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The functions of uterine secretions

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Summary. The likely functions of uterine secretions, often termed histotroph, in the nurture of the early conceptus are reviewed. Particular emphasis has been placed on the pig in which the uterus synthesizes and secretes large amounts of protein in response to progesterone. In this species, which possesses a non-invasive, diffuse type of epitheliochorial placentation, the secretions provide a sustained embryotrophic environment which is distinct from that of serum. A group of basic proteins dominates these uterine secretions after Day 11 of pregnancy and its best characterized member is uteroferrin, an iron-containing acid phosphatase with a deep purple colour. Evidence has accumulated to suggest that uteroferrin, rather than functioning as an acid phosphatase, is involved in transporting iron to the conceptus. Three basic polypeptides which are found non-covalently associated with uteroferrin have been shown to be antigenically closely related to one another and to have arisen by post-translational processing from a common precursor molecule. Their function is unknown. A group of basic protease inhibitors has been identified which bear considerable sequence homology to bovine pancreatic trypsin inhibitor (aprotinin) and may control intrauterine proteolytic events initiated by the conceptuses. The last basic protein so far characterized is lysozyme which is presumed to have an antibacterial role. Finally, two low molecular weight (M_r $\sim 18\,000$) acidic polypeptides have been purified and have sequence homology to a plasma retinol binding protein. Like uteroferrin, these proteins may be responsible for transport of an essential nutrient to the conceptus.

Keywords: uterus, secretions, pregnancy, progesterone, uteroferrin, retinol-binding proteins, lysozyme, iron, vitamin A, protease inhibitor

In this paper we intend to review briefly what is known about the function of uterine secretions during pregnancy. Our emphasis will be on the pig (*Sus scrofa domestica*) since this is the animal mainly used in our laboratories, and probably more is known about the function of the uterine secretions in the pig than in any other species.

The importance of the uterine milieu for proper conceptus development

Although there have been many hundreds of papers documenting the fact that in a variety of species the uterine endometrium is active in secretion, that the proteins in these secretions may be different from those in plasma and that the temporal patterns of protein synthesis are probably correlated with the circulating steroid hormone concentrations of the mother, few uterine secretory proteins have been purified or characterized. There has been considerable speculation as to what roles these proteins might be playing in processes such as attachment, implantation, immunoprotection and nutrition, but few definitive results have emerged (Bazer *et al.*, 1981). A similar ignorance exists about the concentrations of major ions, the amounts of micronutrients and the

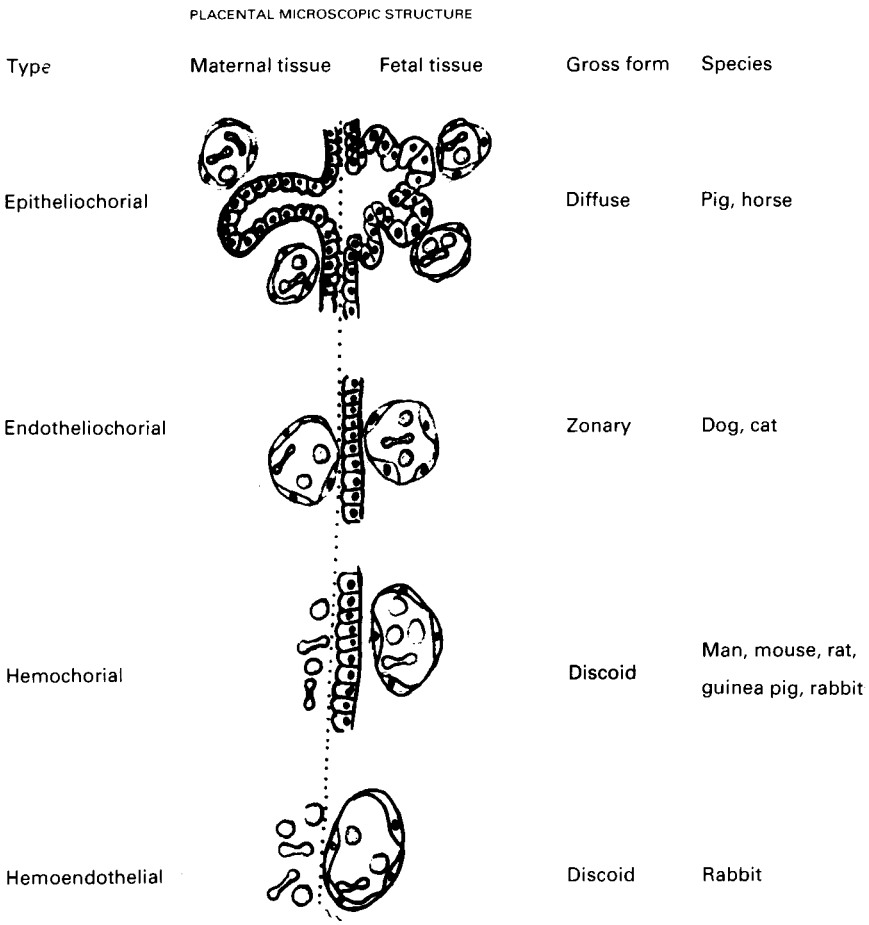

Fig. 1. Classification of mammalian placental structures based on number of tissue layers separating maternal and fetal blood. In the diffuse epitheliochorial type of the pig, a gland and overlying areola are shown. This diagram has been adapted from Amoroso (1951) and Hafez & Jainudeen (1974).

levels of dissolved gases in uterine fluids bathing the conceptus during early pregnancy. It seems likely that these low molecular weight components are also present at concentrations that are different from those in plasma and that their relative compositions also fluctuate in response to maternal hormones. Nevertheless, the fact that ectopic pregnancies can occur in the human and the observation that embryos from many species, once they have passed an early developmental block, can thrive successfully in culture (Brinster, 1973; Wright & Bondioli, 1981; Fishel *et al.*, 1984) and develop beyond the blastocyst stage in media often only slightly modified from ones that were developed primarily for fibroblast growth suggests that uterine secretions might not constitute a unique embryotrophic milieu. By this argument, the uterus might provide little more than a permissive environment within a walled enclosure in which the potentially aggressive fetal allograft can be confined, nurtured and protected.

On the other hand, it is clear that the female genital tract, including the uterus itself, exhibits considerable control over the ability of a conceptus to develop. Embryo transfer experiments in a variety of species have shown that close synchrony must be maintained between the stage of maturity

of the embryo and the stage in the sexual cycle of the recipient uterus (Moore & Shelton, 1964; Rowson & Moore, 1966; Polge, 1982; Pope & First, 1985; Wilmut *et al.*, 1985). Moreover, many species exhibit delayed implantation in which the blastocyst is held for a period of diapause or arrested development within the uterus for several months (see Aitken, 1977; Meade, 1981). The basis of subsequent blastocyst activation is unknown, but is presumed to be triggered by maternal hormones acting on the uterus. Implantation can also be deferred in lactating rodents, but initiated by an injection of oestrogen (McLaren, 1973; Psychoyos, 1973).

For the above reasons it seems likely that the uterine environment of early pregnancy is only narrowly permissive to the early preimplantation conceptus. Even before the pregnancy has been recognized and the normal pattern of ovarian cyclicity interrupted (Heap *et al.*, 1979) the conceptus is advancing rapidly in its development. It must be assumed that the uterine milieu is simultaneously undergoing rapid change as the endometrium responds to the maternal steroids and to the local presence of a conceptus (for brief reviews see Nieder *et al.*, 1987; Morgan *et al.*, 1987). Conceptuses developing slowly or otherwise out of phase with the mother may not be capable of coping with such change and will be lost. The uterine environment, therefore, is likely to place strong selective pressures on conceptuses so that only those developing synchronously with the endometrium will survive. This requirement for synchrony may be the means whereby genetically abnormal embryos which cannot keep pace with events within the uterus are discarded. Certainly this early period of pregnancy is usually associated with high rates of embryonic loss (Polge, 1982).

Once maternal recognition of pregnancy has occurred, the uterus comes under the long term influence of progesterone. For those species in which the conceptuses implant into the uterine wall, the trophoblast has generally by this time come into contact with the maternal blood supply, so that any dependence upon uterine secretions for nutritional support will probably be short-lived (Fig. 1). Rather than being crucial for promoting uterine histotroph production (see next section) the period of long term progesterone dominance may be most important in providing a period of constancy in which the cyclic remodeling of the endometrium is temporarily suspended.

However, in those animal species in which the trophoblast is noninvasive and where there is extensive development of the embryo and its associated membranes before placentation occurs, uterine secretions seem much more likely to play an important role in the maintenance of the conceptus (Amoroso, 1951). Thus, the pig with its diffuse type of epitheliochorial placentation (Fig. 1) is an excellent model for studying the role of uterine secretions supporting the conceptus for an extended portion of pregnancy. However, it would be ingenuous to pretend that the pig model has broad application to all species.

Placentation in the pig and the likely importance of histotroph

The pig conceptuses *in utero* undergo marked morphological changes before their initial attachment to the uterine epithelium (Fig. 2). There is a rapid transition from spherical (3–10 mm in diameter) to tubular (10–50 mm long) and from tubular to elongated filamentous forms between Days 10 and 12 of pregnancy (Perry & Rowlands, 1962; Anderson, 1978; Geisert *et al.*, 1982b), and these blastocysts can eventually attain lengths from 600 to 1000 mm by Day 14 of pregnancy. It should be stressed that blastocysts in different pigs do not all develop at the same rate and that a range of forms, from spherical to filamentous, can often be found within a single uterus at Day 12 or thereabouts. The filamentous blastocysts are placed at regular intervals, end to end, within each uterine horn and do not overlap. Since embryonic losses are high during this period, it is suspected that it is the less developed blastocysts that cannot establish themselves in this pattern and are lost (Anderson, 1978). The high rate of embryonic loss encountered in the European strains of pig (Polge, 1982) may be due more to a high degree of blastocyst asynchrony than to uterine overcrowding *per se*.

