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Regulation of uterine and conceptus secretory activity in the pig

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Summary. Evidence is presented for the involvement of a number of specific uterine- and conceptus-derived proteins in endometrial differentiation and conceptus or fetal development. These secretory proteins include mitogens (insulin-like growth factor-I and -II, epidermal growth factor, uterine luminal fluid mitogen), binding and transport proteins (uteroferrin, insulin-like growth factor and retinol binding proteins, respectively), protease inhibitors (antileukoproteinase, plasmin/trypsin inhibitor), and trophoblastic specific proteins. Using immunological reagents and specific complementary DNA (cDNA) probes, the tissue origins of several of these proteins have now been identified. In addition, the temporal regulation of messenger RNA (mRNA) production for a number of these proteins has been elucidated. The results suggest that although circulating and locally produced steroid hormones may be involved in regulating the synthetic abilities of these tissues during pregnancy, other, as yet undefined, factors may also mediate these activities.

In this paper we present a review of the current knowledge pertaining to the identity, physiological regulation and potential functions of pig maternal and conceptus secretory proteins during pregnancy.

Keywords: uterus; conceptus; pregnancy; growth factors; oestrogen; pig

Introduction

The high incidence of embryonic loss ($\sim 30\%$) observed in the pig in early pregnancy has in the past decade resulted in numerous studies designed to elucidate the mechanisms contributing to prenatal mortality in this species. The fact that, despite the apparently protective environment of the uterus, the proper survival and development of the conceptus can still be compromised suggested to many investigators that abnormalities in the embryo, the uterus or the biochemical interactions between these components exist (see Wilmut *et al.*, 1986). Indeed, the early pregnancy period in the pig is characterized by rapid developmental changes in both the conceptus and uterus, disruption of which may be responsible for arrested conceptus growth (for detailed discussion see Stroband & Van der Lende, 1990). The complexity of these developmental processes is further increased by their demonstrated interdependency. The embryo transfer experiments in pigs reviewed by Pope *et al.* (1990) have shown that the developmental stages of the uterus and the transferred blastocyst must be in synchrony for successful implantation and survival of embryos.

It is likely, therefore, that a signal exists which is responsible for maintaining synchrony of development between the uterus and conceptus. The uterine environment during early pregnancy has limited receptivity to the preimplantation conceptus, and the latter must make its presence

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known immediately. This process, called "maternal recognition of pregnancy", is mediated by blastocyst production of oestrogens which stimulate rapid release of endometrial secretory components into the uterine lumen (see Geisert *et al.*, 1990; Stroband & Van der Lende, 1990).

The identification and characterization of endometrial and blastocyst specific proteins are essential to understand the regulation of conceptus development and pregnancy maintenance. The importance of uterine secretions as a source of nutrients for pig conceptuses before placentation has been recently reviewed (Roberts & Bazer, 1988). Uterine secretions provide a growth medium *in vivo* essential for normal development of the conceptus. Recent work from our own and other laboratories suggests that these secreted macromolecules of embryonic and maternal origin play more active roles in the synchronous growth and development of the conceptus and the uterine endometrium.

Functions and regulation of uterine secretory proteins of pigs

Insulin-like growth factors and binding proteins

Insulin-like growth factor-I (IGF-I) is a polypeptide growth factor that is structurally related to insulin (Zapf & Froesch, 1986). *In vitro*, IGF-I is a potent mitogen for a variety of cell types including fibroblasts, epithelia, myoblasts, chondrocytes and erythroid precursor cells (Zapf & Froesch, 1986). This mitogen acts on its target cells by binding to specific type I and II IGF cell membrane receptors (Nissley *et al.*, 1985). *In vivo*, IGF-I promotes cell division, cell differentiation and tissue morphogenesis by endocrine, autocrine and paracrine mechanisms (Zapf & Froesch, 1986).

The primary amino acid sequence of pig IGF-I (70 amino acids) has been determined from the nucleotide sequence of its mRNA (Tavakkol *et al.*, 1988). Comparison of this sequence with those reported for human (Rotwein, 1986), rat (Roberts *et al.*, 1987), mouse (Bell *et al.*, 1986) and cow (Honegger & Humbel, 1986) IGF-I demonstrates the remarkable evolutionary conservation of this protein. Hybridization analysis of IGF-I mRNAs in pig uterine tissues by using cloned cDNAs as probes has revealed that the highest concentrations occur in early pregnancy (Day 12) with diminished but detectable levels in mid- and late pregnancy (Tavakkol *et al.*, 1988). Although during early pregnancy (Days 8–14), maximal levels of IGF-I mRNAs are observed at Day 12, the tissue content of IGF-I protein remains constant (Letcher *et al.*, 1989a).

IGF-I synthesized by the uterine endometrium is released into the lumen where it presumably comes in contact with the conceptus. IGF-I concentrations in uterine luminal fluids change during the oestrous cycle and early pregnancy with maximal concentrations at Days 10 and 12 (Simmen *et al.*, 1989). The appearance of IGF-I in the uterine lumen therefore temporally coincides with blastocyst oestrogen synthesis and elongation.

