J. Biosci., Vol. 6, *Supplement* 2, July 1984, pp. 63–74.

Biochemical interactions between blastocyst and endometrium in the large domestic animals

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Abstract. In pigs, the blastocyst begins to elongate from a sphere to a long filamentous thread around day 10.5 of pregnancy. At about this time the endometrium secretes large quantities of protein into the uterine lumen. The synthesis of this material which is believed to be required for nutritional support of the conceptus is under the control of progesterone. The release of secretory protein appears to be triggered by the production of estrogens by the elongating blastocyst. Blastocyst estrogens are also involved in the phenomenon of maternal recognition of pregnancy in swine, and their interaction with the maternal system, by a mechanism as yet unknown, prevents a return to reproductive cyclicity. Maternal recognition of pregnancy in the sheep and cow occurs at around the time of blastocyst elongation. Here estrogens do not appear to be involved, and protein products secreted by the conceptus have been implicated. One product of the sheep, ovine trophoblast protein-1, which is produced only during a brief period (days 13–21) of pregnancy, has been purified. It appears to be a hormone whose target tissue is the uterine endometrium.

Keywords. Uterus; endometrium; blastocyst; secretions; proteins; estrogen; progesterone; pregnancy; corpus luteum.

Introduction

Pregnancy depends upon a series of appropriate coordinated interactions between the developing conceptus and the mother (Bazer and First, 1983). The maternal system, for example, must afford an appropriate fluid medium for fertilization within the oviduct. In the case of large domestic animals, where ectopic pregnancies do not occur, only the uterus appears capable of providing the micro- and macromolecular environment and possibly other factors, such as hormones, that are required for continued conceptus development beyond the blastocyst stage. The uterus must also limit the degree of blastocyst invasiveness and the extent of placentation. In turn, signals must originate from the conceptus to protect the corpora lutea (CL) from regression and to prevent the return to reproductive cyclicity. In addition, uterine secretory activity must be maintained, blood flow to the endometrium regulated, immunological privilege of the fetal allograft achieved and mammary gland development promoted. Chemical signals between the conceptus and mother are also responsible for initiating parturition.

In this paper we shall review some of the interactions that occur between the blastocyst and uterine endometrium in early pregnancy of pigs, sheep and cattle. We shall concentrate on those events that occur around the time the blastocyst starts to

Abbreviations used: CL, Corpora lutea; E_1SO_4, estrone sulphate; E_1, estrone, E_2, estradiol; E_3, estriol; E_2V, estradiol valeate; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

elongate, because this period coincides with a series of additional events which appear to be crucial for maintenance of pregnancy. In the pig, for example, it is the time when the maternal endometrium begins to release large quantities of progesterone-induced secretory material into the uterine lumen (Geisert *et al*., 1982a). In all the three species, it coincides with the period during which the phenomenon of maternal recognition of pregnancy occurs (see below). Finally, as the blastocyst begins to expand, it also begins to become active in the production of its own secretory protein products and in steroid metabolism (Flint *et al*., 1979).

Conceptus elongation

In swine, sheep and cattle, blastocysts undergo a dramatic morphological sphere-to-filamentous transition some days prior to their firm attachment to the uterine wall and to the expansion of the allantois (Bazer and First, 1983). Elongation results in a major increase in the surface area to volume ratio of the conceptus and a close association of the trophectoderm (outer epithelial surface) with the maternal uterine endometrium. It is also around this stage of development that the process of maternal recognition of pregnancy occurs, a phenomenon that must result from a chemical signal passing from the conceptus to the maternal system to insure maintainance of CL function and continued progesterone production. In turn, progesterone is responsible for ensuring induction and synthesis of the endometrial secretions upon which the noninvasive conceptuses must rely for their growth and development. Indeed we have compared the secretions produced by the maternal uterus during its formation to a complex embryo culture medium (Bazer *et al*., 1978). Elongation may be necessary in order to provide a sufficient area of trophectoderm surface to allow uptake of nutrients and to facilitate the biochemical interactions between the conceptus and maternal uterus upon which pregnancy depends.

