Embryo–uterine interactions in pigs during week 2 of pregnancy

R. M. Roberts¹,², S. Xie¹ and W. E. Trout¹

Departments of Animal Sciences and Biochemistry, University of Missouri-Columbia, Columbia, MO 65211, USA

The second week of pregnancy is a particularly critical period for embryonic survival in pigs. Within that time, conceptus oestrogen synthesis is initiated, spacing and final placement of conceptuses is completed, and the signal for extending the functional lifespan of the corpora lutea is received by the mother. There is also a marked increase in blood flow to the uterus and the uterine endometrium produces and secretes nutrient histotrophe. Conceptus-derived oestrogen has been implicated in many of these events. It is also during this period that the trophoblast elongates dramatically and the inner cell mass starts to differentiate into the embryo proper. Here, we critically review the evidence that oestrogen is the sole factor initiating long-term corpus luteum maintenance in pigs. We also review the functions and general properties of the major secretory proteins in histotrophe and the role of oestrogen in controlling their expression. It is now generally accepted that asynchrony within a litter underlies much of the losses of conceptuses that are otherwise genetically normal, but which are lagging in their development; however, the underlying mechanisms remain unclear. Here we hypothesize that oestrogenic compounds derived from more advanced conceptuses or provided prematurely, either by injection or in the diet, trigger a massive increase in uterine expression and secretion of retinol-binding protein laden with retinol. We propose that less developed, smaller conceptuses are least able to contend with the sudden exposure to this potential teratogen at a time when they are particularly susceptible to imbalance in retinol supply. Hence, even though their growth proceeds for a few days, their developmental potential is irrevocably compromised.

Introduction

The intent of this paper is to review current information on the biochemical interactions that occur between conceptuses and the maternal endometrium during early pregnancy. We concentrate on the second week after conception, a period that is particularly critical to embryo survival and in which the mother must make appropriate adjustments in her physiology if the pregnancy is to continue. Some of the events that occur during week 2 of pregnancy in swine are listed (Table 1). Not surprisingly, this period is associated with considerable embryonic loss (see below). It is also clear that many of the phenomena listed are sequelae to the production of conceptus oestrogen. This latter topic is therefore discussed in some detail. Finally, porcine embryos rely heavily for their growth and development on provision of uterine secretory proteins, yet cannot tolerate an out-of-phase uterine environment. Since the control of uterine secretory activity and the function of individual protein components have long been an interest of this laboratory, these subjects also receive emphasis in this review.

© 1993 Journals of Reproduction and Fertility Ltd
| Phenomenon                                         | Day of pregnancy | Comments                                                                                           | References                                      |
|---------------------------------------------------|------------------|---------------------------------------------------------------------------------------------------|------------------------------------------------|
| Conceptus oestrogen synthesis                     | 10–12            | Precedes most phenomena described below                                                             | Perry et al., 1976                            |
| Migration, spacing and placement of conceptuses   | 7–12             | Probably mediated in part by conceptus oestrogen                                                    | Pope et al., 1982; Dziuk, 1985; Laforest and    |
|                                                   |                  |                                                                                                    | King, 1992                                     |
| Signal for maternal recognition of pregnancy*     | 12               | Mediated by conceptus oestrogen and possibly other factors                                          | Dhindsa and Dziuk, 1968; Frank et al., 1978    |
| Elongation of conceptuses                         | 12               | Considerable asynchrony between littermates; onset follows initiation of oestradiol synthesis and  |                                                   |
|                                                   |                  | coincides with release of uterine proteins                                                        | Patten, 1948; Perry and Rowlands, 1962;         |
|                                                   |                  |                                                                                                    | Anderson, 1978; Geisert et al., 1982a, b;      |
|                                                   |                  |                                                                                                    | Pope et al., 1988; Xie et al., 1990a           |
| Increased uterine blood flow                      | 13               | Mediated by conceptus oestrogen                                                                    | Ford et al., 1982                             |
| Uterine secretory activity begins                 | 12               | Requires progesterone; modulated by oestrogen                                                       | Geisert et al., 1982a, c; Roberts and Bazer, 1988|
| Secretion of retinol-binding proteins by conceptus| 10–15            | Initiated before conceptus elongation                                                               | Harney and Bazer, 1989; Godkin et al., 1982;   |
|                                                   |                  |                                                                                                    | Trout et al., 1992                            |
| Primitive streak formation and initiation of      | 9–14             | Beginning of embryogenesis                                                                          | Patten, 1948; Anderson, 1978; Jainudeen and    |
| gastrulation and neurulation                      |                  |                                                                                                    | Hafez, 1987                                   |
| Secretion of type I and type II interferons       | 12               | Modulation of maternal immune responses                                                             | LaBonnardière et al., 1991                     |
| Firm adhesion of trophoblast to uterine epithelium| 14               | Appears defective in gilts or sows exposed to oestrogens prematurely                                 | Keys and King, 1990; Morgan et al., 1987       |