Fig. 2. Pig blastocysts at representative stages of development flushed from gilts between Days 10 and 13 of pregnancy. Smallest spherical blastocysts (top) average 3 to 5 mm. Before elongation (row 2) diameters of blastocysts are 8–10 mm. Elongated forms (rows 3 & 4) are about 20 mm in length. The latter rapidly become thread-like (bottom) over the subsequent 6 h or so *in utero* (see Geisert *et al.*, 1982b); Anderson, 1978). However, spherical, elongated and filamentous forms can often be collected from the same uterus at Day 12.

It is during the early elongation phase that pig blastocysts first begin to synthesize appreciable amounts of oestrogens (Perry *et al.*, 1973; Flint *et al.*, 1979; Gadsby *et al.*, 1980; Geisert *et al.*, 1982a). Synthesis peaks at about the time the blastocysts become filamentous and subsequently declines by Day 14. This blastocyst oestrogen is regarded as the diffusible substance responsible for maternal recognition of pregnancy in pigs, since exogenous oestrogen injected daily into unmated pigs between Days 11 and 15 of pregnancy induces a state of pseudopregnancy in which the corpora lutea are maintained for over 100 days (Frank *et al.*, 1977; Bazer *et al.*, 1982). The onset of blastocyst oestrogen synthesis is also accompanied by a synchronized release of secretory material from uterine glandular epithelial cells into the lumen of the gland (Geisert *et al.*, 1982a; Fazleabas *et al.*, 1983). This dumping of secretions, which can also be mimicked by exogenous oestrogen administration (Geisert *et al.*, 1982c), results in a marked change in both the amount and composition of the uterine fluids (Geisert *et al.*, 1982a). In particular, a group of proteins can be detected whose synthesis is regarded as being under the control of progesterone (see next section). Before Day 11, the amount of protein in pig uterine flushes is low (Murray *et al.*, 1972; Geisert *et al.*, 1982a), and the polypeptides themselves seem to be mainly of serum origin. It has been assumed that this sudden change in the uterine milieu is important in the nurturing of the blastocysts at this critical stage of pregnancy.

Although the blastocysts undergo little increase in volume or cell number during the very early rapid phase of elongation (Geisert *et al.*, 1982b), their area exposed to the uterine milieu must increase significantly. Extensive regions of the inner apical surface of the trophectoderm are coated with clathrin, and it is clear that these cells from the spherical blastocyst stage onwards are active in endocytosis (Geisert *et al.*, 1982b; Stroband *et al.*, 1984).

At the time that the secretions are first released into the endometrial glands, the pig blastocysts are not firmly attached to the uterine epithelium. Loose attachment begins around Day 13 (Keys *et al.*, 1986) and is more or less complete by Day 18, when interlocking microvilli on the opposing

surfaces of the trophectoderm and uterine epithelium appear to bind the conceptus in place (see Fig. 7). During the Day 12 to Day 18 period the amount of protein per conceptus increases from around 0·4 mg to 17·5 mg (Anderson, 1978), and the yolk sac begins to produce transferrin, α-fetoprotein and other plasma protein products (Godkin *et al.*, 1985). This phase of rapid growth and development is presumably dependent upon the ability of the trophoblast to acquire nutrients, including trace elements and vitamins, from the uterine fluid in its immediate surrounding.

After Day 20, the allantois, which develops as an extension from the hind gut region of the embryo proper, extends almost the full length of the trophoblast with which it ultimately fuses to form the chorioallantois 'membrane' (Patten, 1948). The allantoic cavity then begins to fill with fluid and inflate like a bladder in a football. The entire conceptus becomes tightly locked within the zone of the uterine horn that was initially occupied by the elongated blastocyst. At no time during pregnancy is the uterine epithelium of the mother eroded. The two blood supplies always remain separated, and, if maternal blood were to supply any of the necessary molecules required by the developing conceptus, these compounds would have to traverse a complex barrier of several cell layers before they could reach the absorptive surface of the chorion. Species which possess such a diffuse, epitheliochorial type of placentation include the pig, horse, camel, elephant, whales and their relatives (Amoroso, 1951), and it seems probable that in each of these species there is an extensive reliance on histotroph, a locally produced uterine secretory product, throughout pregnancy.

In cattle and sheep, the initial stages of placentation are not unlike those of the pig. The spherical blastocyst expands to a filamentous form and initial attachment occurs late. However, in both these species placentomes, consisting of fused fetal cotyledons and maternal caruncles, form and ensure that the fetal and maternal circulations come into close contact (Boshier, 1969; Bjorkman, 1969; King *et al.*, 1980, 1982). There is limited erosion of the uterine epithelium in these regions, and fetal binucleate cells are able to migrate across the breached epithelium to lodge within the maternal tissue (Wooding *et al.*, 1981). Nevertheless, even in these species, in which implantation occurs relatively late, it must be assumed that uterine secretions are utilized by the conceptus for at least the early part of pregnancy.

The importance of uterine secretions as a continued source of nutrients for pig conceptuses was emphasized by Brambel (1933). He postulated that specialized chorionic structures, known as areolae (see Fig. 3), which first develop at about Day 30 of pregnancy in direct apposition to the mouths of uterine glands, were responsible for the absorption of uterine secretions before the transfer of this material to the fetus. Wislocki & Dempsey (1946) supported this conclusion. They also demonstrated the presence of appreciable concentrations of iron and calcium in the cells of the uterine glands, in the secretions within the glands and in the cells of the areolae; they concluded that these sites represented a path of nutrient transport. Finally Wislocki & Dempsey (1946) showed that acid phosphatase activity was associated with the glands, the secretions and the areolae. Thus, a correlation was established between the distribution of iron and the presence of a phosphohydrolase. In a later section of this paper, the function of one major component of porcine uterine secretions, an acid phosphatase that contains bound iron, will be briefly reviewed. The protein, now known generally as uteroferrin, has been postulated to be involved in transplacental iron transport in pigs (see Roberts & Bazer, 1984; Roberts *et al.*, 1986). Such a view has received considerable criticism since there has been reluctance to accept the concept that a protein which has evolved so efficient a phosphohydrolase activity could also fulfil another function, namely that of a translocator of maternal iron to the fetal unit (Davis & Averill, 1982).

Some of the best evidence implicating the areolae in the transport of nutrients to the fetal unit has come from the experiments of Palludan *et al.* (1970) who introduced isotopically labelled iron into the bloodstream of pregnant gilts to label the circulating iron pools. They noted that the iron transported across the placenta remained in non-dialysable and presumably protein-bound form. They were, therefore, able to examine the distribution of iron in the pregnant uterus by auto-radiography of tissue sections. A very high density of silver grains was observed over the uterine glandular epithelium and its associated secretions and over the areolae (Fig. 4a and 4b). Areas of

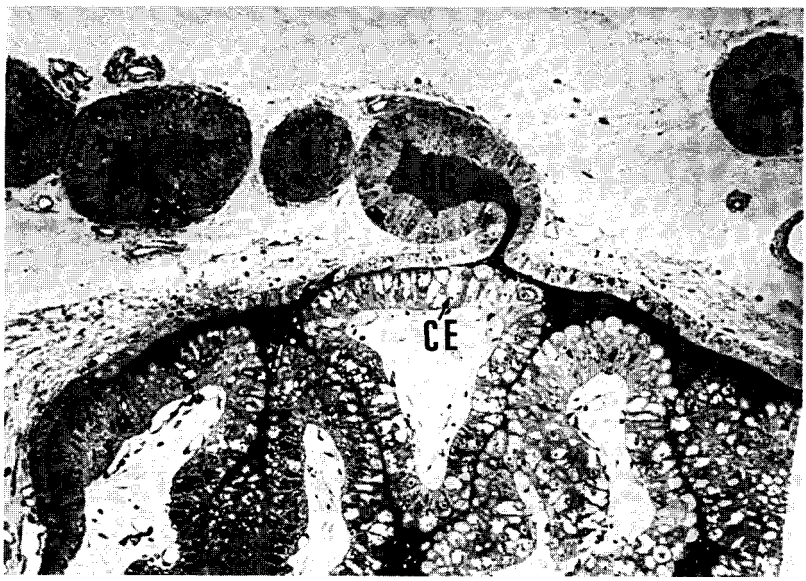

Fig. 3. A histological view across a chorionic areola which has formed opposite the mouth of a uterine gland (UG). This semi-thick section was obtained from a Day 110 placenta. Tissue processing is described by Friess *et al.* (1980). The section was cut across several of the infolding villous structures within the dome-like areolae, and across the upper part of a uterine gland. Note that the chorionic epithelial cells (CE) within the areola are filled with large endocytotic vacuoles. On appropriately fixed tissue uteroferrin has been shown to be present in the secretory vesicles of the glandular epithelium, within the gland lumen, in the space between the mouths of the glands and areolae and in the vacuoles of the chorionic cells (Raub *et al.*, 1985). ×750. Provided by Professor A. Friess, Universitat Bern.

the chorion, other than the areolae, were not heavily labelled. Palludan *et al.* (1970) concluded from their studies that iron is transferred from mother to the conceptus "via the embryotrophic" route, that is, bound to proteins released by the uterine glands. The secretory products are then taken up by the areolae (see Dantzer *et al.*, 1981). Such a concept will be explored in further depth in this review.