In swine, embryos enter the uterus at the 4-cell stage, *i.e.* 60 to 72 h after onset of estrus, reach the blastocyst stage by day 5, shed the zona pellucida between days 6 and 7 and then slowly expand to a 2 to 6 mm diameter sphere by day 10 (Perry and Rowlands, 1962). Between days 10 and 12 the conceptus changes from a sphere of up to 10–15 mm diameter to a tubular from (15 to 50 mm long by 1 to 2 mm wide) and finally achieves a long, thread-like filamentous form about 200 mm long and 0·5 to 1 mm wide (Geisert *et al*., 1982b). These initial stages of blastocyst elongation occur very rapidly. Rates of 30 to 45 mm/h have been estimated, and the morphological transition apparently results from remodelling and not cellular hyperplasia. Indeed the amount of DNA scarcely changes during sphere to thread transition and the volume of the conceptus actually decreases. However, the surface area during the initial transition from a 10 mm sphere to a 200 mm × 1 mm thread, must increase several fold.

Pig conceptuses continue to elongate between days 12 and 16 to reach a final average length of about 800 mm each (Anderson, 1978). During these final stages of elongation mitotic activity is high as reflected by substantial increases in total RNA and DNA per conceptus (Geisert *et al*., 1982b).

Ovine and bovine blastocysts also undergo elongation, but in a less dramatic fashion than in pigs, and they do not achieve as long a final length. In the ewe elongation begins

around days 12–13. They reach a length of ~ 70mm by day 14 (Bindon, 1971) and 150 to 190 mm by day 15 (Chang and Rowson, 1965). In the cow, rapid blastocyst elongation is initiated around day 15, and they are > 50 mm in length by day 18 (Chang, 1952). In neither of these animals has the mechanism of the process received detailed attention, but we assume that the events occur in the progression similar to that described for pigs by Geisert *et al.* (1 982b).

Data are not presently available to elucidate the underlying mechanisms responsible for the cellular remodelling that occurs during blastocyst elongation, although some of the ultrastructural changes have been described for the pig, and a model accounting for the patterns of cellular migration has been presented (Geisert *et al*., 1982b), but will not be discussed further here. Significantly, the outer surface of trophectoderm cells are densely covered with coated pits (Geisert *et al*., 1982b), indicative of endocytosis, and the uptake of components of maternal uterine secretions has been demonstrated in this species (Fazleabas *et al*., 1982; Chen *et al*., 1975). It is also known that the elongating blastocysts are active not only in uptake of protein, but also in secretion of proteins (Godkin *et al*., 1982a).

Relationship between uterine secretory activity and the development of the uterine endometrium

In animals such as the pig which possesses a diffuse, central-type, epitheliochorial placentation (Schlafke and Enders, 1975), there is no erosion of the uterine epithelium throughout pregnancy. Several cell layers, therefore, separate the blood supply of the mother from that of the fetal placenta. It is probably for this reason, therefore, that the pig conceptus receives any macromolecular constituents required for its survival in the form of secretions provided by the glandular epithelium of the maternal endometrium over which it lies (Amoroso, 1952). As anticipated, the pig uterus continues to secrete large quantities of macromolecular secretory components, often known as, histotroph, throughout pregnancy although the rates of secretion change in relation to the endocrine status of the mother (Basha *et al*., 1979). These materials have been investigated in detail over the last decade, and several proteins have been characterized (Bazer *et al.*, 1981; Roberts and Bazer, 1980). Among these are uteroferrin, a purple coloured glycoprotein, which carries iron to the conceptus (Buhi *et al*., 1982; Ducsay *et al*., 1982; Renegar *et al*., 1982), a retinol binding protein, presumably responsible for the transport of that fat soluble vitamin (Adams *et al*., 1981), and a series of protease inhibitors (Fazleabas *et al*., 1982). The functions of the latter are unclear but they may help control the activity of conceptus proteases and even limit the potential invasiveness of the pig conceptus. Alternatively, they may protect the histotroph itself from proteolytic destruction. All of the proteins described above are induced by the hormone of pregnancy, progesterone, although their release from the secretory epithelium may be partially controlled by estrogens produced by the conceptus (see below). Moreover, although estrogens alone do not induce specific uterine proteins, at low concentrations they act synergistically with progesterone to promote increased amounts of secretory protein production while in high amounts they inhibit the production of histotroph (Knight *et al*., 1974 ; Roberts and Bazer, 1980). Finally, an