Exogenous administration of oestrogen or growth hormone to ovariectomized, hypophysectomized rats induces uterine growth and production of IGF-I mRNAs and protein (Murphy & Friesen, 1988). The signal(s) for the uterine synthesis and secretion of IGF-I in cyclic and pregnant pigs has not been elucidated. Plasma oestrogen concentrations remain low until Day 16 of pregnancy (Robertson & King, 1974), which is well after the peak of synthesis of IGF-I mRNAs. Blastocyst-derived oestrogens may, however, influence IGF-I synthesis and secretion into the lumen. The synchronized release of uterine endometrial secretory vesicles is associated with the onset of blastocyst oestrogen production (Geisert *et al.*, 1982b).

The observation that the morphological transformations of pig embryos result from cell proliferation and cellular remodelling (Geisert *et al.*, 1982a) suggests a possible paracrine role for uterine IGF-I in conceptus development. In this regard, preimplantation-stage pig embryos display type-I IGF receptors (A. N. Corps, C. J. Littlewood & K. D. Brown, personal communication) but produce very little IGF-I protein (Letcher *et al.*, 1989a). Similarly, the localization of IGF-I receptors in human uterine and placental tissues (Fant *et al.*, 1986; Rutanen *et al.*, 1988) implicates

IGF-I as a potential mediator of endometrial growth and differentiation. Indeed, IGF-I can promote growth of pig endometrial cells in culture (Y. Ko, F. A. Simmen & R. C. M. Simmen, unpublished results). One may speculate that, in early pregnancy in the pig, both of the above mechanisms are operative, resulting in a uterine environment optimal for the developing conceptus.

Insulin-like growth factor-II (IGF-II) is less growth hormone-dependent than IGF-I and is typically found in serum at highest concentrations during fetal and neonatal development (Zapf & Froesch, 1986). This mitogen has classically been considered a fetal growth factor, although significant expression of IGF-II gene in certain adult stage tissues has also been demonstrated (Murphy *et al.*, 1987). In the non-pregnant adult rat, uterine levels of IGF-II mRNAs are relatively high (Murphy *et al.*, 1987). A specific function(s) for uterine IGF-II has not, however, been identified.

Use of a cloned rat IGF-II cDNA probe (Whitfield *et al.*, 1984) in hybridization studies has demonstrated relatively high IGF-II mRNA levels in the uterus of pregnant pigs (F. A. Simmen, unpublished data). These concentrations approach those noted for IGF-II mRNAs in late-stage fetal pig tissues (e.g. liver and kidney). Major quantitative variations of uterine IGF-II mRNAs during pregnancy are apparent, with lowest concentrations in early pregnancy (pre-implantation), a greater than 10-fold increase by Day 30 and a subsequent decline at Days 90 to 110 (Fig. 1). Pig uterine IGF-II mRNAs are probably not oestrogen-induced *in vivo* since correlations of their tissue concentrations with elevated plasma or blastocyst oestrogens are not evident. Similarly, oestrogen treatment of immature gilts promotes uterine growth without influencing tissue levels of IGF-II mRNA (F. A. Simmen, unpublished data). Studies designed to examine the possible role of progesterone in modulating IGF-II gene expression *in utero* are in progress.

IGF (I and II) actions are mediated via type I and type II IGF receptors present in the plasma membranes of target cells. However, binding of IGFs to receptors is modulated by yet another class of proteins, namely the IGF carrier or binding proteins (Nissley *et al.*, 1985; Rutanen *et al.*, 1988). These secretory proteins inhibit or stimulate IGF bioactivity by mechanisms involving the binding and release of IGFs or the association of the complexed binding proteins with the cell surfaces (Brewer *et al.*, 1988).

There are three or more IGF binding protein genes in the human and rat genomes. These genes encode binding proteins with dissimilar amino acid sequences and tissue distributions (Brewer *et al.*, 1988; Lee *et al.*, 1988; Wood *et al.*, 1988). Studies of one such binding protein (BP-3A of M_r 33 000 (Romanus *et al.*, 1986) during pregnancy in the sow have been initiated. In general, the tissue specificity of BP-3A mRNA closely resembles that of the IGF-II mRNAs (F. A. Simmen, unpublished data). However, several notable differences in the expression of the two genes are observed. BP-3A mRNAs are more abundant than IGF-II transcripts in the pregnant pig uterus (Fig. 1). Additionally, a transient though small increase of BP-3A mRNA, coincident with the increased IGF-I mRNA expression, was observed in uteri at Day 12 of pregnancy. This does not occur for IGF-II mRNAs (Fig. 1).