Progesterone-induced uterine secretions of the pig

The amount of protein that can be flushed from the uterus of a pig changes as the oestrous cycle proceeds (Murray *et al.*, 1972). Between Days 2 and 8, less than 8 mg of protein is present per uterine horn. However, amounts then begin to increase as progesterone comes to dominate the endometrium. At Day 15, which marks the end of the luteal phase of the cycle, up to 50 mg can be recovered per horn, and the flushings are coloured purple due to the presence of uteroferrin. The endocrine status of the pregnant female pig is not obviously distinguishable from that of the cyclic animal until Day 15 (Bazer *et al.*, 1982). About that time progesterone concentrations drop by about two-thirds in the pregnant pig but do not fall to the basal values noted in the follicular phase. The amount of protein in uterine flushings also does not differ significantly between pregnant and non-pregnant animals until about Day 11 (Geisert *et al.*, 1982a). At that time, the conceptuses appear to induce the oestrogen-triggered exocytosis of the contents of secretory vesicles from glandular epithelial cells which was referenced earlier. Release of secretions into the non-pregnant uterus occurs more gradually but the total output of protein over the next 4 days is about the same

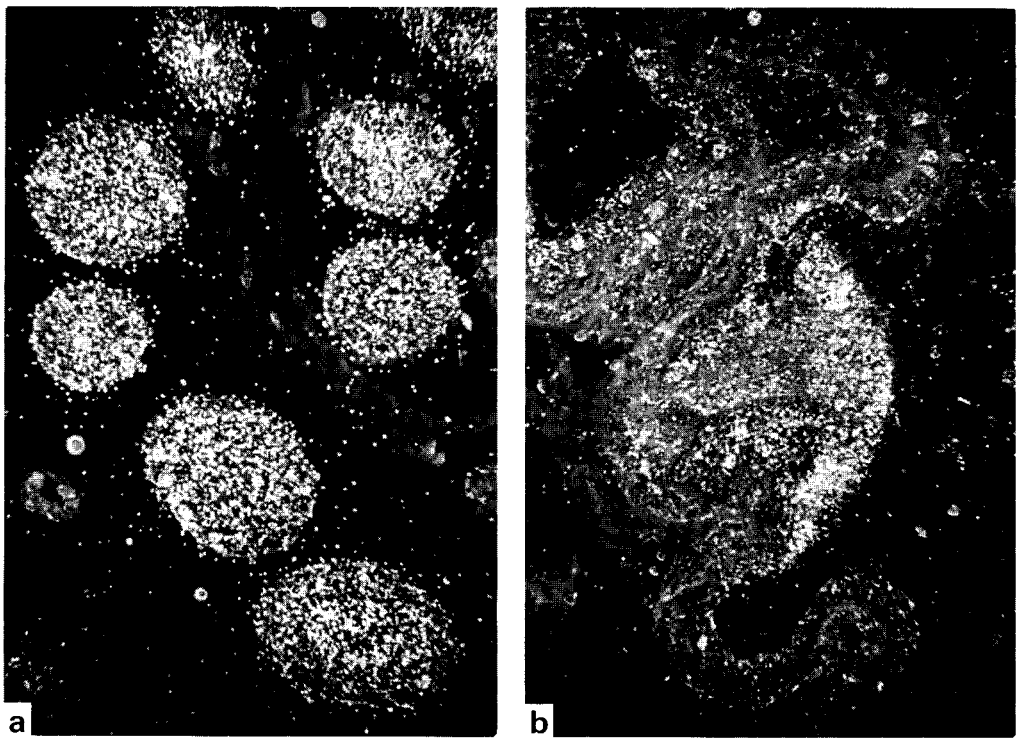

Fig. 4. Histological sections of uterine endometrium 8 h after injection of 4 mCi $^{55}FeCl_3$ into a gilt at Day 95 of pregnancy. In (a) the autoradiograph was photographed under dark ground illumination so that silver grains, indicating the site of iron, appear white. The epithelial cells and luminal contents of the uterine glands are markedly labelled. In (b) the chorionic epithelial cells and the "uterine milk" within the interareolar space are labelled with ^{55}Fe. ×270. Both micrographs are reproduced from Palludan *et al.* (1970) with permission.

as in pregnancy (Geisert *et al.*, 1982a). However, whereas secretory activity of the pregnant endometrium is maintained beyond Day 15, output of protein from the endometrium of non-pregnant gilts falls to low levels as the end of the cycle approaches and as progesterone production by the corpora lutea ceases (Zavy *et al.*, 1984).

Secretory activity of the endometrium increases markedly after Day 30 and appears to reach a maximum between Days 60 and 75 before declining as term approaches (Day 115) (Basha *et al.*, 1979). These changes in uterine synthetic activity are correlated with the endocrine status of the dam. Protein synthesis by the endometrium is high when the ratio of progesterone to oestrogen is at a maximum, but is markedly reduced when oestrogen concentrations increase.

In experiments with ovariectomized gilts (see Chen *et al.*, 1973, 1975; Knight *et al.*, 1974; Roberts & Bazer, 1980; Roberts *et al.*, 1987), it has been found that progesterone causes a significant increase in the amount of protein that can be flushed from the uterus. By contrast, oestradiol alone has no effect relative to controls. Oestradiol, however, acts synergistically with progesterone to promote protein production when it is administered at low concentrations but is inhibitory as the dose is increased (Knight *et al.*, 1974). Prolonged administration of progesterone or of progesterone plus oestrogen over several weeks allows gram quantities of protein to be recovered from a single animal (Schlosnagle *et al.*, 1974; Roberts *et al.*, 1987).

As described earlier, a status referred to as pseudopregnancy can be induced in gilts by administering 5 mg oestradiol valerate/day on Days 11 through 15 of the oestrous cycle (Frank *et al.*, 1977).

These pseudopregnant gilts when examined at Day 45 or thereafter can provide several grams of secretory protein, and the flushings are of a deep purple colour (Basha *et al.*, 1980a).

One other method that can be used to provide uterine secretions in quantity is to confine pregnancy to one uterine horn (Basha *et al.*, 1980a). Collection is then made from the non-gravid horn. Again gram amounts of protein can be recovered.

It has been demonstrated that the major proteins present in uterine secretions which have been obtained by each of the above methods are the same. Moreover, cultured endometrial explants derived from each of the sets of animals, i.e. from progesterone-treated, pseudopregnant and the gravid and non-gravid horns of unilaterally pregnant gilts, each release an identical array of proteins into the medium (Basha *et al.*, 1980b). No effect of the overlying conceptus on the quality of the proteins produced has been noted at Day 60 of pregnancy. Finally, the major proteins present in the secretions in pseudopregnancy are the same as those noted at about Day 12 to 15 of pregnancy and the oestrous cycle. It would appear that the major proteins secreted by the pig endometrium during pregnancy are progesterone-responsive and do not change markedly in quality as pregnancy proceeds. The amounts of protein synthesized during pregnancy, however, change markedly (Basha *et al.*, 1979). In the case of uteroferrin, for example, synthesis at Day 60, when the quantity of uteroferrin produced probably exceeds 1 g/day, has been calculated to be 55-fold higher than at Day 30 and 10-fold higher than at Day 105, about 10 days before term.

The proteins present in uterine flushings from these various groups of animals have been analysed by two dimensional electrophoresis (Fig. 5) (Basha *et al.*, 1980a, b). Since several of the progesterone-induced components are basic, it has been necessary to use both standard isoelectric focussing (for more acidic components) and non-equilibrium pH gradient electrophoresis (NEPHGE) towards the cathode as methods for first dimensional separation. Analysis in the second dimension has been by standard SDS-polyacrylamide gel electrophoresis which separates the polypeptides by size rather than charge. Figures 6(a) and 6(b) illustrate diagrammatically such a separation of the proteins present in the uterine flushings of the progesterone-dominated pig uterus. The main progesterone-induced components have been arrowed and numbered. Each of these components has been at least partly purified and characterized. Their properties are discussed in later sections of this review. The same polypeptides recognized in the flushings are released into the incubation medium when endometrial explants from pseudopregnant or unilaterally pregnant gilts are cultured *in vitro* (Figs 7a,b).

Uteroferrin

Uteroferrin is the best studied component of pig uterine secretions and corresponds to polypeptide 4 in Fig. 6(b) (Roberts & Bazer, 1984; Roberts *et al.*, 1986). It has a deep purple colour which accounts for the purple hue of pig uterine secretions. It is the most abundant of the protein components present, accounting for as much as 15% of the total. It is easily purified by a two-step procedure, has a molecular weight of 35 000 and carries two bound atoms of iron per polypeptide chain. The purple colour of uteroferrin arises from the co-ordination of one of these irons with one or more tyrosine residues (Gaber *et al.*, 1979). The nature of the iron-centre of uteroferrin has been the subject of intense study and continuing controversy (Antanaitis & Aisen, 1984; Roberts *et al.*, 1986). What is clear is that the two iron atoms are in close proximity to one another, that the binding sites are dissimilar and that one of these irons is more easily reduced than the other so that a ferrous–ferric ion pair can result from treatment of the protein with mild reducing agents such as ascorbate or 2-mercaptoethanol. This reduced form of uteroferrin, which is pink with an absorption maximum at 505 nm, is a potent acid phosphatase (Schlosnagle *et al.*, 1976).