estrogen "primed" uterus is probably necessary to provide a necessary number of cells for a subsequent secretory response to progesterone.

The most complete description of the changes that occur in the endometrium of pigs during the estrous cycle and early pregnancy are those of Corner (1921). Detailed ultrastructural observations have also been made throughout pregnancy from day 30 onwards by Sinowatz and Friess (1983) and in early pregnancy by Geisert *et al.* (1982a). In general, these observations are consistent with the concept that the progestational uterus of the pig is highly secretory. Essentially the first 15 days of the estrous cycle and pregnancy have been observed to be quite similar (Corner, 1921). Mitotic activity in the surface epithelium was high around estrus, and the cells become more columnar as the cycle progresses. Mitotic activity in the glands was observed to be highest around days 5 to 6, but mitoses in the deeper glands were evident until about day 11. During the luteal phase the glandular cells gained the appearance of being active in secretion. Data from our laboratory have indicated that a synchronized release of secretions begins around day 11 of pregnancy, while in the nonpregnant conditions this release is somewhat delayed and more gradual (Geisert *et al*., 1982a).

Between day 17 and next estrus (day 21) the surface and glandular epithelial cells gradually revert to an appearance characteristic of estrus. Presumably there is also a loss of cells from the epithelium and glands, but the patterns of cellular and glandular regression have not been well studied. By contrast, in the pregnant condition, the surface and glandular epithelium remains columnar in appearance, and the glands becomes increasingly branched and complex. Between days 30 and 60 of pregnancy the cells of the glands in particular develop massive whorls of rough endoplasmic reticulum (Sinowatz and Friess, 1983). Large secretory vacuoles containing densely staining material are present. These structures can be stained immunocytochemically for uteroferrin (Renegar *et al*., 1982; T. W. Raub, R. M. Roberts and F. W. Bazer, unpublished results) and appear to empty their contents into the lumen of the glands where uteroferrin-positive material can also be detected. The glandular cells maintain this striking appearance until term (day 115).

In the sheep and the cow such a reliance of the conceptus on uterine secretory activity is believed to be much less than in the pig due to the development of placentomes (fused fetal cotyledon and maternal caruncle) which become evident soon after the chorioallantois (placenta) has spread over the available surface of the placenta (Hafez and Jainudeen, 1974). Development of the cotyledons on the placental side is presumably initiated by the presence of opposing maternal caruncles (King *et al*., 1980). In these regions the uterine epithelium is eroded (Boshier, 1969). The placentomes are believed to be the major sites of gas and nutrient exchange after about day 30 of pregnancy. The caruncles, for example, receive over 80% of the maternal uterine blood supply in the sheep and the cotyledons over 90 % of the placental circulation (Hafez and Jainudeen, 1974). In addition, when the placental surface area is decreased either by twinning or by surgical restriction the size of the placentomes increases to accommodate the growth needs of the fetus (Caton *et al.*, 1983). It is assumed that these structures are also major sites of exchange of endocrine information between the mother and conceptus. It is within the fetal cotyledons, for example, that placental lactogens are believed to be formed (Chen *et al*., 1975; Martal and Djiane, 1977; Kensinger *et al.*, 1982) and where estrogen production takes place.