The ontogeny of IGF gene expression in the pig uterus can be divided into two distinct phases. The period corresponding to blastocyst elongation is characterized by high levels of IGF-I mRNAs, low levels of IGF-II mRNAs and low to moderate levels of BP-3A mRNA. The later period corresponding to fetal growth and development is characterized by high concentrations of both IGF-II and BP-3A mRNAs. Uterine and endometrial tissues also express IGF receptors (Rutanen *et al.*, 1988). Presumably, the cumulative expression of both IGFs, their receptors and their binding proteins contributes to the sustained, constant growth of the uterus throughout pregnancy. IGF-II and BP-3A may constitute major secretory proteins of the pig endometrium, although this is only speculative at the present time. A question of major interest raised by these findings is whether uterine IGF-II and BP-3A cross the placental barriers (embryotrophic route) and contribute to fetal tissue growth in any direct way. A related question pertains to the known ability of the IGFs to mediate secretion of other proteins in reproductive tissues (Thrailkill *et al.*, 1988). These factors may well represent major mediators of uterine secretory activity in the pig.

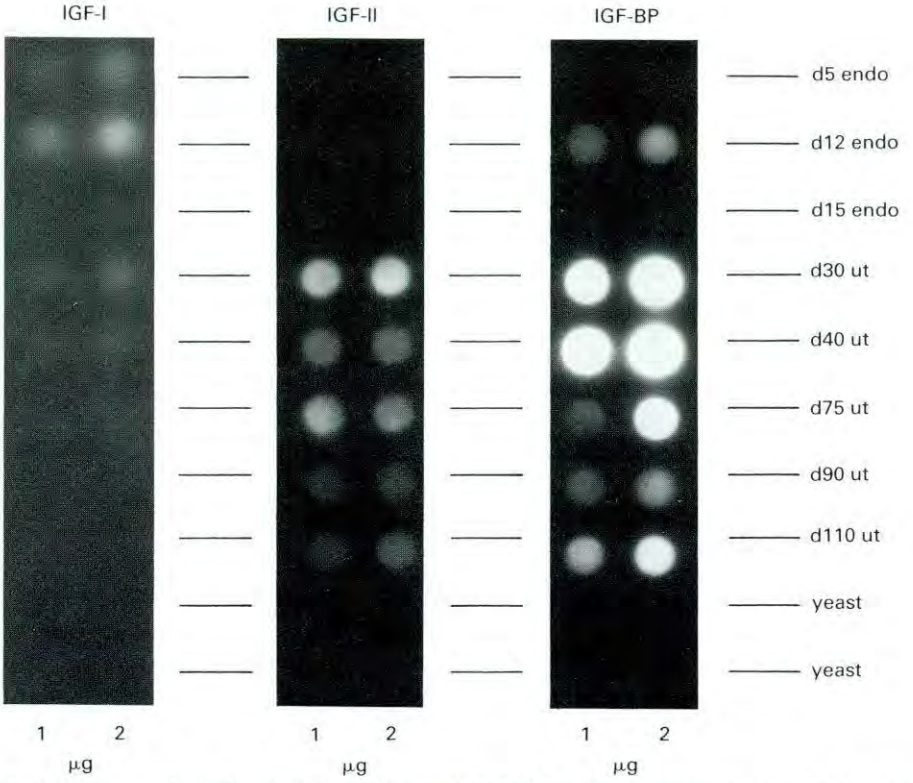

Fig. 1. Dot blot analysis for IGF-I, IGF-II and IGF-BP mRNA expression in whole uterine (ut) or endometrial (endo) tissues of pregnant sows. Poly(A)$^+$ RNA was isolated from endometrial or whole uterine tissues and blotted onto BioTrans nylon membranes. The membranes were hybridized to ^{32}P-labelled probes to detect the mRNA levels for the specific genes, as a function of the day of pregnancy. Yeast RNA was included as a negative control in the study.

Other growth factors

Uterine luminal fluids from early pregnant (Days 8–12) sows contain factors that stimulate DNA synthesis in a variety of animal cells (Simmen *et al.*, 1988a). The major growth factor component in these fluids has been partly purified and termed uterine luminal fluid mitogen (ULFM). This is a small molecular weight (M_r ~4800) polypeptide, biologically distinct from epidermal growth factor (EGF) and other growth factors characterized to date. ULFM is mitogenic in primary cultures of pig uterine stromal cells (corresponding to Day 12 of pregnancy). ULFM is probably of endometrial origin, and its absence in blood of early pregnant pigs has been noted (Simmen *et al.*, 1988a).

ULFM is also present in uterine secretions of cyclic sows (Simmen *et al.*, 1988a). In these and early pregnant animals, the activity is highest at Day 8 (the earliest time point examined) and is diminished by Day 14 (Simmen *et al.*, 1989). This decrease in activity is more pronounced in uterine luminal fluid of pregnant than cyclic animals at Days 11 and 12. Thus, local oestrogens may affect the synthesis and/or secretion of the mitogen.