*Maternal recognition of pregnancy here refers to the extension of the functional lifespan of the corpora lutea.
Basis of Embryonic Loss in Pigs

The majority of corpora lutea present on the ovaries of gilts are represented by viable blastocysts at day 9 to 10 of pregnancy (Polge, 1982; Xie et al., 1990a). However, only about 70-80% of these blastocysts are estimated to survive until day 25 (Pope and First, 1985). Losses are higher in sows than in gilts and are greatest when large numbers of ovulations occur (Perry, 1960). A recent study (Lambert et al., 1991) indicated that most embryonic loss occurred before day 10 of pregnancy, but the observations were made on young, first cycle gilts, and peripubertal gilts tend to have an extended pro-oestrus with a prolonged oestrogen peak. Consequently, the oocytes of first cycle gilts may become developmentally defective owing to overexposure to the steroid (Archibong et al., 1987). Nevertheless, whatever the timing of embryonic death, it remains poorly explained and has a major impact on productivity and hence profitability in the industry.

Although it is still not completely clear exactly when embryos unaccounted for at the end of the first month of pregnancy die, it has been proposed that the loss results from asynchronous development among littersmates, a phenomenon that leads to poorly coordinated biochemical interactions between the conceptuses and the maternal systems (Pope, 1988; Pope et al., 1990). Specifically, smaller, less advanced conceptuses are presumed to be lost because they become out of phase with the uterine environment. It is well established, for example, that embryos transferred to more advanced uteri quickly die (Jarrell et al., 1990; Geisert et al., 1991). Thus, some early losses may simply result from the inability of embryos to maintain their rates of development in the face of an increasingly hostile uterine environment, although the nature of the toxic response remains poorly understood. The situation is a little clearer at day 10 to 12 of pregnancy. Here most investigators have suggested that production of oestrogen by the larger conceptuses, as well as being responsible for extending the functional lifespan of the corpus luteum, causes a sudden change in the uterine milieu that cannot be tolerated by those conceptuses already lagging in development (Pope and First, 1985; Morgan et al., 1987; Pope, 1988). This concept is discussed in greater detail later.

It seems unlikely that the more slowly developing conceptuses die because they are all genetically defective since both small and large day 7 blastocysts transferred to separate, surgically isolated uterine horns of a recipient female at day 6 of her cycle survive and appear normal 6.5 days later. However, attrition of small blastocysts is high if a similar transfer is made to a day 7 recipient (i.e. a synchronous, surrogate mother) with larger blastocysts placed in the contralateral horn (Table 2; Wilde et al., 1988). Such an experiment illustrates two points. First, as stated above, genetically normal embryos probably comprise the majority of those lost during early pregnancy in the pig, although a selection against lagging embryos may be very useful in minimizing any possibility of a sow bearing defective young. Second, the embryos in one uterine horn can influence the development of others in the contralateral horn. Thus, an explanation for the findings is that the synchronous transfer of day 7 blastocysts to a day 6 uterus temporarily retards the more advanced embryos and allows the smaller ones to catch up. In the asynchronous transfer, however, the larger embryos retain their advantage, secrete oestrogen earlier and, by mechanisms still unclear, trigger changes in the other uterine horn.