Initiation of blastocyst estrogen secretion by conceptuses

Pig blastocysts begin to produce estrogens soon after day 10 of pregnancy (Flint *et al*., 1979; Fischer, 1981; Geisert *et al*.,1982a). The two reports from our laboratory (Fischer, 1981; Geisert *et al*., 1982a) have shown that production begins at around the 10 mm spherical stage (days 10·5 to 11) just preceding the rapid transition from sphere to thread. Based on concentrations of estrone sulphate (E_1SO_4) in maternal plasma, estrogen secretion by pig conceptuses is triphasic with major increases between days 10 and 12 (Stoner *et al*., 1981), days 16 and 30 (Stoner *et al.*, 1981) and day 60 and term (Knight *et al*.,1977). The mechanisms whereby conceptus estrogen production is regulated is not known, nor is much evidence available to indicate what functions these estrogens, particularly those formed during the later two phases, play during pregnancy, although they may regulate uterine secretory activity. In this review we shall be concerned only with estrogen production by early expanding blastocysts where some functional relationships between the conceptus and mother have been established.

Total recoverable estradiol (E_2), estrone (E_1) and estriol (E_3), E_1SO_4 and E_2SO_4 in uterine flushings of gilts increase markedly as blastocysts begin to increase in size from ~ 5 mm spheres (at day 10) elongated filaments at day 12. These estrogens then decrease by day 14 (Geisert *et al*., 1982a). A corresponding increase in utero-ovarian vein plasma E_1SO_4 is observed during the day 10 to 12 period and is assumed to have originated from conceptus estrogen production (Stoner *et al*., 1981).

Data concerning estrogen production by bovine or ovine conceptuses are very limited. Shemesh *et al.* (1979) reported that bovine blastocysts between days 13 and 16 produced small quantities of E_2, and Eley *et al.* (1979), have demonstrated metabolism of progesterone and androstenedione to estrogens. However, there is no evidence for a corresponding increase in utero-ovarian vein estrogens during this period. Steroid production by ovine blastocysts has not been established. Ellinwood *et al.* (1978) reported that E_1 concentrations in maternal utero-ovarian vein were higher than in nonpregnant controls in the day 13 to 17 period, but Reynolds *et al.* (1982) were unable to show such differences.

Clearly, systematic studies for steroid production by ovine and bovine blastocysts have not been carried out. Such studies will be necessary if transient periods of estrogen production, as occur in pigs, are to be detected.

Estrogen-induced secretion by endometrial epithelium

Geisert *et al.* (1982a) reported the accumulation of secretory vesicles in endometrial gland epithelium of the pig between days 10·5 and 12 of gestation. With onset of estrogen production by tubular and filamentous blastocysts between days 11 and 12 of gestation, there was a marked increase in total recoverable calcium, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), PGE_2, uteroferrin and protease inhibitor levels in uterine flushings. There were no significant.changes in these same components in uterine flushings, collected from nonpregnant gilts during a corresponding time period. In these animals the release of secretory materials was delayed and occurred more gradually. However, administration of 5 mg estradiol valerate (E_2V) to nonpregnant gilts on day 11 resulted in increases in total recoverable calcium, prostaglandin and total protein, uteroferrin and protease inhibitor activity by 24 h post-injection which were similar to those for

pregnant gilts with respect to quantitative values and temporal patterns of change of each component (Geisert *et al*., 1982c). It has been proposed that blastocyst estrogens may be responsible for this synchronous release of secretory material. Rubin and Laychock (1978) have suggested that increased calcium activates phospholipase A_2 which releases arachadonic acid from membrane phospholipids. This event, in turn, triggers exocytosis of secretory vesicles and formation of prostaglandins *via* the arachidonate pathway. Conceivably blastocyst estrogen causes the initial release of calcium from the plasma membrane leading to the above cascade of events. Pietras and Szego (1979a, b) have reported that a site of estrogen action in the uterus of the rat is the plasma membrane and that there is a very rapid cytological response to the steroid (Rambo and Szego, 1983). Alternatively, the secretory mechanism may involve intracellular estrogen receptors.

