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Identification, Characterization, and Possible Functions During Early Pregnancy of Uterine-Derived Peptide Growth Factors

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Introduction

The establishment and maintenance of pregnancy requires complex and precise interactions among biochemical, immunological, and hormonal factors. Prenatal mortality during early pregnancy is a significant problem in all mammalian species. In particular, porcine embryos exhibit a high degree of mortality during early pregnancy (1). The molecular mechanisms underlying this reproductive problem remain to be elucidated.

Pigs, sheep, cows, and horses exhibit nonivasive types of placentation in which embryos are dependent upon uterine secretions as a nutritional source during early pregnancy (2). Uterine secretions which accumulate in the uterine lumen include a complex array of molecules ranging from inorganic ions to macromolecules, such as proteins (3, 4). Several classes of proteins of conceptus and uterine origins in uterine secretions of domestic animals have been identified and characterized. These are thought to mediate not only maternal-fetal communication but also conceptus and uterine development. One class of endometrial regulatory proteins are the polypeptide growth factors. An increasing body of evidence indicating the involvement of polypeptide growth factors in reproductive function has facilitated our understanding of the mechanism for conceptus and uterine development (5-10). However, more studies are required to clarify the nature of peptide growth factors and their involvement in endometrial and conceptus development of domestic animal species during early pregnancy. This is required to expand our current knowledge regarding the potential involvement of peptide growth factors in abnormal endometrial function and conceptus development contributing to prenatal mortality in mammals during early pregnancy. In this regard, studies on the identification, characterization, and possible functions of peptide growth factors for conceptus and uterine development during early pregnancy were conducted.

A Uterine Cell Mitogen in Porcine Uterine Luminal Fluids

Recent interest in peptide growth factors and the role of uterine secretions in conceptus development was responsible for the search for and characterization of growth promoting factors in porcine uterine luminal fluids. The partial purification and

initial characterization of a growth factor in porcine uterine luminal fluids, termed uterine luminal fluid mitogen (ULFM), was conducted. Results obtained from this study are summarized as follows: (i) ULFM is a small Mr polypeptide with a Mr of 4,800 and a pI of about 6.4 that is inactivated by trypsin, proteinase K and β-mercaptoethanol, but heat-stable; (ii) ULFM is not a pregnancy-specific protein since a similar mitogenic factor was present in ULFs from cyclic gilts; (iii) ULFM is apparently distinct from other known peptide growth factors, such as IGF-I, IGF-II, EGF, TGFs, FGFs, PDGF, and CSF-1, which suggests that ULFM is a novel mitogen; and (iv) partially purified ULFM is a potent mitogen for both fibroblastic and epithelial cells with activities comparable to those for EGF and IGF-I in proliferation and mitogen assays utilizing both cell types.

Insulin-like Growth Factors in Sheep Uterine Luminal Fluids

The identification of a mitogen distinct from known growth factors in porcine ULF led to the studies of sheep ULF growth factors and comparisons with porcine ULF, since both sheep and pig conceptuses are characterized by rapid morphological changes during the peri-implantation period of development and both have noninvasive placentation. The results of this study are summarized as follows: (i) ULFs from cyclic and pregnant ewes contain both IGF-I and IGF-II. ULF IGF-I levels for cyclic ewes were higher than those for pregnant ewes and changes were affected by day in cyclic and pregnant ewes. ULF IGF-II content was not different between cyclic and pregnant ewes, but IGF-II levels varied depending on the day of the estrous cycle or pregnancy. IGF-II levels were generally higher than IGF-I levels in the ovine ULFs examined; (ii) Ovine ULFs contain mitogenic factor(s) distinct from the IGFs. A gradual increase in mitogen activity from Day 10 to 14 and a decrease on Day 16 were observed for ULFs from both cyclic and pregnant ewes. The mitogenic activity in the ULFs from cyclic ewes was heat-sensitive, but the mitogenic activity in ULFs from pregnant ewes at each day of pregnancy was differentially affected by heat-treatment, showing a greater percentage reduction for those ULFs with higher mitogenic activity. Overall, the temporal variation in AKR-2B mitogenic activity in ULFs mimicked the developmental profile for ULF IGF-II in cyclic and pregnant ewes and for ULF IGF-I in pregnant ewes; (iii) Ovine ULFs do not contain PDGF or porcine ULFM-like activity. The differences in Mr and in degree of inactivation by heat-treatment of the ovine and porcine mitogenic factor(s) suggested the absence of ULFM in ovine ULFs; (iv) The secretion of ovine trophoblast protein-1 (oTP-1) was stimulated or at least maintained by the combination of IGF-I and IGF-II, but not by IGF-I or IGF-II alone. The more advanced conceptuses, as judged by their size and morphology, secreted more oTP-1; and (v) ULFs from cyclic and pregnant ewes contained at least four different mitogenic factors, namely, IGF-I, IGF-II and two additional, as yet unidentified, mitogenic factors. Results of this study also indicated a possible regulatory role for IGFs in synthesis and/or secretion of oTP-1 during the period of maternal recognition of pregnancy in sheep.

Uterine Epithelial Cell Development by Peptide Growth Factors

The results obtained from the above studies indicate the presence of several growth factors in uterine secretions of domestic species. Since peptide growth factors are multifunctional, subsequent interest was focused on their actions and interactions during uterine cell growth and differentiation. Therefore, the studies were initiated to examine the in vitro effects of peptide growth factors identified in the uterus focusing in particular on DNA and protein synthesis. Rabbit endometrial epithelial cells (HRE-H9) transformed with an origin-defective, temperature-sensitive SV40 mutant at 33 C were used since this cell line retains characteristics of the tissue of origin, but with an unlimited life span. At present, there are no established endometrial epithelial cell lines from any domestic species. Results of this study are summarized as follows: (i) Peptide growth factors, such as IGF-I, IGF-II, EGF, and FGFs, stimulated DNA synthesis of non-transformed cells in a dose-dependent manner, but not that of transformed cells. This result supports the autocrine mode of growth factor action to render cells transformed and possible in vivo regulation by these growth factors of normal uterine endometrial epithelial cell proliferation; (ii) TGF-β1 inhibited uterine epithelial cell growth, a result that indirectly confirmed the epithelial nature of the HRE-H9 cell line; (iii) TGF-β1 stimulated protein secretion, probably due to its stimulatory effects on synthesis, deposition, and turnover of extracellular matrix proteins and cell adhesion molecules; (iv) The synergistic and antagonistic interactions of growth factors during uterine epithelial cell growth were also demonstrated. The most stimulatory effects on DNA and protein synthesis were the combination of IGF-I and EGF, effects abrogated by TGF-β1; (v) The mitogenic actions of IGF-I and IGF-II on HRE-H9 cells were shown to be mediated by Type I IGF receptors because of the relative absence of Type II IGF receptors on these cells; and (vi) The presence of IGFBPs in cell lysates and culture medium of HRE-H9 cells was confirmed. A putative IGFBP-1 with a Mr of 31,000 was secreted. The higher levels of IGFBPs synthesized and secreted at 37 C than at 33 C may account for the lower basal mitotic activity of HRE-H9 cells at 37 C than at 33 C, as IGFBP-1 is generally inhibitory in IGF-mediated physiological processes. Overall, results of this study demonstrated the complex interactions that are likely to occur in vivo among stimulatory and inhibitory growth factors to modulate the growth and differentiation of endometrial epithelial cells during the estrous cycle and pregnancy.

Conclusion

The studies have identified peptide growth factors in the uterine secretions of domestic species and have demonstrated their effects on conceptus and endometrial functions, and their synergistic and/or antagonistic interactions with regard to endometrial epithelial cell growth. Based on this information, a model was proposed which related progesterone, ULFM, and IGFs as mediators of coordinated conceptus and uterine

development during early pregnancy. Future studies regarding the structure and functions of ULFM, the possible interactions of ULFM and the IGFs with estrogens and/or progesterone, and the in vivo effects of growth factors on conceptus and endometrial activities are required to critically examine the validity of the proposed model. This in turn may provide clues as to the basis for prenatal mortality during early pregnancy in the domestic animal species and lead to development of rational, biotechnology-based schemes for alleviating this significant reproductive problem.

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