In a normal pregnancy, considerable developmental asynchrony can be readily noted at about days 11–12 of pregnancy. It is not uncommon, for example, to find small spherical blastocysts <5 mm in diameter along with tubular and filamentous forms (Perry and Rowlands, 1962; Anderson, 1978; Pope et al., 1988). There are several possible causes of such variability. For example, different embryos may be genetically programmed to develop at different rates. Another possibility is that the oocytes shed last during ovulation, a process that occurs over several hours in pigs, give rise to the smaller embryos noted 12 days later (Pope et al., 1988). This last hypothesis has been based on three observations: heterogeneity in follicle/oocyte maturation right before ovulation (Ainsworth et al., 1980; Xie et al., 1990b, Biggs et al., 1993); skewed ovulation with a few oocytes shed later during the process of ovulation (Pope et al., 1988) and slower cleavage embryos being the less developed conceptuses on day 11 (Xie et al., 1990a). However, the relationship between the later matured follicles/oocytes and the later ovulators has not been established; nor has the developmental potential of the later matured oocytes been evaluated. Evidence has also emerged that ovulation intervals are not the only causes for embryo diversity (Pope, 1992; Soede...
Table 2. Embryonic survival (mean ± SEM) after synchronous (day 7 recipient) and asynchronous (day 6 recipient) transfer of small and large day 7 littermate blastocysts

| Type of transfer | Small day 7 blastocysts | Large day 7 blastocysts |
|------------------|-------------------------|------------------------|
| Synchronous      | 38.3 ± 5.8<sup>a</sup>  | 73.9 ± 5.8<sup>b</sup>  |
| (n = 9)          |                         |                        |
| Asynchronous     | 75.4 ± 6.6<sup>b</sup>  | 70.7 ± 6.6<sup>b</sup>  |
| (n = 7)          |                         |                        |

<sup>a</sup>Means with different superscripts are significantly different (P < 0.01).

Data from Wilde et al. (1988) with permission.

Nevertheless, there seems little doubt that it is these more poorly developed embryos that are subsequently lost.

Timing and Extent of Conceptus Oestrogen Production

As discussed in a later section, oestrogen, first produced by pig conceptuses at about day 11 of pregnancy, is considered to be critical for ensuring that the corpora lutea do not regress as they do at the end of a non-fertile oestrous cycle (Bazer et al., 1982). By day 11, spherical blastocysts 5–7 mm in diameter can synthesize oestrogen from a variety of substrates (Perry et al., 1976; Heap et al., 1979; Fischer et al., 1985), although a pathway involving Δ<sup>4</sup>-3-ketosteroids, and particularly progesterone, seems most likely (see Conley et al., 1992 for discussion). The oestrogen is secreted transiently, with maximal output per mass of tissue detected at the time of early elongation. This burst of activity is correlated with a transient increase in concentrations of oestrogens (oestradiol and oestrone) and particularly oestrone sulfate in maternal plasma (Stoner et al., 1981; Bazer et al., 1982). Oestradiol is oxidized to oestrone in a reaction catalysed by a specific dehydrogenase. Free oestrone is probably then sulfated by endometrial sulfotransferase. The activity of both enzymes reaches peak values in porcine endometrial tissue between days 5 and 13 (Pack and Brooks, 1974).

The rate-limiting enzyme during this first period of conceptus oestrogen production is probably 17α-hydroxylase cytochrome P<sub>450</sub>, the expression of which reaches peak values during the tubular phase of development and declines as conceptuses become filamentous (Conley et al., 1992). Oestradiol concentrations in conceptus tissues are approximately fifty-fold higher at day 12 than at day 14 of development (67.5 versus 1.3 pg in 100 μg protein, respectively) (Conley et al., 1992). Moreover, total content of oestrogens in flushings from uterine horns containing early elongating conceptuses at about day 12 of pregnancy is considerably higher than at day 14, when all the conceptuses are filamentous (about 20 ng versus about 2 ng) (Geisert et al., 1982a).

It is difficult to determine exactly how much oestrogen such conceptuses are elaborating in vivo. Measurements from tissue extracts and uterine flushings do not take into account how efficiently steroid is removed from the uterine lumen. Estimates made on production by cultured conceptuses are also likely to be low since the tissues are in general metabolic decline and do not necessarily have access to all the precursor molecules needed to produce oestrogens efficiently.