A very similar series of events has been reported in the cat by Bareither and Verhage (1980). There, however, it is estrogen which induces the accumulation of intracellular secretory product, and progesterone which triggers rapid exocytosis of this material from secretory vesicles. In the roe deer there is also a marked accumulation of secretory vesicles in uterine epithelium during the period of embryonic diapause (July to December) (Aitken, 1979). These vesicles undergo exocytosis in association with with the termination of delayed implantation which is accompanied by an increase in calcium and α-amino nitrogen in the uterine lumen. There is also evidence that the blastocyst of the roe deer begins to produce estrogen prior to the termination of the delay period. In the preceding examples, therefore, steroid hormones synthesized by the blastocyst cause the release of secretory products from the maternal endometrium which are presumably required for continued growth and development of the conceptus.

Blastocyst estrogens and maternal recognition of pregnancy in pigs

The life span of the CL determines the duration of progesterone production and therefore the period of histotroph production by the uterine endometrium. It is therefore essential for continuation of pregnancy that the conceptus should provide a "signal": which will result in the maintenance of the CL and thus allow for the establishment of pregnancy. This period of early pregnancy (days 11–14 in swine) when the conceptus acts to prevent CL regression is recognized as the period in which "maternal recognition of pregnancy" occurs. In the domestic farm animals it is now generally agreed that $PGF_{2\alpha}$ produced by the uterine endometrium is the luteolytic agent which will cause CL regression and the cessation of progesterone production (Bazer *et al*., 1981a). In the pig, therefore, the conceptus must produce some substance or substances that directly or indirectly prevent the luteolytic effects of uterine $PGF_{2\alpha}$. The theory has been developed that estrogens produced by the embryo prevents $PGF_{2\alpha}$ release from the uterine endometrium into the uterine venous drainage where it would gain access to the CL and cause luteolysis (Bazer and Thatcher, 1977). This theory has been discussed in detail elsewhere (Bazer *et al*., 1981). In support of this concept it has been noted that whereas utero-ovarian vein plasma concentrations of $PGF_{2\alpha}$ and metabolites are elevated during the period of luteolysis in non-pregnant gilts, no significant changes were noted at equivalent time periods in pregnant gilts. Similarly if

nonpregnant gilts were treated with estradiol valerate (5 mg/day) on days 11 through 15 after onset of estrus, the increase in PGF levels did not occur (Frank *et al.,* 1977). Moreover these animals became pseudopregnant in the sense that luteal function along with continued endometrial secretory activity was prolonged on average for more than 100 days.

The precise mechanism whereby blastocyst estrogen prevents the secretion of $PGF_{2\alpha}$ in an endocrine direction (*i.e.* towards the uterine vasculature) remains unknown. One possibility is that $PGF_{2\alpha}$ is diverted in an exocrine direction (*i.e.* towards the uterine lumen), since $PGF_{2\alpha}$ concentrations are high in uterine flushings of pseudopregnant and pregnant gilts in the day 11 to 14 period (Geisert *et al*., 1982a). The concept that $PGF_{2\alpha}$ is the luteolytic agent in swine and that estrogens are luteostatic will not be reviewed in further detail here since the concepts have been discussed thoroughly elsewhere (Bazer *et al*., 1981). Nevertheless, it should be emphasized that other interpretations of the results are possible. One is that the blastocyst produces some other substance, such as a protein, which acts locally on the endometrium, which in turn responds by producing estrogens or other substances to reduce output of $PGF_{2\alpha}$ into the utero-ovarian vein.

Maternal recognition of pregnancy in the sheep and cow

In the sheep, the CL regresses at the end of a 16–17 day estrous cycle as the result of the action of a uterine luteolysin which, as in the pig, is probably $PGF_{2\alpha}$ (McCracken *et al*., 1972). The $PGF_{2\alpha}$ is again believed to be produced by the uterine endometrium and transported to the ovary in a local fashion. The presence of viable conceptuses within the uterus prevents CL regression, but the nature of the blastocyst product which prevents CL regression is unknown, but is thought not to be estrogen. However, a transient period of estrogen production has not yet been definitively ruled out.