The role of ULFM *in vivo* may be related to the enhanced DNA synthesis observed in the rat endometrial stroma before the development of uterine sensitivity to deciduogenic stimuli (Moulton & Koenig, 1984). In the rat, oestrogen is synergistic with progesterone in stimulating the induction of this event (Moulton & Koenig, 1984). If one considers the uterine endometria as the major tissue source of ULFM, a mechanism may be proposed in which the targetting of this mitogen is redirected from the lumen to the stroma under the influence of steroid hormones. Oestrogen has

been implicated to alter the secretion of prostaglandin F-2α from an endocrine to an exocrine pathway for maintenance of corpora lutea (Bazer & Thatcher, 1977). At Days 11–14, the diminished ULFM content of luminal fluids may be explained by a similar re-routing of this factor to stromal cells. Complete elucidation of the mechanism of control awaits purification of ULFM to homogeneity and the availability of immunological or molecular probes.

Gel filtration chromatography of uterine luminal fluids from Day-12 pregnant sows has also identified other small molecular weight mitogens (M_r < 10 000) distinct from ULFM. One of these components is predicted to be EGF or an EGF analogue, based on anti-mouse EGF (mEGF) IgG reduction of mitogenic activity of crude uterine luminal fluid (Simmen *et al.*, 1988a). The role of EGF in early pregnancy may be 2-fold. The mitogen may induce endometrial growth and differentiation to create an environment favourable for the developing conceptus. Indeed, intraluminal administration of anti-mouse EGF antibody can inhibit growth of the mouse uterine epithelium *in vivo* (G. Stancel, personal communication). Alternatively, conceptuses may utilize luminal EGF for their own developmental needs. Specific binding of ^{125}I-labelled mouse EGF to Day-12 and -16 pig conceptuses has been reported (Letcher *et al.*, 1989b), suggesting a biological function for this or related mitogens (e.g. transforming growth factors) in conceptus development. The source of this EGF is most probably the uterine cells (DiAugustine *et al.*, 1988), which implies autocrine and paracrine mechanisms of EGF action in early pregnancy.

The mechanism controlling EGF production in early pregnancy is not known. Studies relating to the synthesis and secretion of this growth factor in pig reproductive tissues are limited by the unavailability of homologous immunoassays for pig EGF. EGF is less structurally related than IGF-I across species, and the use of an RIA with mouse EGF as the standard has not demonstrated this growth factor in pig tissue extracts or biological fluids (R. C. M. Simmen, unpublished observations). In the mouse uterus, oestrogen has been demonstrated to induce EGF mRNA concentration although the induction is only 2-fold (DiAugustine *et al.*, 1988). However, since oestrogen similarly increases the synthesis of EGF receptors (Lingham *et al.*, 1988), the total oestrogen effect via EGF on uterine tissue growth and differentiation may be highly significant.

Uteroferrin

Uteroferrin is one of the most abundant glycoproteins synthesized and secreted by the uterine glandular epithelium of the pig (Roberts & Bazer, 1980, 1985). Uteroferrin is initially translated from its mRNA as a preprotein of M_r 31 000 (Simmen *et al.*, 1988b) and, upon glycosylation, is secreted as a single polypeptide chain of M_r 35 000. In uterine extracts a slightly larger form of the protein (M_r 37 000) is detected (Baumbach *et al.*, 1986). This is thought to represent newly synthesized uteroferrin, although the processing events leading from this form to mature uteroferrin are presently unclear.

The proposed biological function of uteroferrin is related to its high binding affinity for iron. The protein can bind two atoms of iron per molecule and is postulated to act as a major iron carrier from the uterus to the developing fetus (Roberts *et al.*, 1986) and this embryotrophic route of uteroferrin transport has been elucidated (Renegar *et al.*, 1982). Uteroferrin is taken up by specialized cells of the areolae, is released into the chorioallantoic capillaries and transported to the fetus via the umbilical vein. Within the fetus, the protein is sequestered by the liver or spleen which are sites for iron metabolism. Receptors that can recognize the high mannose carbohydrate chains of uteroferrin have been demonstrated on the reticuloendothelial cells lining the sinusoids of the fetal liver (Saunders *et al.*, 1985) and are presumed to aid in the intracellular routing of uteroferrin for fetal haemoglobin synthesis (Renegar *et al.*, 1982).

The temporal expression of uteroferrin during pregnancy has been characterized at the levels of its mRNA and protein (Simmen *et al.*, 1988b). Highest concentrations of uteroferrin mRNA quantitated using uteroferrin cDNAs as probes, are observed at mid- and late pregnancy, with detectable but greatly diminished levels in early pregnancy. Uterine content of uteroferrin protein

follows the pattern of uteroferrin mRNA from early to mid-pregnancy; however, in late pregnancy (Days 75–110), immunoreactive uteroferrin is low, despite maximal concentrations of uteroferrin mRNA (Simmen *et al.*, 1988b). The secretory levels of uteroferrin as measured in uterine secretions and/or allantoic fluids also decline in late pregnancy (Basha *et al.*, 1979; Buhi *et al.*, 1982). Based on these results, which suggest differential rates of uteroferrin mRNA and protein production, it is likely that the regulation of uteroferrin synthesis involves control at transcriptional and post-transcriptional levels.

