ABSTRACTS
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Early canine pregnancy a battle for successful growth and angiogenesis

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OBJECTIVES: A row of studies was undertaken to understand some of the mechanisms regulating proliferation and angiogenesis in the uterus of the pregnant bitch. Our studies comprised immunological changes inside the uterus as well as the preimplantation and invading embryos, furthermore the role of progesterone and apoptosis.

RESULTS AND DISCUSSION: Preimplantation canine embryos were found to express mRNA for matrix metalloproteinases (MMP) 2,9, well known to promote trophoblast invasion in other species. The embryos furthermore expressed mRNA for cytokines like tumor necrosis factor (TNF)α and Interleukin (IL)6 known to increase the gelatinolysis of maternal endometrial tissue; and increased activity of MMP 2,9 has been detected in canine maternal uterine tissue during the preimplantation and placentaation stage (1,2). The preimplantation embryos in addition expressed transforming growth factor (TGF)β and leucemina inhibiting factor (LIF), factors known to inhibit and probably regulate gelatinolysis, which might indicate a self-regulatory mechanism (2). Secretory products were not isolated and their function not proven, however, the described intrauterine changings are dependant on the presence of preimplantation embryos – during the preimplantation stage, they are so strong that in serum of pregnant animals, significantly higher MMP2,9 activity was found then in non-pregnant bitches (3). Interesting was the strong expression of COX 2 in the embryos, probably indicating PGF2α synthesis. This prostaglandin is supposed to initiate cytokine synthesis inside the embryos and the maternal endometrium. The embryos furthermore expressed mRNA for CD4, a receptor that interacts with immune cells; investigations concerning its functions are ongoing. A further defence mechanism could be the lack of MHC I and II since no mRNA was detected in canine preimplantation embryos. This is supposed to provide shelter from maternal cytotoxic cells. The role of hormones on embryo production of cytokines, growth factors and enzymes still has to be investigated. We did not find mRNA for progesterone (P4) receptor (PR) (2) in preimplantation embryos. However, other hormone receptors like estradiol- and GnRH-R as well as receptors for growth factors would be of interest. - The pre-implantation uterus expressed similar factors as the diestrus uterus, however, factors exclusively expressed in the pregnant uterus were interleukin(IL)-4, CD8 and interferone (INF)YT (2). These factors are known to modulate the intrauterine milieu towards a predominance of Th2 cells, which is essential for maintenance of pregnancy in other species. During the implantation and placentaation stage, a significant up-regulation of LIF was striking. Furthermore the expression of insulin-like growth factor (IGF)2 and granulocyte macrophage colony stimulating factor (GM-CSF) at placentaation sites. We in addition found that PR was down regulated inside the uterus except at placentaation sites (4), which is supposed to be essential for maintenance of pregnancy. When an anti-progesterone was given during the implantation/placentaation stage, we measured a significantly increased activity of endometrial collagenases (MMP2,9). Receptor bound P4 is therefore supposed to participate in the regulation of these enzymes. However, the activity was significantly higher during natural abortions probably indicating a more inflammatory mechanism (5). We therefore at present investigate the role of apoptosis as a further regulatory mechanism during implantation, as has been described in humans. During a pilot study, expression of FAS and FAS-L mRNA was assessed in uterine tissue from pregnant bitches until the placentaation stage. In uterine preimplantation tissues, expression of FAS-L exceeded that of non pregnant bitches and then decreased significantly. Expression of FAS did not change significantly. Immunohistochemistry so far revealed the expression of FAS-L protein in uterine tissue of pregnant bitches. In preimplantation embryos, only FAS-L but not FAS could be detected (personal communication). These changings might indicate increased defence against apoptosis during early pregnancy. In human decidual stromal cells, GnRH-R was recognized to be involved in the regulation of apoptosis, since blockade with an GnRH antagonist induced apoptosis in vitro (6). The role of GnRH as a regulatory hormone during early canine pregnancy has not been investigated yet. However, only recently we assessed expression of mRNA for GnRH-R at canine implantation sites (personal communication). At last, we investigated uterine tissue of pregnant bitches for the expression of mRNA for certain growth and angiogenesis factors (platelet activating factor PAF, epithelial growth factors EGF, vascular endothelial growth factor VEGF) and their receptors. In the course of pregnancy, significantly higher expression of PAF and PAFR as well as VEGF and VEGFR2 during the preimplantation stage than in all other stages, and a strong upregulation of EGF
during implantation was characteristic. In the embryos, mRNA from all factors except VEGF was detected. This may be explained by an increased need of the investigated factors during preimplantation and implantation (7).

CONCLUSIONS: Pregnancy specific immunological changings can be found in the early pregnant canine uterus, probably initiated by the preimplantation embryo. Expression of PR is conserved at placentation sites only, which is supposed to be essential for implantation. Gelatinolysis is among others regulated by receptor bound P4. Until implantation an increased expression of factors important for growth and angiogenesis was assessed inside the uterus (PAF and PAF-R, VEGF und VEGFR2, EGF). The FAS and FAS-L apoptosis system seems to participate in the regulation of implantation; since GnRH-R was detected at implantation sites, a probable regulatory function of GnRH should be further investigated.