Embryo transfer experiments with sheep have shown that a conceptus must be present in the uterus by days 12–13 of pregnancy for CL to remain functional and for pregnancy to be maintained (Moor, 1968). It was also shown that conceptus homogenates from days 14–15 of pregnancy maintained luteal function when infused into the uterus of nonpregnant, day 12, recipient ewes (Rowson and Moor, 1967). Extracts derived from day 25 sheep conceptuses or homogenates from day 14 pig conceptuses were ineffectual as was the transfer of heat-treated days 12–14 conceptuses. Repeated daily infusions of days 14–15 sheep homogenates were more effective than a single infusion at day 12. Similar results were obtained by Martal *et al.* (1979) who reported that daily intrauterine injections of extracts of days 14–16, but not days 21–23 conceptuses into day 12 recipients were sufficient to extend luteal function for up to several months in over one-half of recipient ewes tested. The active component was proteinaceous and was called trophoblastin. Although it is not completely resolved whether factors from the conceptus can affect the CL directly (Godkin *et al*., 1978), most available evidence is consistent with the view that the conceptus proteins responsible for maternal recognition of pregnancy must be introduced into the uterine lumen, where they appear to exert their effect (Moor, 1968; Ellinwood *et al*., 1979). Presumably, their main site of action is on the endometrium which, in turn, may

respond by altering the production or release of $PGF_{2\alpha}$ or alter the ability of $PGF_{2\alpha}$ to cause luteolysis.

Considerably less is known about maternal recognition of pregnancy in the cow. Northey and French (1980) reported that pregnant cows from which embryos were removed on day 17 and 19 had interestrous intervals of 25 ± 1·2 and 26·2 6 days compared with those having embryos removed on day 13 (20·2 ± 8 days) or not mated 20 to 21 days. The nature of the active substances remains unknown, but by analogy with the sheep it may be a blastocyst protein.

Polypeptides released by incubated blastocysts

Pigs: Pig conceptuses secrete a number of proteins, beginning as early as day 10.5 of pregnancy (Godkin *et al*., 1982a). These results were obtained by culturing conceptuses of increasing age and development in a culture medium containing radioactive amino acids. Protein synthesis continued for at least 24 h when the experiments were terminated. Tissue proteins, as well as secreted products, were analyzed by two-dimensional polyacrylamide gel electrophoresis and detected by fluorography. Proteins in the medium were also analyzed by gel permeation and ion-exchange chromatographic methods.

The proteins released into the medium were relatively few and were clearly distinguishable from the major tissue proteins. We believe that they represent secretory products(Godkin *et al.*, 1982a). The pattern of these secreted proteins changed markedly in both a qualitative and quantitative manner with increasing age of the conceptuses. Between days 10·5 and 12 a group of acidic low molecular weight proteins (pl 5·6 to 6·2; M_r 20 to 25 K) predominated. At days 13 and 16 the small acidic proteins were still present but the major product was a basic protein (M_r 35 to 50K; pI ~ 8·0). With time this protein also disappeared from the gels and was not a detectable product of day 20 to 35 chorionic tissue.

In addition to the above, the cultured pig conceptuses throughout the elongation stage produce a glycoprotein which does not enter polyacrylamide gels. This molecule is 50 % by weight carbohydrate and of very high molecular weight (~ 700,000) (Masters *et al*., 1982). Preliminary experiments have shown that this glycoprotein was not produced by day 10·5 spherical blastocysts and that it was released only during the elongation phase (W. Grey, F. W. Bazer and R. M. Roberts, unpublished results).