Uteroferrin and other iron-containing phosphatases have been the subject of two recent reviews (Antanaitis & Aisen, 1983; Roberts & Bazer, 1984). The enzymes have a number of characteristic features which distinguish them from other acid phosphatases. These include molecular weights

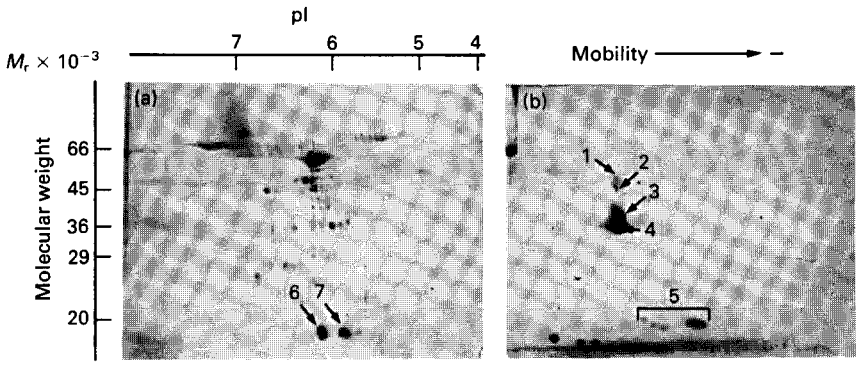

Fig. 5. Two-dimensional electrophoresis of the uterine flushings from a pseudopregnant gilt. Gels were stained by Coomassie blue. (a) Separations by standard two-dimensional polyacrylamide gel electrophoresis. The arrows show the two progesterone-responsive polypeptides with isoelectric points of 6·3 and 6·1 (from left to right) which have molecular weights of around 18 000. (b) Analysis by non-equilibrium pH gradient electrophoresis. The numbers show the position of the various progesterone-responsive polypeptides in the secretions. Proteins 1, 2 and 3 are the uteroferrin-associated polypeptides; 4 is uteroferrin; 5, the plasmin inhibitors. Lysozyme is also probably associated with component 5 in this separation.

within the 30 000–40 000 range, basic pI, purple colouration, a poor ability to hydrolyse aliphatic phosphate esters, phosphoprotein phosphatase activity and insensitivity to tartrate inhibition (see Ketcham *et al.*, 1985). Uteroferrin, though secreted, also carries the so-called lysosomal recognition marker, mannose 6-phosphate, when it is first synthesized (Baumbach *et al.*, 1984). It has been postulated that the uteroferrin class of acid phosphatase, which appears to be extremely widespread, is normally confined to lysosomes (Roberts & Bazer, 1984). However, in the pig uterus that is under the influence of progesterone uteroferrin is redirected along a secretory pathway.

Uteroferrin-like acid phosphatases have also been purified from uterine secretions of the horse (McDowell *et al.*, 1982) and cow (C. A. Ketcham, W. Clark, F. W. Bazer & R. M. Roberts, unpublishd results). Both are purple and cross-react with antibodies to pig uteroferrin (unpublished results), but are considerably less stable than pig uteroferrin so that their iron and purple colour are easily lost during purification and storage.

The name uteroferrin was originally coined because of evidence that the protein is involved in iron metabolism within the pregnant uterus (Roberts & Bazer, 1980). Uteroferrin is synthesized and secreted by epithelial cells of the uterine glands for subsequent transport to the fetal placental unit. It is taken up by the specialized absorptive cells of the placental areolae (Chen *et al.*, 1975; Renegar *et al.*, 1982; Raub *et al.*, 1985) and ultimately enters the fetal circulation (Renegar *et al.*, 1982) whence the protein can be distributed in sites of iron metabolism such as the liver and spleen. Receptors recognizing the 'high mannose' carbohydrate chains of uteroferrin are present on the reticuloendothelial cells lining the sinusoids of the fetal liver and seem to be responsible for uteroferrin clearance from the hepatic circulation (Saunders *et al.*, 1985). Excess uteroferrin is cleared by the fetal kidney and enters the allantoic sac (Renegar *et al.*, 1982) which seems to serve as a temporary site from iron storage and iron exchange (Buhi *et al.*, 1982, 1983). Based on available data it is likely that uteroferrin serves, not as an acid phosphatase, but as an intermediary in the transport of iron from the maternal uterine endometrium to the conceptus (Roberts *et al.*, 1986).

The observation from our laboratory (Buhi *et al.*, 1982) that uteroferrin can donate its iron to apotransferrin in the presence of low molecular weight chelating substances, a mechanism which Buhi *et al.* (1982) suggested to be important in fetal iron metabolism within the allantoic sac, has recently been challenged (Doi *et al.*, 1986). However, these experiments have been repeated by us and our original observation confirmed (unpublished results).

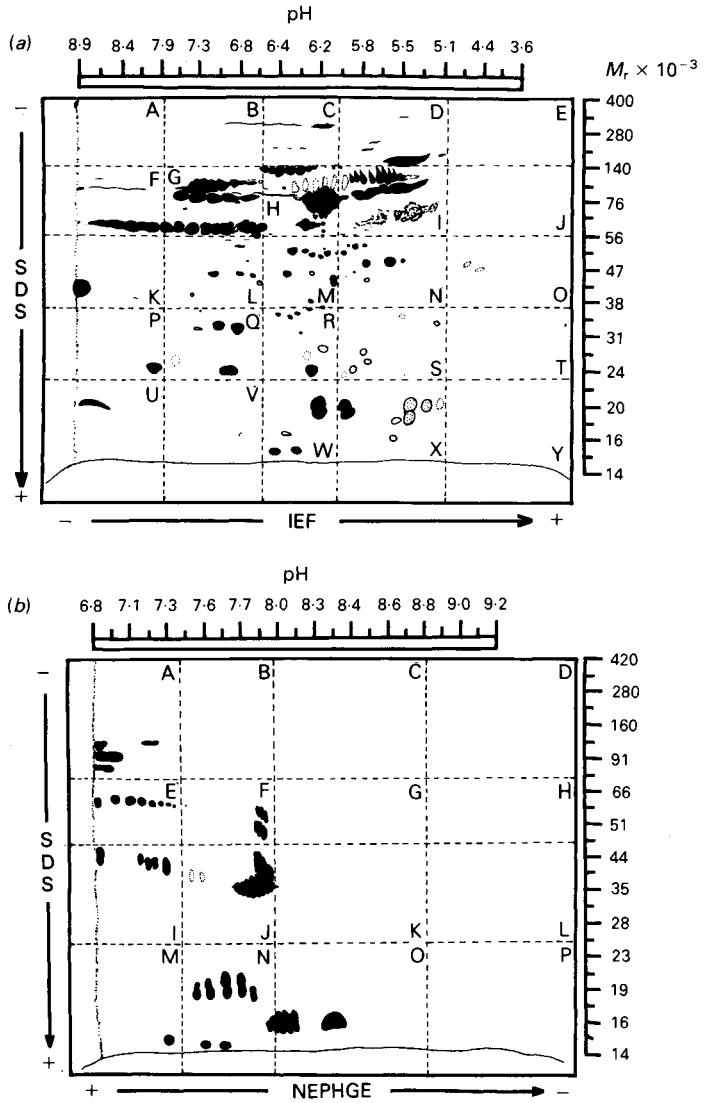

Fig. 6. Diagrams showing the positions on (a) two-dimensional electrophoretic gels of the more acidic group of polypeptides found in uterine flushings and (b) NEPHGE gels of the major basic polypeptides found in uterine flushings. In (a) the pH gradient in the IEF gel is drawn along the horizontal axis, and a molecular weight scale is shown for the second dimension on the right. Major polypeptides are shown in black. Analysis was as described by Basha *et al.* (1980a). In (b) the pH gradient existing at the time electrophoresis was terminated in the first dimension is shown by the horizontal scale. A molecular weight scale for the second dimension is shown on the right. Major polypeptides are indicated in the solid areas. Analysis was by the method of O'Farrell *et al.* (1977) as described by Basha *et al.* (1980a).

The primary amino acid sequence of uteroferrin has largely been elucidated (Hunt *et al.*, 1987), but has given no insight into the nature of the iron centre. Although there is a striking homology with the purple phosphoprotein phosphatase of beef spleen (see Cambell & Zerner, 1973; Debrunner *et al.*, 1983, for a description of this protein), with many regions showing complete conservation of structure, there is no resemblance to other known iron-binding proteins such as transferrin.

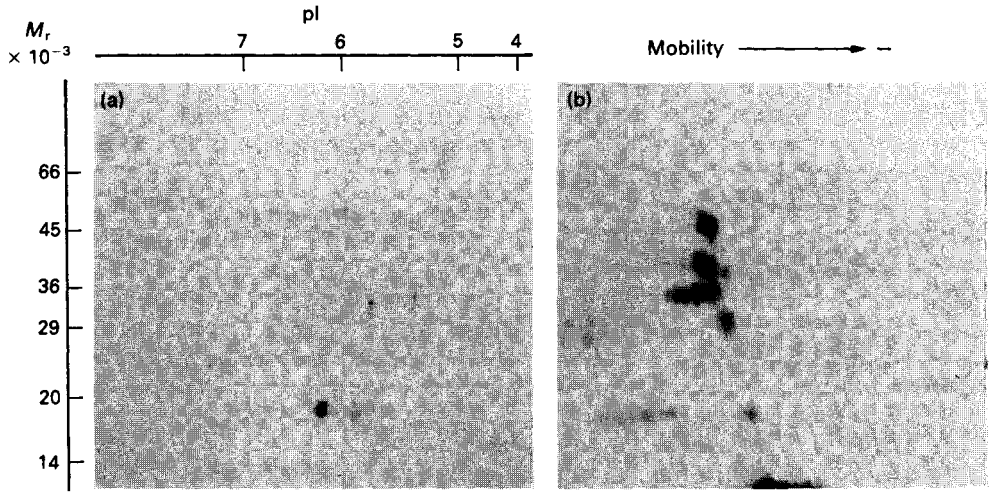

Fig. 7. Two-dimensional electrophoresis of the radioactive polypeptides secreted into the medium during culture of endometrial explants for 24 h in the presence of L-[^{35}S]methionine. The polypeptides were separated by two-dimensional polyacrylamide electrophoresis, the gels were dried and radioactive spots were detected by autoradiography (exposure time 2 weeks). Each gel was run with 75 000–150 000 d.p.m. of radioactive sample. (a) Proteins released by endometrium from a Day 60 pseudopregnant gilt and analysed by standard two-dimensional PAGE. (b) Proteins released by endometrium from a unilaterally pregnant gilt and analysed by non-equilibrium pH gradient electrophoresis (NEPHGE). Data from Basha (1980b).

