

GROWTH AND ANGIOGENESIS IN THE PREGNANT CANINE UTERUS

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Aims

The aim of the present study was to investigate whether platelet activating factor (PAF), PAF-Rezeptor (PAF-R), epidermal growth factor (EGF), EGF-R, vascular endothelial growth factor (VEGF), VEGF-R1 and VEGF-R2 are expressed in the pregnant canine uterus. Since these factors contribute to growth and angiogenesis during early pregnancy in other mammals, they might be of interest as target antigens for the development of contraceptives

Materials and Methods

Pregnant bitches (n=21) were ovariohysterectomized at days 10-12 (preimplantation, n=7), 18-25 (implantation, n=7) and 28-45 (placentation, n=7) days after mating, respectively. The preimplantation group was proven pregnant by embryo flushing of the uterus after the operation (Fig.1). Four non-pregnant bitches in diestrus served as controls. Tissue samples from the uterus (placentation sites, interplacental sites, Fig. 2; non-pregnant animals: middle of horn) were excised and snap frozen in liquid nitrogen after embedding in Tissue Tek®. Embryos (morula stage) and tissues were stored at -86°C until analyses. mRNA extraction for RT-PCR was performed with Trizol®-Reagent. For quantification, real-time RT-PCR methodology was used (housekeeping genes: β -Actin, GAPDH).



Fig. 1

Each uterine horn was flushed with PBS from the tip of the horn and into the embryo filter. Embryos were observed by monocular microscope, washed in PBS and snap frozen at -86°C.

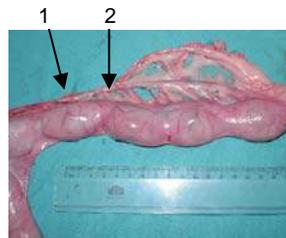


Fig. 2

Pregnant uterine horn, day 25 of gestation. A sample was excised from one placentation site (1) and one interplacental site (2). Material obtained was whole uterine wall with/without placenta

Fig. 4

Quantitative gene expression in the uterine wall of pregnant and non-pregnant animals; VEGF and VEGF-R1. Results are given as average values.

*# Bars with equal indices differ significantly ($p < 0.05$). Lines connect results from pregnant animals.

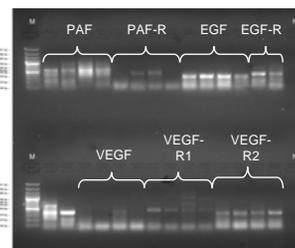
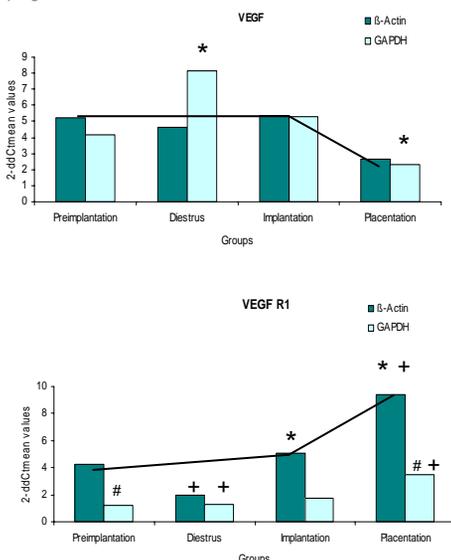


Fig. 3

Gel electrophoresis with mRNA obtained from preimplantation embryos out of 4 bitches. Gel pieces were cut out and sequenced.

Results and Discussion

In the course of pregnancy, mainly the receptors underwent significant changes. During pregnancy, VEGF and EGF expression slightly increased until implantation and markedly decreased during placentation (n.s., Fig. 4), whereas PAF increased during placentation (n.s.). The course of the receptors in samples from pregnant animals was in diametrical opposition to the course of the related factors, with significant increases towards placentation concerning VEGF-R1,-2 (Fig. 4) and EGF-R ($p < 0.05$), and significant decreases concerning PAF-R ($p < 0.05$). In comparison to the non-pregnant animals, expression of VEGF-R1,-2 and EGF-R was much higher during placentation, whereas it was lower concerning PAF (all $p < 0.05$). In the embryos, mRNA from all factors except VEGF was detected (Fig. 3). **Conclusions:** Results are similar to those observed in other mammals. Control of local receptor expression might be more interesting as to non-surgical sterilization than manipulation of the investigated growth factors itself.