Progesterone control of uteroferrin biosynthesis and secretion has been well documented (Roberts & Bazer, 1980). Under the influence of progesterone, increasing amounts of uteroferrin are secreted into the uterine lumen (Basha *et al.*, 1980). Oestrogen alone has no detectable effect on uteroferrin synthesis or secretion although at low doses it acts synergistically with progesterone to promote total endometrial protein and uteroferrin production. In studies utilizing cultured explants of pig endometrium (Basha *et al.*, 1979), uteroferrin production increased markedly after Day 30, peaked at Day 60 and declined in late pregnancy (Day 105). These changes in rates of uteroferrin synthesis are correlated with ratios of progesterone to oestrogen concentrations in sow plasma. In contrast, production of the uteroferrin mRNA transcript is dependent on progesterone only until mid-pregnancy (Day 75). High levels of uteroferrin mRNA are maintained at late term, despite a low progesterone to oestrogen ratio.

The uteroferrin chromosomal gene has recently been isolated from a pig genomic DNA library (Srinivas & Simmen, 1989). Analysis of the organization of this gene should facilitate identification of the structural elements involved in its hormonal and tissue-specific regulation at the molecular level. Induction by iron occurs at the levels of transcription and translation for other iron-binding proteins such as transferrin and ferritin (McKnight *et al.*, 1980; Bomford *et al.*, 1981; Rouault *et al.*, 1987). Although uteroferrin is distinct in structure and function from these proteins (Hunt *et al.*, 1987), its expression may be similarly regulated by iron concentration.

Protease inhibitors

A plasmin/trypsin inhibitor (PI) of M_r 14 000 has been purified from uterine secretions of early pregnant pigs (see review, Roberts & Bazer, 1988). PI is one of the Kunitz class of protease inhibitors (Laskowski & Kato, 1980) and is proposed to control the activity of the protease plasminogen activator (PA), whose substrate plasminogen is also present in uterine secretions (Fazleabas *et al.*, 1983). Pig blastocysts isolated between Days 10 and 16 of pregnancy release high amounts of PA (Fazleabas *et al.*, 1983), and the coincident increase in the activity of the inhibitor around this period may help to maintain the integrity of the uterine epithelium despite the invasive potential of the trophoblast (Samuel & Perry, 1972).

PI synthesis and secretion are influenced by progesterone (Fazleabas *et al.*, 1982). However, oestrogen acts synergistically with progesterone to modulate these events further (Fazleabas *et al.*, 1982). Therefore, in early pregnancy the mechanism of control of PI synthesis appears to parallel that of uteroferrin, although their respective sites of synthesis within the uterine epithelium differ (Fazleabas *et al.*, 1985). Since maximal levels of PI in uterine secretions occur at Day 12 (Fazleabas *et al.*, 1983), oestrogens of blastocyst origin may be the stimulus for PI release into the lumen. These same oestrogens may also initiate the enhanced production and secretion of PA by the blastocysts and the increased uptake of serum plasminogen by the uterus (Fazleabas *et al.*, 1983).

A protease inhibitor distinct from the PI of early pregnancy has been identified by nucleotide sequence analysis of pig uterine cDNA clones (Farmer *et al.*, 1989). This uterine inhibitor is the pig counterpart of human antileukoproteinase, a mucosal secretory protein which can inhibit the activities of the enzymes elastase and cathepsin G (Seemuller *et al.*, 1986). The pig and human proteins exhibit 70% amino acid sequence homology, and each contains 16 cysteine residues located at exactly the same positions within their respective protein sequences (Farmer *et al.*, 1989).

Analysis of pig antileukoproteinase (ALP) expression in various tissues of maternal origin has demonstrated highest concentrations of ALP mRNA in uterine tissues in mid- and late pregnancy, with low but detectable levels in early (Day 12) pregnancy (Fig. 2). This pattern of mRNA expression closely resembles that of uteroferrin mRNA (Simmen *et al.*, 1988b), although in mid- and late gestation the relative abundance of ALP mRNA is almost 4-fold that of uteroferrin (R. C. M. Simmen, unpublished results). Based on the above, it can be assumed that the mechanisms regulating mRNA synthesis for the two proteins are very similar if not identical. However, it remains to be determined what controls the secretion rate of the ALP protein or whether regulation of ALP synthesis at the level of translation, as has been postulated for uteroferrin, exists.