A series of cDNA clones corresponding to uteroferrin mRNA have recently been produced from a lambda gt11 cDNA library prepared from pig endometrium at mid-pregnancy (Simmen *et al.*, 1987). The availability of such cDNA probes will allow the levels of uteroferrin mRNA to be assessed during the oestrous cycle, during pregnancy and during the course of hormone replacement therapy to ovariectomized gilts, and provide further insight into the controls operating over uteroferrin synthesis.

The uteroferrin-associated basic polypeptides

Two molecular weight classes of uteroferrin have long been recognized in crude uterine washings and in allantoic fluid (Chen *et al.*, 1973; Bazer *et al.*, 1975). The first is purple and elutes from gel filtration columns with an apparent molecular weight of 35 000; it corresponds to the form discussed in the previous section. Gel filtration has revealed a second type of uteroferrin of M_r ~80 000 (Bazer *et al.*, 1975) which is pink and possesses the full enzymic activity characteristics of mercaptoethanol-reduced uteroferrin (Baumbach *et al.*, 1986). The pink colour, unlike that of reduced low molecular weight uteroferrin, is also stable for several months in the presence of oxygen.

Baumbach *et al.* (1986) concluded that this high molecular weight form of uteroferrin is a heterodimer in which one molecule of uteroferrin is associated non-covalently with a second polypeptide. The latter seems to maintain the acid phosphatase 'subunit' in a stable, active conformation. The heterodimer is easily dissociated by pH conditions below 5, by SDS-electrophoresis and by treatment with antibodies raised either against uteroferrin or the associated polypeptide. At least three molecular weight forms of the uteroferrin-associated polypeptide (M_r = 40 000, 46 000 and 50 000) have been identified. Each of these polypeptides is recognized by a single monoclonal antibody, and they are, therefore, antigenically related. These three polypeptides correspond to 1, 2 and

3 in Fig. 5(b). Like uteroferrin, they are synthesized in response to progesterone administration, are abundant components of pig uterine secretions, and appear in allantoic fluid, an observation which suggests they too are transported across the placenta. It has been shown (see Roberts *et al.*, 1987) that all three of these polypeptides arise from a single translation product of M_r 46 000 and that they probably are formed by differential glycosylation (polypeptides 1 and 2) and a proteolytic cleavage event (polypeptide 3).

The function of the uteroferrin-associated basic proteins is unknown and the implication of their association with uteroferrin remains puzzling. Limited amino acid sequencing of their NH_2-termini has revealed no clear-cut similarity to other proteins of known sequence (P. V. Malathy, M. K. Murray & R. M. Roberts, unpublished results). Several cDNA clones for the proteins have been isolated after immunological screening of a cDNA library (unpublished results). These materials should lead to an elucidation of the entire primary amino acid sequences of the polypeptides and provide a better insight into their possible relationship to other proteins.

Plasmin inhibitors

A group of plasmin/trypsin inhibitors has been demonstrated in uterine secretions of pigs during the luteal phase of the oestrous cycle, and during pregnancy (Mullins *et al.*, 1980; Fazleabas *et al.*, 1983) and pseudopregnancy (Fazleabas *et al.*, 1982). These are shown as components 5 in Fig. 5(b). One inhibitor has been purified (Fazleabas *et al.*, 1982). It is a low molecular weight ($M_r \sim 14\,000$) basic polypeptide and appears to belong to a family of at least four immunologically related isoforms which differ slightly in pI. The B form, purified by us, is present in the highest amounts in uterine secretions and appears to be synthesized predominantly by the surface epithelium of the uterus and not by the deeper glandular epithelium where uteroferrin is formed (Fazleabas *et al.*, 1984). The inhibitor binds to trypsin and plasmin in a tight 1:1 complex but less tightly to chymotrypsin and seems to belong to the so-called Kunitz class of protease inhibitor (Laskowski & Kato, 1980). The inhibitor also binds urokinase, the urinary plasminogen activator (R. Lottenberg, A. T. Fazleabas & R. M. Roberts, unpublished results). Limited N-terminal amino acid sequencing of the dominant form of the uterine plasmin inhibitor has shown it to have striking homology with aprotinin, a basic protein of M_r 6512 and the active ingredient of the commercial product Trasylol, which can be extracted from a variety of cattle organs, including the lungs and pancreas (Fritz & Wunderer, 1983).

We have proposed that the uterine plasmin inhibitors of the pig serve to control proteolytic activity within the uterus (Fazleabas *et al.*, 1982, 1983). The elongating pig conceptus, during the Day 11–14 period, releases high amounts of plasminogen activator (PA) (Mullins *et al.*, 1980; Fazleabas *et al.*, 1983). Moreover, plasminogen is present in uterine secretions, presumably as a serum transudate, so that there is the potential for the generation of a damaging cascade of proteolytic activity in the early pregnant uterus (Fazleabas *et al.*, 1983). The pig conceptus is noninvasive within the uterus, but the trophoblast does show invasive characteristics when transplanted to ectopic sites where protease inhibitors may not be locally produced (Samuel, 1971; Samuel & Perry, 1972). Proteases, particularly PA, have been implicated in implantation (Strickland *et al.*, 1976; Denker, 1980) and outgrowth of embryos (Kubo *et al.*, 1981), in tissue remodelling and morphogenesis (Beers *et al.*, 1975; Ossowski *et al.*, 1979; Bode & Dziadek, 1979) and in the invasive spread of malignant (Ossowski *et al.*, 1973; Unkeless *et al.*, 1973; Pollack *et al.*, 1974) as well as normal cells (Unkeless *et al.*, 1974).

PA acts by generating plasmin from plasminogen. Plasmin has broad specificity and can hydrolyse components of basement membrane and connective tissue as well as fibrin (Werb *et al.*, 1980). Protease inhibitors are likely to function in controlling such events. Such inhibitors may protect the uterus from proteases released by the conceptus and help resist the potentially invasive pig trophoblast. The superficial type of implantation seen in the pig may be, in part, the result of

inhibitor production by the endometrium. Application of protease inhibitors, for example, prevents implantation in mice (Dabitch & Andary, 1974) and rabbits (Denker, 1980). Endogenous uterine inhibitors may also protect nutritionally important proteins from breakdown in the uterine lumen or during transplacental transport.

Lysozyme

Lysozymes are hydrolytic enzymes which cleave the β1,4-glycosidic linkages of bacterial peptidoglycan. They are typically found in exocrine secretions of animals where they function as a primary defence against bacterial infestation. Lysozyme activity appears in the uterine secretions of pigs in response to progesterone treatment (Roberts *et al.*, 1976) and is detectable in cervical secretions during the luteal phase of the human menstrual cycle (Schumacher, 1974). In both situations it is generally assumed that the role of the enzyme is antibacterial.

Although lysozyme appears to be synthesized and secreted in response to progesterone, it is unclear in which cells it is produced. Like uteroferrin, it may be a product of the glandular epithelium or, like the plasmin inhibitor, formed largely by non-glandular epithelial cells. Alternatively, uterine lysozyme may be a product of migratory cells of the immune system which may colonize the endometrium in response to progesterone.

Low molecular weight acidic proteins

These proteins correspond to spots 6 and 7 of Fig. 6(a). They have molecular weights of about 20 000 and isoelectric points of 6·3 and 6·1, respectively. Until recently they have received little attention, although like uteroferrin they are progesterone-responsive and are relatively abundant components of flushings from pseudopregnant pigs (Fig. 5a). These proteins have been purified (J. Clawitter & R. M. Roberts, unpublished results) by means of anion-exchange chromatography on a TSK-DEAE-5PW anion exchange column (LKB Instruments, Uppsala, Sweden) and subjected to limited N-terminal amino acid sequencing. Both polypeptides are dissimilar in sequence over their first 9 amino acids, but differ at only three positions over the subsequent 12. Polypeptide 6 also has a region (residues 5–20) which differs at only four positions from a stretch of sequence (residues 21–36) of a retinol-binding protein of human plasma (Colantuoni *et al.*, 1983). These results suggest that the low molecular weight acidic proteins 6 and 7 may play a role in vitamin A transport to the fetus. It is not yet clear whether they correspond to the retinol and retinoic acid binding proteins described by Adams *et al.* (1981).

Other progesterone-responsive proteins

A number of additional, but relatively minor, protein spots are present on two dimensional electrophoretic gels when uterine flushings of pigs are analysed that are not components of serum. In addition, several hydrolytic enzyme activities have been detected in flushings in addition to lysozyme and uteroferrin. Most of these appear to be present in small amounts, and their presence may have little significance. However, two glycosidases, β-hexosaminidase and β-galactosidase, are found in appreciable quantities in flushings derived from pseudopregnant gilts (Hansen *et al.*, 1985). These enzymes have acid pH optima and appear to be of the typical lysosomal type of acid hydrolase. Their function is unclear.

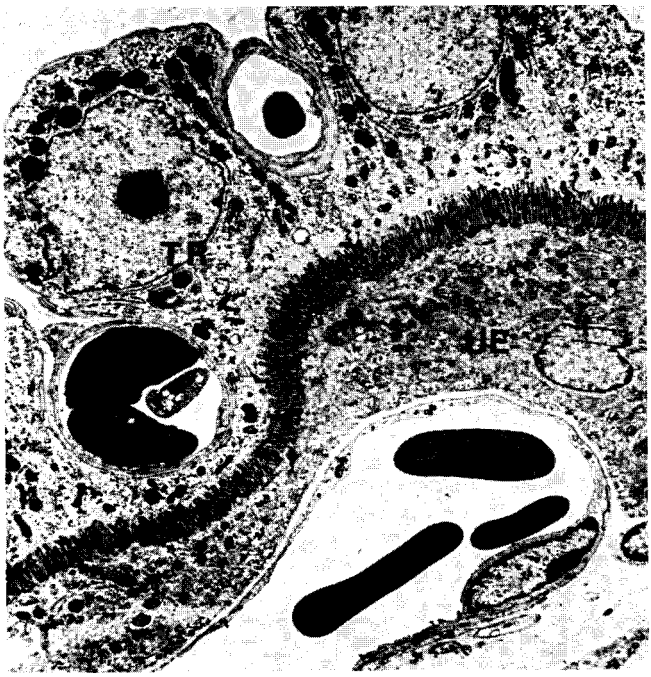

Fig. 8. A region of pig placenta at Day 58 of pregnancy. Fetal capillaries protrude deeply into the trophectoderm. The distance between the fetal and maternal capillaries and the microvillous contact zone (seen as a region of interlocking microvilli) is reduced to 1 μm or less. UE, uterine epithelium; TR, trophoblast. × 15 000. Photograph provided by courtesy of Professor A. Friess, Universität Bern.

Functions of uterine proteins in pigs: a summary

As described earlier in this review, secretions obtained from the progesterone dominated uterus of the pig contain a diverse group of proteins which are synthesized by the endometrium in response to progesterone. Some of these components, for example uteroferrin and the retinol-binding protein, appear to play a nutritional role. Indeed, uterine secretory material has historically been termed histotroph and assumed to nourish the developing embryo. Such a role is expected to be more important in those species in which implantation occurs late or in which placentation is of the superficial non-invasive type.

The secretions may also play a part in directing or limiting the growth and development of the conceptus. The plasmin inhibitor, for example, could prevent the trophoblast from invading the uterine wall. Simmen *et al.* (1986) have reported that uterine secretions of the pig contain factors which might promote proliferation of specific cell types. The uterus clearly provides an environment in which remarkable rates of growth are achieved, and uterine secretions may be a rich source of growth factors and other regulatory polypeptides.

Other functions of uterine proteins may include antibacterial, antiviral or immunoprotective activities. Lysozyme, a common component of exocrine secretions, clearly falls into the first of these categories. Uterine secretions of the cow, pig and ewe have also been shown to inhibit the incorporation of [^{3}H]thymidine into mitogen-stimulated lymphocytes and into mixed lymphocyte cultures, two common tests that supposedly indicate immunosuppression (Roberts, 1977; Murray *et al.*, 1978; Segerson, 1981; Hansen *et al.*, 1987).

Alternative routes of transplacental transfer of nutrient molecules

During the phase of conceptus development that occurs before firm attachment to the uterine epithelium is observed, gases and small nutrient molecules, such as sugars and amino acids, presumably reach the absorptive surface of the trophectoderm in solution by diffusion through the luminal fluids. As pregnancy proceeds, mechanisms must come into play which allow more efficient transfer of such molecules between the blood supplies of the uterus and placenta. At first sight the epitheliochorial placenta would appear to present a formidable barrier to such transfer. It seems unlikely that the uterine glands and areolae could be involved to any major extent, since in these regions the two blood supplies are well separated (Friess *et al.*, 1981; Fig. 8). However, in the areas away from the glandular openings, particularly along the crests and flanks of the villous folds (or ridges) of the endometrium, the capillaries on the maternal and fetal sides come within a few micrometres of each other (Friess *et al.*, 1980; Fig. 3). In a sense, both epithelia become vascularized because the capillaries, particularly on the fetal side, protrude downwards as far as the tight junctions between adjacent epithelial cells permit.

What is not clear is whether this close contiguity of the maternal and fetal blood supplies over much of the placental surface permits the passage of necessary larger molecules, such as those which must carry metals and vitamins. Such a process would require selective transcellular trafficking of proteins, since there is no evidence that maternal plasma proteins as a group ever reach the fetal blood circulation. The pig neonate, unlike the human, for example, carries no maternal immunoglobulins at birth. The alternative to this process is the embryotrophic route as exemplified by the transplacental transport of uteroferrin and its bound iron. However, it still remains questionable whether the elaboration of histotroph and the embryotrophic route alone can account for the iron requirements and other nutritional needs of the fetus in late pregnancy.

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References

Adams, K.L., Bazer, F.W. & Roberts, R.M. (1981) Progesterone-induced secretion of a retinol-binding protein in the pig uterus. *J. Reprod. Fert.* **62,** 39–47.

Aitken, R.J. (1977) Embryonic diapause. In *Development in Mammals*, Vol. 1, pp. 307–360. Ed. M. H. Johnson. North-Holland, Amsterdam.

Amoroso, E.C. (1951) Placentation. In *Marshall's Physiology of Reproduction*, 3rd edn, Vol. II, pp. 127–311. Ed. A. S. Parkes. Longman Green, London.

Anderson, L.L. (1978) Growth, protein content and distribution of early embryos. *Anat. Rec.* **190,** 143–154.

Antanaitis, B.C. & Aisen, P. (1983) Uteroferrin and the purple acid phosphatases. In *Advances in Inorganic Biochemistry*, Vol. 7, *Iron Binding Proteins*, pp. 111–136. Eds E. C. Theil, G. L. Eichorn & L. Marzilli. Elsevier, New York.

Antanaitis, B.C. & Aisen, P. (1984) Stoichiometry of iron binding by uteroferrin and its relationship to phosphate content. *J. biol. Chem.* **259,** 2066–2069.

Basha, S.M.M., Bazer, F.W. & Roberts, R.M. (1979) The secretion of a uterine specific, purple phosphatase by cultured explants of porcine endometrium: dependency upon the state of pregnancy of the donor animal. *Biol. Reprod.* **20,** 431–441.

Basha, S.M.M., Bazer, F.W., Geisert, R.D. & Roberts, R.M. (1980a) Progesterone-induced uterine secretions in pigs. Recovery from pseudopregnant and unilaterally pregnant gilts. *J. Anim. Sci.* **50,** 113–123.

Basha, S.M.M., Bazer, F.W. & Roberts, R.M. (1980b) Effects of conceptus on quantitative and qualitative aspects of uterine secretion in pigs. *J. Reprod. Fert.* **60,** 41–48.

Baumbach, G.A., Saunders, P.T.K., Bazer, F.W. & Roberts, R.M. (1984) Uteroferrin has N-linked, high mannose oligosaccharides which contain mannose 6-phosphate. *Proc. natn. Acad. Sci. U.S.A.* **81,** 2985–2989.

Baumbach, G.A., Ketcham, C.M., Richardson, D.E., Bazer, F.W. & Roberts, R.M. (1986) Isolation and characterization of a high molecular weight, stable pink form of uteroferrin from uterine secretions and allantoic fluid of pigs. *J. biol. Chem.* **261,** 12869–12878.

Bazer, F.W., Chen, T.T., Knight, J.W., Schlosnagle, D.C., Baldwin, N.J. & Roberts, R.M. (1975) Presence of a progesterone-induced uterine specific acid phosphatase in allantoic fluid in gilts. *J. Anim. Sci.* **41,** 1112–1119.

Bazer, F.W., Roberts, R.M., Sharp, D.C. & Thatcher, W.W. (1981) Uterine proteins synthesized during the progestative period and pregnancy. In *Uterus et*

Fecondité, pp. 17–32. Eds C. Boury-Heyler, P. Mauléon & Y. Rochet. Masson Publishing Co., Paris.

Bazer, F.W., Geisert, R.D., Thatcher, W.W. & Roberts, R.M. (1982) The establishment and maintenance of pregnancy. In *Control of Pig Reproduction*, pp. 227–252. Eds D. J. A. Cole & G. R. Foxcroft. Butterworth Scientific, London.

Beers, W.M., Strickland, S. & Reich, E. (1975) Ovarian plasminogen activator: relationship to ovulation and hormonal regulation. *Cell* **6**, 387–394.

Bjorkman, N.H. (1969) Light and electron microscopic studies on alteration in the normal bovine placentome. *Anat. Rec.* **163**, 17–29.

Bode, V.C. & Dziadek, M.A. (1979) Plasminogen activator secretion during mouse embryogenesis. *Devl Biol.* **73**, 272–289.

Boshier, D.P. (1969) A histological and histochemical examination of implantation and early placentome formation in sheep. *J. Reprod. Fert.* **19**, 51–61.

Brambel, C.E. (1933) Allantochorionic differentiation of the pig studied morphologically and histochemically. *Am. J. Anat.* **52**, 397–459.

Brinster, R.L. (1973) Nutrition and metabolism of the ovum, zygote and blastocyst. In *Handbook of Physiology-Endocrinology* II, Part 2, pp. 165–185. Eds R. O. Greep, E. G. Astwood & S. R. Geiger. American Physiological Society, Washington, D.C.

Buhi, W.C., Ducsay, C.A., Bazer, F.W. & Roberts, R.M. (1982) Iron transfer between the purple phosphatase uteroferrin and transferrin and its possible role in iron metabolism of the fetal pig. *J. biol. Chem.* **257**, 1712–1721.

Buhi, W.C., Ducsay, C.A., Bartol, F.F., Bazer, F.W. & Roberts, R.M. (1983) A function of the allantoic sac in the metabolism of uteroferrin and maternal iron by the fetal pig. *Placenta* **4**, 455–470.

Campbell, H.D. & Zerner, B. (1973) A low molecular weight acid phosphatase which contains iron. *Biochem. Biophys. Res. Commun.* **54**, 1498–1503.

Chen, T.T., Bazer, F.W., Cetorelli, J.J., Pollard, W.E. & Roberts, R.M. (1973) Purification and properties of a progesterone-induced basic glycoprotein from the uterine fluids of pigs. *J. biol. Chem.* **248**, 8560–8566.

Chen, T.T., Bazer, F.W., Gebhardt, B.M. & Roberts, R.M. (1975) Uterine secretions in mammals: synthesis and placental transport of a purple acid phosphatase in pig. *Biol. Reprod.* **13**, 304–313.

Colantuoni, V., Romano, V., Bensi, G., Santoro, C., Constanzo, F., Ruugei, G. & Cortese, R. (1983) Cloning and sequencing of a full length cDNA coding for human retinol binding protein. *Nucleic Acids Res.* **11**, 7769–7776.

Dabitch, D. & Andary, T.J. (1974) Prevention of blastocyst implantation in mice with proteinase inhibitors. *Fert. Steril.* **25**, 954–957.

Dantzer, V., Bjorkman, N. & Hasselager, E. (1981) An electron microscopic study of histotroph in the interareolar part of the porcine placenta. *Placenta* **2**, 19–28.

Davis, J.C. & Averill, B.A. (1982) Evidence for a spin-coupled binuclear iron unit at the active site of the purple acid phosphatase from beef spleen. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4623–4627.

Debrunner, P.G., Hendric, M.P., deJersey, J., Keough, D.T., Sage, T. & Zerner, B. (1983) Mossbauer and EPR study of the binuclear iron center in purple acid phosphatase. *Biochim. Biophys. Acta* **745**, 103–106.

Denker, H.W. (1980) Role of proteinases in implantation. *Prog. Reprod. Biol.* **7**, 28–42.

Doi, K., Antanaitis, B.C. & Aisen, P. (1986) Absence of iron transfer from uteroferrin to transferrin. *J. biol. Chem.* **261**, 14936–14938.

Fazleabas, A.T., Bazer, F.W. & Roberts, R.M. (1982) Purification and properties of a progesterone-induced plasmin/trypsin inhibitor from uterine secretions of pigs and its immunochemical localization in the pregnant uterus. *J. biol. Chem.* **257**, 6886–6897.

Fazleabas, A.T., Geisert, R.D., Bazer, F.W. & Roberts, R.M. (1983) The relationship between the release of plasminogen activator and estrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig. *Biol. Reprod.* **29**, 225–238.

Fazleabas, A.T., Bazer, F.W., Hansen, P.J., Geisert, R.D. & Roberts, R.M. (1984) Differential patterns of secretory protein localization within the pig uterine endometrium. *Endocrinology* **116**, 240–245.

Fishel, S.B., Edwards, R.G. & Evans, C.J. (1984) Human chorionic gonadotrophin secreted by preimplantation embryos cultured *in vitro*. *Science, N.Y.* **223**, 816–817.

Flint, A.P.F., Burton, R.D., Gadsby, J.E., Saunders, P.T.K. & Heap, R.B. (1979) Blastocyst oestrogen synthesis and maternal recognition of pregnancy. In *Maternal Recognition of Pregnancy* (Ciba Fndn Symp. No. 64), pp. 208–209. Ed. J. Whelan. Excerpta Medica, Amsterdam.

Frank, M., Bazer, F.W., Thatcher, W.W. & Wilcox, C.J. (1977) A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine: III Effects of estradiol valerate on prostaglandin F, progestins, estrone and estradiol concentrations in the utero-ovarian vein of non-pregnant gilts. *Prostaglandins* **14**, 1183–1196.

Friess, A.E., Sinowatz, F., Skolek-Winnisch, R. & Trautner, W. (1980) The placentation of the pig. I. Fine structural changes of the placental barrier during pregnancy. *Anat. Embryol.* **158**, 179–191.

Friess, A.E., Sinowatz, F., Skolek-Winnisch, R. & Trautner, W. (1981) The placenta of the pig. II. The ultrastructure of the areolae. *Anat. Embryol.* **163**, 43–53.

Fritz, H. & Wunderer, G. (1983) Biochemistry and applications of aprotinin, the kallikrein inhibitor from bovine organs. *Arzneim-Forsch. Drug Res.* **33**, 479–496.

Gaber, B.P., Sheridan, J.P., Bazer, F.W. & Roberts, R.M. (1979) Resonance raman scattering from uteroferrin, the purple glycoprotein of the porcine uterus. *J. biol. Chem.* **254**, 8340–8342.

Gadsby, J.E., Heap, R.B. & Burton, R.D. (1980) Oestrogen synthesis by blastocyst and early embryonic tissue of various species. *J. Reprod. Fert.* **60**, 409–417.

Geisert, R.D., Renegar, R.H., Thatcher, W.W., Roberts, R.M. & Bazer, F.W. (1982a) Establishment of pregnancy in the pig: I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions. *Biol. Reprod.* **27**, 925–949.

Geisert, R.D., Brookbank, J.W., Roberts, R.M. & Bazer, F.W. (1982b) Establishment of pregnancy in the pig:

II. Cellular remodelling of the porcine blastocyst during elongation on day 12 of pregnancy. *Biol. Reprod.* **27,** 941–955.

Geisert, R.D., Thatcher, W.W., Roberts, R.M. & Bazer, F.W. (1982c) Establishment of pregnancy in the pig III. Endometrial secretory response to estradiol valerate administered on day 11 of the estrous cycle. *Biol. Reprod.* **27,** 957–965.

Godkin, J.D., Bazer, F.W. & Roberts, R.M. (1985) Protein production by cultures established from Day 14–16 sheep and pig conceptuses. *J. Reprod. Fert.* **74,** 377–382.

Hafez, E.S.E. & Jainudeen, M.R. (1974) Gestation prenatal physiology and parturition. In *Reproduction in Farm Animals*, 3rd edn, pp. 166–202. Ed. E. S. E. Hafez. Lea & Feibiger, Philadelphia.

Hansen, P.J., Bazer, F.W. & Roberts, R.M. (1985) Appearance of β-hexosaminidase and other lysosomal-like enzymes in the uterine lumen of gilts, ewes and mares in response to progesterone and oestrogens. *J. Reprod. Fert.* **73,** 411–424.

Hansen, P.J., Segerson, E.C. & Bazer, F.W. (1987) Characterization of immunosuppressive substances in the basic protein fraction of uterine secretions from pregnant ewes. *Biol. Reprod.* **36,** 393–404.

Heap, R.B., Flint, A.P. & Gadsby, J.G. (1979) Role of embryonic signals in the establishment of pregnancy. *Br. med. Bull.* **35,** 129–135.

Hunt, D.F., Yates, J.R., Shabanowitz, J., Zhu, N., Zirino, T., Averill, B.A., Daurat-Larroque, S.T., Shewate, J.G., Roberts, R.M. & Brew, K. (1987) Sequence homology in the melalloproteins: purple acid phosphatase from beef spleen and uteroferrin from porcine uterus. *Biochem. Biophys. Res. Commun.* **144,** 1154–1160.

Ketcham, C.M., Baumbach, G.A., Bazer, F.W. & Roberts, R.M. (1985) The type 5, acid phosphatase from spleen of patients with hairy cell leukemia. Purification, properties, immunological characterization and comparison with porcine uteroferrin. *J. biol. Chem.* **260,** 5768–5776.

Keys, J.L., King, G.J. & Kennedy, T.G. (1986) Increased uterine vascular permeability at the time of embryonic attachment in the pig. *Biol. Reprod.* **34,** 405–411.

King, G.J., Atkinson, B.A. & Robertson, H.A. (1980) Development of the bovine placentome from Days 20 to 24 of gestation. *J. Reprod. Fert.* **59,** 95–100.

King, G.J., Atkinson, B.A. & Robertson, H.A. (1982) Implantation and early placentation in domestic ungulates. *J. Reprod. Fert., Suppl.* **31,** 17–20.

Knight, J.W., Bazer, F.W., Wallace, H.D. & Wilcox, C.J. (1974) Dose response relationships between exogenous progesterone and estradiol and porcine uterine secretions. *J. Anim. Sci.* **39,** 747–751.

Kubo, H., Spindle, H. & Pederson, R.A. (1981) Inhibition of mouse blastocyst attachment and outgrowth by protease inhibitors. *J. exp. Zool.* **216,** 445–451.

Laskowski, M. & Kato, I. (1980) Protein inhibitors of proteinases. *Ann. Rev. Biochem.* **49,** 593–626.

McDowell, K., Sharp, D.C., Zavy, M.T., Fazleabas, A., Roberts, R.M. & Bazer, F.W. (1982) Partial characterization of the equine uteroferrin-like protein. *J. Reprod. Fert., Suppl.* **32,** 329–334.

McLaren, A. (1973) Blastocyst activation. In *The Regulation of Mammalian Reproduction*, pp. 321–328. Eds. S. J. Segal, R. Crozier, P. A. Corfman & P. G. Condliffe. Charles C. Thomas, Springfield.

Mead, R.A. (1981) Delayed implantation in mustelids with special emphasis on the spotted skunk. *J. Reprod. Fert., Suppl.* **29,** 11–24.