The function of the above proteins produced by the pig blastocysts remain unknown although it is tempting to speculate that their appearance reflects some specific but as yet mysterious role in the maintenance of pregnancy.

Sheep: Sheep conceptuses between days 13 and 23 also secrete a variety of proteins into the medium when they are cultured *in vitro* (Godkin *et al.*, 1982b). Using a modified Eagle's Minimal Essential medium supplemented with L-[^{3}H]-leucine as a polypeptide precursor it was found that the release of proteins was linear for at least 24 h and that day 16 blastocysts could convert up to 8% of the radioactivity supplied into non-dialysable macromolecules in the medium. Two-dimensional polyacrylamide gel electrophoresis revealed that at day 13 there was only one major product produced.

Initially this protein was called Protein X (Godkin *et al*., 1982b), but we have since named it ovine trophoblast protein-1 (or oTP-1) (Godkin *et al*., 1981 a and 1984). Ovine oTP-1 consists of three closely similar isoelectric species (pI's around 5·3 to 5·7) each with molecular weights of 17,000 (Godkin *et al.*, 1982b). Between days 14 and 21 additional proteins were detected. One of these was of high molecular weight (> 660,000) and did not appear on the two-dimensional gels. Its properties have been discussed in detail elsewhere (Masters *et al*., 1982). Ovine oTP-1, however, continued to predominate as a major secreted product until day 23 when it could no longer be detected (Godkin *et al.*, 1982b). Explants of chorion from day 30 of pregnancy failed to secrete oTP-1. It was shown that oTP-1 was a product of the trophoblast and not of the yolk-sac by dissecting out the two tissues from day 16 conceptuses and culturing them separately.

The ovine oTP-1 is produced in significant amounts by conceptuses in the middle of the day 13–21 period. For example, day 14 to 16 conceptuses release 50 to 100 μg of oTP-1 in 24 h, although very little of the protein is detectable in the tissues themselves. Because it was a major protein product of the conceptus it was possible to purify oTP-1 from culture medium by a two step procedure involving successive DEAE-ion exchange chromatography and gel filtration on a column of Sephacryl S-200 (Godkin *et al*., 1982b). Because oTP-1 and some of the other proteins appear to be produced transiently during the critical day 13 to 21 period, it has been suggested that they may play a role in maternal recognition of pregnancy in the sheep (Godkin et *al*., 1984a). If so it seemed likely that they would either enter the maternal system and act on the ovaries directly, or alternatively, assert their action *via* the endometrium. The latter alternative was considered more likely since conceptus homogenates only prolong the cycle if they are introduced into the uterine lumen.

To test whether secreted conceptus protein could extend CL function Godkin *et al.* (1984b) infused proteins released by day 15-16 conceptuses into the uterine lumen of cyclic ewes. Beginning on day 12 either a concentrated solution of total secreted proteins or diluted sheep serum was introduced daily *via* an indwelling catheter into the uterine lumen of 3 ewes for 7 days (days 12–18). Peripheral blood samples were collected daily for 14 days (days 12–25). On day 25, all ewes were laparotomized and ovaries observed to determine whether CL, previously marked with India ink, were maintained. All controls had ovulated and formed new CL. By contrast, none of the ewes treated with conceptus proteins had ovulated, and their peripheral progesterone levels remained elevated. One ewe maintained a functional CL until day 52, when she was hysterectomized. Light microscopy of histological sections prepared from the endometrium revealed glandular development comparable to endometrium of day 60 pregnant animals. The cells of the CL were similar to those from cyclic animals during mid to late diestrus.

oTP-1 was also introduced into the uterine lumen of 3 animals. In these, plasma progesterone concentrations were maintained for an average of 4 days longer than in control animals (Godkin *et al*., 1984b).