The function of ALP in pregnancy has not been previously explored. In humans, highest concentrations of this protein occur in seminal plasma, cervical, bronchial and nasal secretions and tears (Seemuller *et al.*, 1986). Its absence in human endometrium and high levels in the cervical

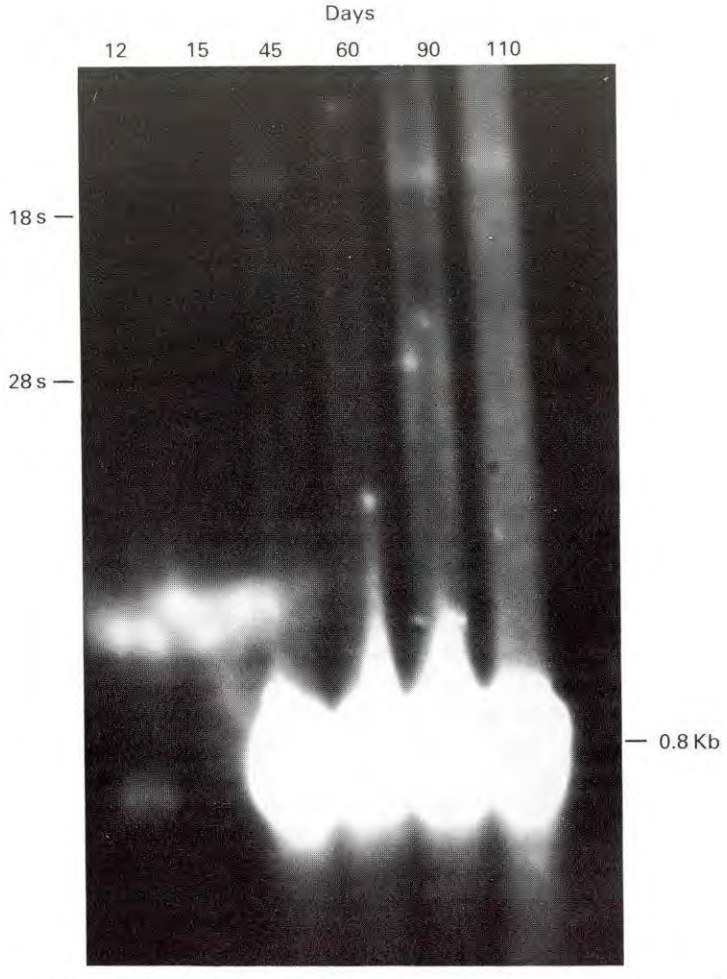

Fig. 2. Northern blot analysis of poly(A)$^+$ RNA from pig whole uterine tissues for expression of antileukoproteinase (ALP). Poly(A)$^+$ RNA was isolated from whole uterine tissues at the days of pregnancy indicated. RNA samples (3 μg/lane) were electrophoresed through a 1·5% agarose gel containing 2·2 M-formaldehyde and transferred to a BioTrans membrane. The filter was probed with ^{32}P-labelled ALP cDNA probe, and the resultant autoradiogram is shown.

mucus during the menstrual cycle have been noted (Casslen *et al.*, 1981). The limitation of sources from human uterine tissues precludes examination of ALP function in human pregnancy. In the pig, ALP may function in the maintenance of the integrity of the placental membrane. Protease activities control the synthesis and degradation of the extracellular matrix, and ALP may modulate these activities to prevent premature rupture of fetal membranes, which can lead to fetal death.

Other progesterone-induced proteins

Several less characterized uterine secretory proteins have been identified as progesterone-dependent, based on their appearance in uterine secretions of sows with elevated serum progesterone concentrations. These include the uteroferrin-associated basic proteins (Baumbach *et al.*, 1986), the enzyme lysozyme whose function is presumed to be antibacterial (Roberts *et al.*, 1976), and retinol binding protein of M_r 17 000 which may be involved in vitamin A transport to the fetus (Adams *et al.*, 1981).

Among these, the uteroferrin-associated proteins are the most extensively characterized in terms of biochemical properties, site of synthesis and intracellular processing events (Baumbach *et al.*, 1986). Their functions, however, remain unclear. The lack of appropriate biochemical or immunological probes for the other proteins currently limits the elucidation of their properties and functions. However, these proteins are found at high concentrations in allantoic fluids and it may therefore be assumed that their function is related more to fetal than conceptus growth and that their regulation may be similar to that of uteroferrin.

Functions and regulation of conceptus secretory proteins

Interferon-related pig trophoblast proteins

Conceptuses from sheep and cows in early pregnancy release a low molecular weight (M_r 21 000–24 000) protein that constitutes the major secretory product of the trophoblast (Godkin *et al.*, 1982; Helmer *et al.*, 1987). These trophoblastic proteins have been postulated to function as a signal for maternal recognition of pregnancy. The ovine trophoblast protein (oTP-1) is synthesized between Days 13 and 21 of pregnancy (Godkin *et al.*, 1982). Within this period high embryonic expression of oTP-1 mRNA is observed at Day 16 (Hansen *et al.*, 1985), with maximal protein production and release at Day 17 (Hansen *et al.*, 1985) coinciding with the rescue of the corpora lutea from luteolysis. Similarly, in cattle, the secretion of the analogous protein (bTP-1) (Bartol *et al.*, 1985) and its appearance in uterine flushings (Kazemi *et al.*, 1988) are noted at Days 15–25 which is the critical time for embryo signalling to the mother.