Moore, N.W. & Shelton, J.N. (1964) Egg transfer in sheep. Effect of degree of synchronization between donor and recipient, age of egg and site of transfer on survival of transferred eggs. *J. Reprod. Fert.* **7,** 145–152.

Morgan, G.L., Geisert, R.D., Zavy, M.T., Shawley, R.V. & Fazleabas, A.T. (1987) Development of pig blastocysts in a uterine environment advanced by exogenous oestrogen. *J. Reprod. Fert.* **80,** 125–131.

Mullins, D.E., Bazer, F.W. & Roberts, R.M. (1980) Secretion of a progesterone-induced inhibitor of plasminogen activator by the porcine uterus. *Cell* **20,** 865–872.

Murray, F.A., Segerson, E.C. & Brown, F.T. (1978) Suppression of lymphocytes *in vitro* by porcine uterine secretory protein. *Biol. Reprod.* **19,** 15–25.

Murray, M.K., Bazer, F.W., Wallace, H.D. & Warnick, A.C. (1972) Quantitative and qualitative variation in the secretion of protein by the porcine uterus during the estrous cycle. *Biol. Reprod.* **7,** 314–320.

Nieder, G.L., Weitlauf, H.M. & Suda-Hartman, M. (1987) Synthesis and secretion of stage-specific proteins by peri-implantation mouse embryos. *Biol. Reprod.* **36,** 687–699.

O'Farrell, P.Z., Goodman, H.M. & O'Farrell, P.H. (1977) High resolution two dimensional electrophoresis of basic as well as acidic proteins. *Cell* **12,** 1133–1142.

Ossowski, L., Unkeless, J.C., Tobia, A., Quigley, J.P., Rifkin, D.B. & Reich, E. (1973) An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. II. Mammalian fibroblast cultures transformed by DNA and RNA tumor viruses. *J. exp. Med.* **137,** 112–126.

Ossowski, L., Biegel, D. & Reich, E. (1979) Mammary plasminogen activator: Correlation with involution, hormonal modulation and comparison between normal and neoplastic tissue. *Cell* **16,** 929–940.

Palludan, B., Wegger, J. & Moustgard, J. (1970) Placental transfer of iron. *Danish Roy. Vet. Agric. Univ. Yearbook*, Copenh. 62–90.

Patten, B.M. (1948) *Embryology of the Pig*, pp. 227–270. Mcgraw-Hill, New York.

Perry, J.S. & Rowlands, I.W. (1962) Early pregnancy in the pig. *J. Reprod. Fert.* **4,** 175–188.

Perry, J.S., Heap, R.B. & Amoroso, E.C. (1973) Steroid hormone production by pig blastocysts. *Nature, Lond.* **245,** 45–47.

Polge, C. (1982) Embryo transplantation and preservation. In *Control of Pig Reproduction*, pp. 277–291. Eds D. J. A. Cole & G. R. Foxcroft. Butterworth Scientific, London.

Pollack, R., Risser, R., Conlon, S. & Rifkin, D.B. (1974) Plasminogen activator production accompanies loss of anchorage regulation in transformation of primary rat embryo cells by simian virus 40. *Proc. natn. Acad. Sci. U.S.A.* **71,** 4792–4796.

Pope, W.F. & First, N.L. (1985) Factors affecting the survival of pig embryos. *Theriogenology* **23,** 91–105.

Psychoyos, A. (1973) Endocrine control of egg implantation. In *Handbook of Physiology-Endocrinology* II, Part 2, pp. 187–215. Eds R. O. Greep, E. G. Astwood & S. R. Geiger. American Physiological Society, Washington D.C.

Raub, T.J., Bazer, F.W. & Roberts, R.M. (1985) Localization of the iron transport glycoprotein, uteroferrin, in the porcine endometrium and placenta by using immunocolloidal gold. *Anat. Embryol.* **171,** 253–258.

Reneger, R.H., Bazer, F.W. & Roberts, R.M. (1982) Placental transport and distribution of uteroferrin in the fetal pig. *Biol. Reprod.* **27,** 1247–1260.

Roberts, G.P. (1977) Inhibition of lymphocyte stimulation by bovine uterine proteins. *J. Reprod. Fert.* **50,** 337–339.

Roberts, R.M. & Bazer, F.W. (1980) The properties, function and hormonal control of synthesis of uteroferrin: the purple protein of the pig uterus. In *Steroid-Induced Proteins*, pp. 133–149. Ed. M. Beato. Elsevier-North Holland, Amsterdam.

Roberts, R.M. & Bazer, R.W. (1984) Uteroferrin: a protein in search of a function. *Bio Essays* **1,** 8–11.

Roberts, R.M., Bazer, F.W., Baldwin, N. & Pollard, W.E. (1976) Induction of lysozyme and leucine aminopeptidase activities in the uterine flushings of pigs by progesterone. *Archs Biochem. Biophys.* **177,** 499–507.

Roberts, R.M., Raub, T.J. & Bazer, F.W. (1986) Role of uteroferrin in transplacental iron transport in the pig. *Fedn Proc. Fedn Am. Socs exp. Biol.* **45,** 2513–2518.

Roberts, R.M., Murray, M.K., Burke, M.G., Ketcham, C.M. & Bazer, F.W. (1987) Hormonal Control and Function of Secretory Proteins. In *Cell Biology of the Uterus*, (in press). Ed. W. Leavitt. Plenum Press, New York.

Rowson, L.E.A. & Moor, R.M. (1966) Embryo transfer in the sheep: the significance of synchronizing oestrus in the donor and recipient animal. *J. Reprod. Fert.* **11,** 207–212.

Samuel, C.A. (1971) The development of the pig trophoblast in ectopic sites. *J. Reprod. Fert.* **27,** 494–495.

Samuel, C.A. & Perry, J.S. (1972) The ultrastructure of the pig trophoblast transplanted to an ectopic site in the uterine wall. *J. Anat.* **113,** 139–149.

Saunders, P.T.K., Renegar, R.H., Raub, T.J., Baumbach, G.A., Atkinson, P.H., Bazer, F.W. & Roberts, R.M. (1985) The carbohydrate structure of porcine uteroferrin and the role of the high mannose chains in promoting uptake by the reticuloendothelial cells of the fetal liver. *J. biol. Chem.* **260,** 3658–3665.

Schlosnagle, D.C., Bazer, F.W., Tsibris, J.C.M. & Roberts, R.M. (1974) An iron containing phosphatase induced by progesterone in the uterine fluids of pigs. *J. biol. Chem.* **249,** 7574–7579.

Schlosnagle, D.C., Sander, E.G., Bazer, F.W. & Roberts, R.M. (1976) Requirement of an essential thiol group and ferric iron for the activity of the progesterone-induced porcine uterine purple phosphatase. *J. biol. Chem.* **251,** 4680–4685.

Schumacher, G.F.B. (1974) Lysozyme in human genital tract secretions. In *Lysozyme*, pp. 427–447. Eds F. Osserman, R. E. Canfield & S. Beychok. Academic Press, New York.

Segerson, E.C. (1981) Immunosuppressive effect of ovine uterine secretory protein upon lymphocytes *in vitro*. *Biol. Reprod.* **25,** 77–84.

Simmen, R.C.M., Wilde, M.H., Pope, W.F. & Simmen, F.A. (1986) Porcine uterine luminal fluid contains epithelial cell growth stimulating factors. *J. Cell Biol.* **103,** 154a, Abstr. 565.

Simmen, R.C.M., Liu, X.H., Brew, K. & Roberts, R.M. (1987) Isolation and characterization of cDNA clones encoding porcine uteroferrin. *Endocrinology* **120,** *Suppl.* 133, Abstr. 450.

Strickland, S., Reich, E. & Sherman, M.I. (1976) Plasminogen activator in early embryogenesis: enzyme production by trophoblast and parietal endoderm. *Cell* **9,** 231–240.

Stroband, H.W.J., Taverne, N. & Bogaard, M.V.D. (1984) The pig blastocyst: its ultrastructure and the uptake of protein macromolecules. *Cell Tissue Res.* **235,** 347–356.

Unkeless, J.C., Tobia, A., Ossowski, L., Quigley, J.P., Rifkin, D.B. & Reich, E. (1973) An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblast cultures transformed by avian RNA tumor viruses. *J. exp. Med.* **137,** 84–111.

Unkeless, J.C., Gordon, S. & Reich, E. (1974) Secretion of plasminogen activator by stimulated macrophages. *J. exp. Med.* **139,** 834–850.

Werb, Z., Banda, M.J. & Jones, P.A. (1980) Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins and collagen by proteinases isolated from macrophages. *J. exp. Med.* **152,** 1340–1357.

Wilmut, I., Sales, D.I. & Ashworth, C.J. (1985) The influence of variation in embryo stage and maternal hormone profiles on embryo survival in farm animals. *Theriogenology* **23,** 107–119.

Wislocki, G.B. & Dempsey, E.W. (1946) Histochemical reactions of the placenta of the pig. *Am. J. Anat.* **78,** 181–225.

Wooding, F.B.P., Flint, A.P.F., Heap, R.B. & Hobbs, T. (1981) Autoradiographic evidence for immigration and fusion of cells in the sheep placenta: resolution of a problem in placental classification. *Cell Biol. Int. Rep.* **5,** 821–827.

Wright, R.W. & Bondioli, K.R. (1981) Aspects of *in vitro* fertilization and embryo culture in domestic animals. *J. Anim. Sci.* **53,** 702–729.

Zavy, M.T., Roberts, R.M. & Bazer, F.W. (1984) Acid phosphatase and leucine aminopeptidase activity in the uterine flushings of nonpregnant and pregnant gilts. *J. Reprod. Fert.* **72,** 503–507.