: inseminated only (control group; n = 5), induced ovulation and inseminated (n = 5), and superovulated and inseminated (n = 5). The onset of estrus (day 0) was determined as the day of occurrence of a standing reflex in the presence of a boar. Gilts of the inseminated group received 100 mL of diluted semen, via a transcervical catheter, 12 and 24 h after detection of their third estrus. Gilts assigned to the induced ovulation and inseminated group were injected with 750 IU eCG (Folligon; Intervet, Boxmeer, The Netherlands), followed by 500 IU hCG (Chorulon; Intervet) 72 h later between days 12 and 16 of their second estrus. The superovulated and inseminated group of gilts received 1,500 IU eCG followed by 1,000 IU hCG 72 h later between days 12 and 16 of their second estrus. Then, gilts of both the latter groups (induced ovulation and inseminated and superovulated and inseminated) received 100 mL intrauterine infusions of diluted semen; via transcervical catheter 24 and 48 h after hCG administration (as described by Małysz-Cymborska et al [18]).

Table 1
Concentration of E_2 and P_4 in blood serum of gilts on day 3 after insemination.

	Inseminated	Induced ovulation and inseminated	Superovulated and inseminated
E_2 (pg/mL)	37.57 ± 3.6	34.45 ± 1.93	52.73 ± 4.4[a]
P_4 (ng/mL)	7.78 ± 2.2	14.02 ± 3.6	18.85 ± 4.3[b]

Abbreviations: E_2, estradiol; P_4, progesterone.
[a] $P < 0.01$.
[b] $P < 0.05$.