The mechanism of action of oTP-1 has been examined. The protein interacts with the uterus via specific receptors present in the endometrium (Godkin *et al.*, 1984) to alter the tissue's protein synthetic capacity (Godkin *et al.*, 1984) and ability to produce prostaglandins (Fincher *et al.*, 1986; Vallet *et al.*, 1988). The nature of the specific protein changes is not clear, nor how they can delay luteal regression (Godkin *et al.*, 1984), although reduced levels of circulating PGF-2α from the uterus have been correlated with maintenance of the corpora lutea (Bazer & Thatcher, 1977).

The significant sequence similarity of oTP-1, bTP-1 and the interferon (IFN-α) family of proteins (Imakawa *et al.*, 1987, 1989) also suggests an immunoprotective role for these proteins in early pregnancy. The fetal allograft can trigger maternal interactions which may lead to loss of pregnancy, if left uncontroiled. The interferon-like proteins of conceptus origin, by analogy with IFNs, can delay allograft rejection and inhibit lymphocyte activation (Friedman *et al.*, 1986), thus allowing the conceptus to be nurtured within the uterine environment.

In the pig, the presence of a group of conceptus proteins related to interferons has been demonstrated (Cross & Roberts, 1988). These low molecular weight (M_r 24 000) acidic proteins are secreted by elongating conceptuses at Days 11–17 of pregnancy. The sequence similarity of these

proteins and oTP-1 appears to be limited (Cross & Roberts, 1988), but their respective interferon-like activities suggest functional relationships.

Although the period of maximal synthesis of the pig trophoblastic proteins has not been extensively characterized during early pregnancy, their expression within the time of blastocyst oestrogen synthesis raises the question of the possible role of local oestrogen production in their synthesis and secretion. Changes in blastocyst, rather than plasma, oestrogen concentrations are temporally correlated with the induction of uterine endometrial secretory activity (Geisert *et al.*, 1982b), and proteins of conceptus origin may respond similarly to this signal. Alternatively, the endometrial proteins secreted in response to local oestrogen may act in a paracrine fashion to trigger the synthesis and release of these conceptus-derived macromolecules. However, since studies to date have utilized blastocysts cultured *in vitro* in the absence of oestrogen, it is also possible that the trophoblastic proteins represent gene products whose expression is regulated as a function of embryological stage rather than by specific hormones *per se*. The nature of these signals at best remains speculative.

Other blastocyst proteins

Pig blastocysts recovered between Days 10 and 18 of pregnancy and cultured *in vitro* also release a number of other proteins whose identities and functions are currently unknown (Godkin *et al.*, 1982; Powell-Jones *et al.*, 1984). These proteins are differentially expressed as a function of conceptus development. While the major secreted products at Days 10·5–12 are acidic, low molecular weight (M_r 20 000–25 000) polypeptides, the major proteins detected between Days 13 and 16 are basic and in the molecular weight range of 35 000–50 000 (Godkin *et al.*, 1982; Powell-Jones *et al.*, 1984). After Day 18, still another group of polypeptides in the 50 000–70 000 molecular weight range are synthesized.

One of the pig blastocyst proteins released at Day 16 is a high molecular weight glycoprotein which is also a major secretory product of cow and sheep blastocysts (Masters *et al.*, 1982). In the sheep and the pig, this protein is also synthesized during the period of rapid blastocyst elongation (Godkin *et al.*, 1982; W. G. Gray & R. M. Roberts, unpublished results). The function of this glycoprotein in early pregnancy is not known although, on the basis of its extremely high carbohydrate composition, it is postulated to provide a protease-resistant or immunologically tolerant coating over the expanding blastocysts (Masters *et al.*, 1982).

Temporal relationships: secreted proteins and hormonal environment

The molecular mechanisms regulating the synthesis and secretion of maternal and conceptus secretory proteins, although largely unclear, have been partly elucidated by using biological reagents and specific cDNA probes. For maternal secreted proteins, correlation of their temporal expression with the hormonal environment of the uterus suggests two general mechanisms of control (Fig. 3). The first mechanism relates to the control by blastocyst-derived oestrogen and appears to be operative in the synthesis and secretion of the growth factor IGF-I, the plasmin inhibitor PI and of ULFM. The second mechanism, largely under progesterone control, although possibly modulated by oestrogen, appears to be responsible for the induction of the synthesis of IGF-II, IGF-BP, uteroferrin, ALP and other endometrial proteins (e.g. lysozyme, uteroferrin-associated proteins).

