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Expression of hypoxia-inducible factors and vascular endothelial growth factor during pregnancy in the feline uterus

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ABSTRACT

Hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF) have critical roles during the development of the fetomaternal unit. The HIFs regulate placentation and vascularization by stimulation of VEGF gene expression. This study aimed to investigate the expression profiles of HIF gene family and VEGF in the cat uterus during pregnancy. Tissue samples of the whole uterine wall were collected after ovariohysterectomy and allocated to the following groups: embryo positive (group 1 [G1], n = 7, 7 days after mating), early pregnancy (group 2 [G2], n = 7, 20 days after mating), mid-pregnancy (group 3 [G3], n = 7, 24 days after mating), late pregnancy (group 4 [G4], n = 7, 30–45 days after mating), and oocyte positive groups (group 5 [G5], n = 7, 7 days after induction of ovulation with GnRH analog). Relative mRNA levels were determined by real-time polymerase chain reaction. As housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase was used. The relative gene expression of HIF1A in G5 was found to be significantly higher than that of other groups (G1, G2, G3, and G4) ($P < 0.05$). In addition, the expression of HIF2A in G5 was higher than that of G1 and HIF2A gene expression at placentation sites of G4 was higher than in G1, G2, and G3 ($P < 0.05$). Immunohistochemistry indicated that HIF1A, HIF2A, and VEGF expressions were observed in different cell types of uterine and placental tissues in late pregnancy and oocyte groups. The expression of HIF3A did not change significantly in any group investigated. These observations suggest that HIFs and VEGF may play a role in the establishment and development of pregnancy.

1. Introduction

Development of placenta is a complex process and occurs as a result of a complex interaction between embryo and uterus. This process is regulated by many factors, such as cytokines [1], growth factors [2], steroid hormones [3], prostaglandins [4], and environmental conditions [5] of

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Table 1
Primers used in real-time polymerase chain reaction analyses.

Gene	Primer sequence (5′–3′)		PCR product (bp)	Accession number
	Forward	Reverse		
GAPDH	5′-ATCACCATCTTCCAGGAGCGAGA-3′	5′-GTCTTCTGGGTGGCAGTGATGG-3′	341	AF157626.1
HIF1A	5′-TTGGCAGCAATGACACAGACACTG-3′	5′-TTGAGTGCAGGGTCAGCACTACTT-3	175	XM_001493206
HIF2A	5′-ACACACAAGCTCCTGTCCTCAGTT-3′	5′-ACCCTCCAAGGCTTTCAGGTACAA-3′	96	XM_001498442
HIF3A	5′-CAATGCCTGGTGCTCATCTGTGAA-3′	5′-AGGTGAACTTCATGTCCAGGCTGT-3′	109	XM_001500780
VEGF	5′-TTTCTGCTCTCTTGGGTGCATTGG-3′	5′-TGCGCTGGTAGACATCCATGAACT-3′	139	AB071947.1

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor.

the uterus. As for environmental conditions, oxygen is limited when implantation and placentation develop during early pregnancy, and this is particularly important for placental development and during invasion [5]. Cells can respond to hypoxia with series of events that include regulation of gene expression [6].

The transcription factors activated during low oxygen conditions are called hypoxia-inducible factors (HIFs) and they are involved in the process of placentation and vascularization [5]. The HIF proteins are members of a larger group of proteins known as Per-ARNT-Sim proteins [6,7]. The HIF gene family comprises three α (HIF1A, HIF2A, and HIF3A) and three β (HIF1B, HIF2B, and HIF3B) subunits [8]. Important events related to implantation and placentation include increased vascular permeability and angiogenesis; the balance between these events is regulated by HIFs [8,9]. The HIFs play important roles in the regulation of angiogenesis during the implantation period [10]. Most of these genes related to angiogenesis are known to be activated by HIFs. Especially HIF1A is the most effective subunit for angiogenesis [11].