As a result of the above experiments we suggest that secreted proteins of the conceptus are involved in the maintenance of luteal function during early pregnancy. It is not clear why oTP-1 was not as effective as total secreted conceptus protein in prolonging the cycle. Possibly it was introduced at too low a concentration to provoke a

complete and effective response from the maternal system. Moreover, oTP-1 may have been partly degraded during purification. Another possibility is that because we applied only small amounts of pure oTP-1 it was not protected adequately from proteolytic destruction within the uterus. The infusion of greater quantities of oTP-1 or the addition of protein carrier might reduce the amount of oTP-1 degradation and allow greater quantity of active peptide to be available for interaction with maternal tissues. Finally, it is conceivable that oTP-1 constitutes just one component of an antiluteolytic complex, and that other conceptus proteins make up the remainder of this complex.

Some initial experiments have also been conducted to determine how oTP-1 interacts with the maternal system. Using a specific antiserum raised against oTP-1, Godkin *et al.* (1984a) demonstrated that the protein was associated with trophectoderm cells of the elongating blastocyst and with the surface and upper glandular epithelium of the maternal uterus. Receptors which bound oTP-1 with high affinity (k_d = 2 $\times$ 10^{-10} M) were present in crude membrane fractions prepared from homogenates of endometrium. Uterine infusion of [^{125}I]-labelled oTP-1 into day 12 nonpregnant ewes showed that the majority of the radioactivity was retained in the uterus and did not enter the maternal vasculature. There was no significant association with the CL, ovaries or other tissues tested. oTP-1 failed to compete with ovine prolactin for rabbit mammary receptors or with human chorionic gonadotrophin or bovine luteinizing hormone for sheep luteal cell receptors; nor did oTP-1 stimulate progesterone production by dispersed luteal cells from day 12 cycling ewes. Incubation of endometrial explants from day 12 nonpregnant ewes with 5 μg/ml oTP-1 resulted in increased rates of release of newly synthesized protein into the medium. Two-dimensional Polyacrylamide gel electrophoresis revealed that the synthesis of at least 6 polypeptides was stimulated selectively by the presence of oTP-1.

The above data suggest that oTP-1 acts on the maternal endometrium and not directly on the CL or other extrauterine tissues. It is suggested that these biochemical interactions with the endometrium may elicit maternal responses which contribute to the maintenance of pregnancy in the sheep.

Cow: Maternal recognition of pregnancy in the cow is believed to occur around day 16. Intrauterine injections of embryonic homogenates (17 to 18 days of age) during days 15 to 17 of the estrous cycle extended the interestrous interval to 24 days from 21·1 days for controls and delayed the decline in progesterone associated with CL regression (Northey and French, 1980). Bartol *et al.* (1982) have shown that cow conceptuses isolated during this critical period of pregnancy secrete two major polypeptide products. As with the sheep and pig, one was a high molecular weight glycol-protein (Masters *et al.*, 1983). The other was of much lower molecular weight (~ 20,000–25,000) and fairly acidic in nature. Like the similar sheep protein it consisted of several isoelectric species. Unlike oTP-1, however, this protein has not yet been purified. Nevertheless the introduction of total secreted proteins from cow conceptuses into day 16 nonpregnant cows led to an average nine day extension of their estrous cycles (Knickerbocker *et al.*, 1984). It seems possible, therefore, that the mechanisms for maternal recognition of pregnancy in the cow and the sheep may be similar, and that the chemical signal is a protein secreted by the early elongating blastocyst.

Concluding remarks

It has become clear that although the elongating blastocysts of the pig, sheep and cow depend upon an appropriate uterine milieu for their growth and development, they are not passing occupants but are involved in controlling this intrauterine environment by generating chemical signals which cause appropriate responses in the maternal system. These signals may include the production of steroid hormones and the release of secreted proteins which presumably also have a hormone-like function. Available evidence suggests that this type of endocrine signaling from the conceptus begins at about the time the blastocyst begins to elongate from a sphere to a long thread-like form.

Acknowledgements

We thank Drs. J. Godkin, F. Bartol and J. Knickerbocker whose unpublished research contributed to this review. Research was supported by NIH grants HD 08560 and HD 10436.

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