Obviously, the postulated mechanisms are not as straightforward as suggested. Protein production can be regulated at the levels of transcription and translation, and both levels of control may be differentially influenced by the same hormonal environment. Similarly, the secretory process is a complex event which may be altered in its timing and/or direction by its environment. Uteroferrin represents an excellent example of a uterine-derived protein whose expression at the levels of

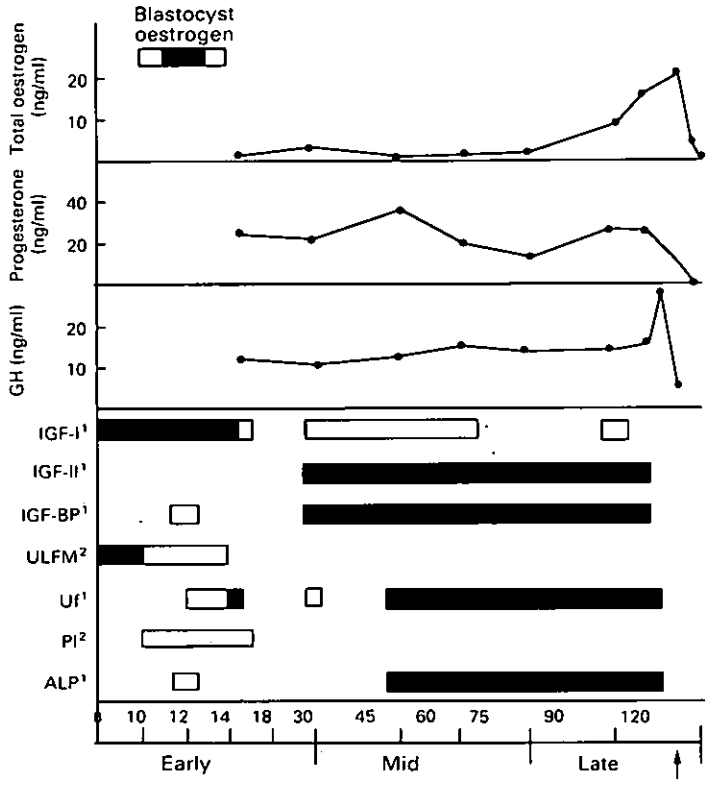

Fig. 3. Temporal expression of specific uterine mRNAs or proteins at pregnancy. The expression of mRNAs (Superscript 1) for specific proteins was detected by corresponding cDNA probes. Amounts of ULFM and of plasmin/trypsin inhibitor (PI) were quantified by [^{3}H]-thymidine incorporation into mouse AKR-2B fibroblastic cells and by inhibition of fibrinolysis (Fazleabas *et al.*, 1982), respectively (Superscript 2). Shaded bars indicate higher relative levels of expression compared to open bars. Plasma concentrations of oestrogen, progesterone and growth hormone are derived from the data of De Hoff *et al.* (1986). Days of pregnancy are indicated by numbers preceding vertical lines and the day of parturition (Day 115) is indicated by the arrow.

mRNA and protein is differentially regulated by progesterone. Although uteroferrin protein secretion is directly related to the ratio of progesterone to oestrogen concentrations in the serum, uteroferrin mRNA synthesis appears not to be so. The growth factor IGF-I which is expressed throughout pregnancy, is another example. Whereas in early pregnancy, IGF-I mRNA synthesis and protein secretion appear to be induced by blastocyst-derived oestrogen, IGF-I mRNA expression in mid- and late pregnancy cannot be directly correlated with the circulating concentrations of oestrogen or progesterone (De Hoff *et al.*, 1986). Uterine IGF-I mRNA levels also do not parallel the serum concentrations of growth hormone in sows (De Hoff *et al.*, 1986), despite direct demonstration of growth hormone-induced synthesis of IGF-I mRNAs in other species (Roberts *et al.*, 1987; Murphy & Friesen, 1988).

The above discrepancies may be related in part to the dependence of uterine endometrial and conceptus secretory activities on other proteins which are synthesized in response to steroid hormones. Indeed, it has been suggested that the secretion of specific uterine proteins may be associated more with the progesterone-dependent differentiation state of endometrial cells than to a direct effect of progesterone. To date there is little information as to the nature of the proteins involved in these processes. However, understanding of the programme of uterine endometrial

differentiation and gene expression is ultimately required to elucidate its subsequent effects on secretory activity.

It is important to note that, despite the apparent major role of blastocyst-derived oestrogen in controlling endometrial secretory activity in early pregnancy, the endometrial tissue is also under progesterone regulation. The functional interaction of these steroid hormones is reflected in the differentiation of the uterus and the synthesis of its associated secretory products at this stage of pregnancy. Similarly, in late pregnancy, although progesterone may be the major factor in the synthesis and secretion of uterine proteins, placental or fetal-derived factors acting systemically may also be involved.

The current limited studies on the nature of conceptus-derived proteins preclude elucidation of their regulation. Based on their differential expression in early pregnancy, however, their functional importance during the period of maternal recognition of pregnancy and implantation is implied. It is possible that synthesis is related to the progesterone-dependent differentiation of endometrial cells whose secretory proteins may act as signals for the initiation of the programme of embryonic differentiation. It is equally possible that secretory activity is initiated by factors implicated in the activation of gene expression in other systems (Braude *et al.*, 1988).

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