Because the major target of ovarian steroids is the endometrium, it is possible that HIF gene expression is regulated by progesterone and estrogen [10]. The relationship between HIFs and pregnancy has been investigated in the mouse uterus during the preimplantation period, and HIFs were shown to be expressed in response to ovarian steroid hormones [10,12]. In addition, during early pregnancy, expression of HIF1A was detected in the cow uterus, specifically in the caruncules [13]. Expression of HIFs in the uterus has been identified in the early pregnancy period in sheep [14] and mare [15]. The HIFs regulate angiogenesis by stimulation of vascular endothelial growth factor (VEGF) expression [16,17]. The HIF1A and HIF2A are major transcriptional activators of VEGF gene [18]. Angiogenesis is physiologically increased in the pregnant uterus [19]. Steroid hormones such as estrogen and progesterone modulate uterine angiogenesis [20]. Vascular endothelial growth factor is expressed in uterine tissue in women and rats during the cycle [21] and during implantation stage in mice [22], rabbits [23], ewes [24], pigs [25], and bitches [2]. Vascular endothelial growth factor induces physiological development of blood vessels [26]. It is also involved in angiogenesis in the cyclic ovaries and uterus [27]. Furthermore, VEGF increases vascular permeability and induces proliferation of endothelial cells [28,29].

In cats, 5 to 6 days after fertilization, the embryos migrate from the oviduct to the uterus [30]. After Day 20 of pregnancy, formation of a chorioallantoic placenta begins [31]. Between Days 25 and 30 of pregnancy, the cat placenta begins vascularization and development. Up to 30 to 45 days of cat pregnancy, the placenta increases in size and develops vasculature [32]. This study was designed to determine (1) expression profiles and (2) localizations of HIFs and VEGF in cat uterus during pregnancy.

2. Materials and methods

2.1. Animal groups and sampling

A total of 35 healthy female cats of different breeds and ages were used in this study. All animals were submitted to physical examination and proved to be clinically healthy. Cats were divided into five groups. They were identified as embryo positive (group 1 [G1], 7 days after mating, n = 7), early pregnancy (group 2 [G2], 20 days after mating, n = 7), mid-pregnancy (group 3 [G3], 24 days after mating, n = 7), late pregnancy (group 4 [G4], 30–45 days after mating), and oocyte positive groups (group 5 [G5], 7 days after induction of ovulation with GnRH analog, n = 7). In G5, estrus was determined based on the reports from owners of cats and ovulations induced by vaginal stimulation with sterile cotton swab for 5 to 7 minutes and GnRH analog administration (Buserelin, 25 μg/cat, im, Receptal). The G1 and G5 were determined by verifying an embryo or oocyte after flushing the uterine horns with lactate ringer solution after ovariohysterectomy. In other groups, the stage of pregnancy was determined based on the reports from the owners regarding the time of the last

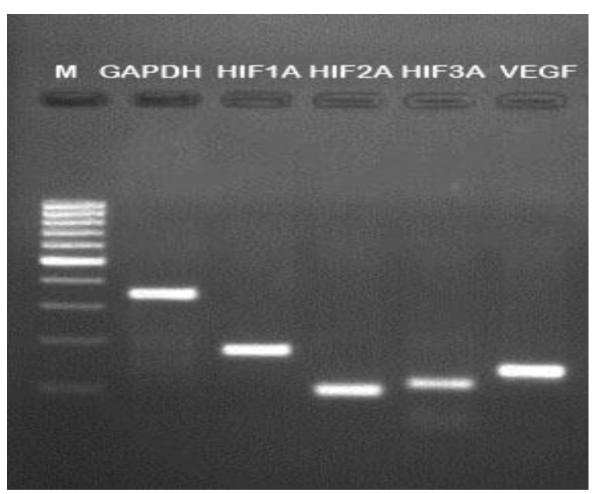

Fig. 1. Expression of HIF genes and VEGF at mRNA level. Lane M, 100 bp DNA ladder. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor.