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Effects of vascular endothelial growth factor on porcine preimplantation embryos produced by *in vitro* fertilization and somatic cell nuclear transfer

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Abstract

This study examined the effects of vascular endothelial growth factor (VEGF) on porcine embryos produced by *in vitro* fertilization (IVF) and somatic cell nuclear transfer (SCNT) at different developmental stages. Four sets of experiments were performed. In the first, supplementation of the *in vitro* culture medium with 5 ng/mL VEGF was suitable for porcine IVF embryo development, and the blastocyst formation rate was significantly higher than the control and other groups (57.73 ± 6.78% (5 ng/mL VEGF) vs. 43.21 ± 10.22% (control), 42.16 ± 10.24% (50 ng/mL VEGF) and 41.91 ± 11.74% (500 ng/mL VEGF); $P < 0.05$). The total cell number after supplementation with 5 ng/mL VEGF was significantly higher than the control and other groups (151.85 ± 39.77 (5 ng/mL VEGF) vs. 100.00 ± 34.43 (control), 91.2 ± 31.51 (50 ng/mL VEGF), and 112.53 ± 47.66 (500 ng/mL VEGF); $P < 0.05$). In the second experiment, when VEGF was added at different developmental stages of IVF derived embryos (early stage, days 1–3, late stage, days 4–7), the blastocyst formation rate and total cell number were significantly higher at the late stage (47.71 ± 9.13% and 131.5 ± 20.70, respectively) than in the control (34.32 ± 7.44% and 85.50 ± 20.41, respectively) and at the early stage (33.60 ± 5.78% and 86.75 ± 25.10, respectively; $P < 0.05$). There was no significant difference in the blastocyst development rate or total cell number between the whole culture period (days 1–7) and the late stage culture period after supplementation with 5 ng/mL VEGF ($P > 0.05$). In the third experiment, the cleavage rate was significantly higher when SCNT embryos were cultured with VEGF during the whole culture period than in the late stage (63.56 ± 15.52% vs. 39.72 ± 4.94%; $P < 0.05$), but there was no significant difference between the control and the early stage culture period ($P > 0.05$). The blastocyst formation rate was significantly higher at the late stage culture period with VEGF than at the early stage culture period (34.40 ± 15.06% vs. (16.07 ± 5.01%; $P < 0.05$). There was no significant difference in the total cell number between the groups ($P > 0.05$). In experiment 4, using real-time PCR, VEGF mRNA expression was detected in all the developmental stages of IVF and SCNT embryos, but the expression level varied according to the developmental stage. VEGF receptor, KDR mRNA was detected in all stages IVF and SCNT embryos. However, flt-1 mRNA was not expressed in all embryonic stages of IVF and SCNT embryos. These data suggest that VEGF supplementation at the late embryonic developmental stage might improve the developmental potential of both IVF and SCNT preimplantation porcine embryos through its receptors.

1. Introduction

Successful implantation requires functionally active embryos [1] and it involves a complex sequence of signaling events that are crucial for the establishment of

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pregnancy. Many molecular mediators participate in this early feto-maternal interaction, including adhesion molecules, cytokines, growth factors, and lipids [2]. Growth factors involved include leukemia inhibitory factor (LIF), macrophage colony-stimulating factor-1 (CSF-1), interleukin-1 (IL-1), prostaglandin G (PG), vascular endothelial growth factor (VEGF), glycodelin-A, and insulin-like growth factors (IGFs) and their binding proteins, and heparin binding-epidermal growth factor (HB-EGF) [3]. Most of these growth factors are derived from or produced by the endometrium or embryo. Absence of these factors can lead to implantation failure or, in rare cases, severe developmental abnormalities.

The biological effects of VEGF are almost exclusively mediated by two receptor tyrosine kinases that were isolated from a placental cDNA library in 1990. The *fms*-like tyrosine kinase (flt-1 or VEGFR-1; 180 kDa) is a high affinity receptor for VEGF-A, VEGF-B, and PIGF [4] and *flt-1* mRNA is expressed in vascular endothelial cells. The second high affinity receptor is fetal liver kinase-1/kinase insert domain-containing receptor (flk-1/KDR or VEGFR-2). The expression of both receptors is largely restricted to the vascular endothelium [5].

The highest peak of VEGF expression in the proliferative stage of the estrus cycle, and the steady rate of vessel growth during the mid- and late-luteal phases, are important for implantation [6]. The VEGF has been detected in human oviductal luminal epithelium [7], and VEGF mRNA expression was greatest during the peri-ovulatory stage, and in the ampullary and infundibular regions [7]. Although VEGF mRNA is expressed more in the infundibulum and ampulla, VEGF supports fertilization and early embryo development [7].

Although VEGF is produced primarily during the peri-ovulatory period [8], and human VEGF mRNA can be detected in the unfertilized oocyte to the blastocyst stage [9], the effects of VEGF on preimplantation embryos are poorly understood. Additionally, the numbers of blastomeres in the blastocyst increased after VEGF supplementation in the *in vitro* maturation (IVM) medium, suggesting that, in addition to the known angiogenic effects, VEGF may affect early embryonic viability [10]. Growth factors within tubal fluid have the potential to influence embryonic growth and development [11]. Thus, the present study compared the stage-specific embryotropic effects of VEGF on porcine preimplantation embryos derived by IVF and SCNT.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA), unless otherwise stated.

2.2. Ovary collection, recovery and in vitro maturation of oocytes

Ovaries of prepubertal gilts were collected from a commercial abattoir and transported to the laboratory within 2 h in 0.9% (w/v) NaCl solution supplemented with penicillin-G (100 U/mL) and streptomycin sulfate (100 mg/mL) at 30 to 35 °C. The follicular fluid with oocytes was aspirated from 3- to 6-mm antral follicles with a 10-mL disposable syringe and 20-gauge needle and collected in a 15-mL centrifuge tube. Cumulus-oocyte complexes (COC) were recovered under a stereoscope microscope; those with at least three layers of compact cumulus cells and with homogenous cytoplasm were selected for in vitro maturation. The selected COCs were transferred and cultured in 500 μL of tissue culture medium 199 (Life Technologies, Rockville, MD, USA) supplemented with 26 mM sodium bicarbonate, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 10 ng/mL epidermal growth factor, 0.5 IU/mL porcine luteinizing hormone, 0.5 IU/mL porcine follicle stimulating hormone, 10% (v/v) pFF, 75 μg/mL penicillin-G, and 50 μg/mL streptomycin. The pFF was aspirated from 3–7-mm follicles of prepubertal gilt ovaries. After centrifugation at 1600× g for 30 min, the supernatants were collected and filtered sequentially through 1.2- and 0.45-μM syringe filters (Gelman Sciences, Ann Arbor, MI, USA). The prepared pFF was then stored at −20 °C until use. For maturation, the selected COCs were washed three times in oocyte maturation medium containing hormone supplements, and approximately 50–60 oocytes were transferred into each well of a 4-well Nunc dish (Nunc, Roskilde, Denmark) containing 500 μL of culture medium and equilibrated at least 2 hrs with 5% CO_2 at 39 °C in a humidified atmosphere. After 20–22 h of maturation with hormones, the oocytes were washed two times in a maturation medium without hormone supplements and then cultured for 20–22 h without hormone supplements at 39 °C under 5% CO_2 in air.

2.3. In vitro fertilization

A 0.25 ml straw of frozen boar semen was thawed at 39 °C for 1 min in a water bath and then washed with 5 mL of phosphate-buffered saline (PBS) by centrifug-