Nevertheless, Wilde and Pope (1987) incubated individual day 11.5 conceptuses for only 6 h. Large quantities (20–40 ng) of oestradiol were produced by both tubular and filamentous conceptuses. If a litter contains 10–15 conceptuses, 1–3 μg oestradiol will be produced daily. These data are somewhat in
agreement with those of Ford et al. (1982), who calculated both total blood flow to the uterus and the difference between concentrations of oestriadiol and oestrone in uterine vein and uterine artery. On the basis of their data, it can be estimated that approximately 1.4 μg of free oestrogens leave the uterus daily at about day 13 of pregnancy. Export from the uteri of nonpregnant gilts was negligible. However, differences of 100 pg ml⁻¹ in utero-ovarian concentrations of oestrone sulfate, which was not measured by Ford et al. (1982), have been observed between pregnant and nonpregnant gilts at day 11-12 of gestation (Stoner et al., 1981; Bazer et al., 1982). Similarly, Stone and Seamark (1985) reported that plasma concentrations of oestrone sulfate in mated gilts were 100-200 pg ml⁻¹ higher than those in uninfected gilts at about this stage of gestation. If the blood volume of these gilts is assumed to be 7 litres, the total content of water soluble oestrone sulfate at any time would exceed 700 ng. As oestrone sulfate would be expected to have a half-life of minutes in serum rather than hours, it seems likely that during this stage of pregnancy large amounts of sulfated oestrone are being fabricated. The question arises, therefore, as to whether the conceptuses are the source of all this oestrogen. Conceivably some is of uterine or even ovarian origin, although the ability of the former to synthesize oestrogen during early pregnancy has been reported to be limited (Fischer et al., 1985). Clearly these data on oestrogen concentrations in blood need to be re-evaluated, particularly as there are inconsistencies in some of the values that have been obtained for individual steroids and their sulfated derivatives.

Conceptus Oestrogens and the Signal for Maternal Recognition of Pregnancy

The conceptus signal that initiates luteal maintenance in pregnant sows must be produced by at least day 12 of pregnancy and conceptuses must also occupy each uterine horn by that time for the signal to be effective (Dhindsa and Dzuik, 1968). There seems little doubt that PGF₂α, originating from the uterine endometrium, is the luteolytic substance that limits the lifespan of the corpora lutea during the oestrous cycle. This topic has been reviewed extensively elsewhere (Bazer et al., 1986, 1989) and will not be covered here. However, the hypothesis that a relatively brief exposure of the uterus to conceptus-derived oestrogen at about day 11-14 provides the protective basis for this extension of the lifespan of the corpus luteum (Bazer and Thatcher, 1977) deserves some further consideration.

Exogenous oestrogens administered in large amounts to nonpregnant gilts at about day 12 have long been known to be luteotrophic (Gardner et al., 1963), and pseudopregnancy accompanied by prolonged bilateral maintenance of corpora lutea can be induced by daily injections of milligram quantities of oestradiol between days 11 and 15 after the onset of oestrus (see Frank et al., 1978; Bazer et al., 1982). However, such doses cannot reasonably be regarded as physiological. Provision of oestradiol at the site where it is presumed to be most effective, i.e. in the uterus, in amounts designed to mimic those released by conceptuses during this period have had little or no effect on luteal lifespan. For example, van der Meulen et al. (1991) observed that injection of 380 ng of oestradiol every 6 h from day 11 to day 15 into both uterine horns resulted in a slight but nonsignificant increase (21.7 ± 1.0 days versus 20.5 ± 1.5 days in controls) in inter-oestrous interval. Similar data were obtained in an unpublished study cited by Ford et al. (1982) in which gilts receiving 375 ng oestradiol every 6 h on days 10-14 experienced a 3-day delay in luteolysis and then returned to oestrus.

Using higher doses of oestradiol (100 μg day⁻¹; days 10-14), which were either infused into the uterine lumen or injected subcutaneously, Saunders et al. (1983) observed that both treatments gave a modest 4-5 day extension of the oestrous cycle. Finally, Laforest and King (1992) inserted Silastic beads impregnated with oestradiol or oestradiol benzoate into the uterine lumen of gilts on day 10 of the oestrous cycle. The beads released enough oestradiol to day 16 to maintain intraluminal concentrations at or above those observed in pregnant gilts. These authors also observed only a 2-6 day extension of the oestrous cycle in these gilts.

The above results may at first appear to contrast with the results of Ball and Day (1982), who developed the unilaterally pregnant pig model to examine the mechanisms of luteal maintenance. These authors successfully maintained bilateral corpora lutea to day 19 in 67% of unilaterally pregnant gilts infused with conceptus extracts. The extracts, containing 15 ng total oestrogens ml⁻¹ were introduced infused (15 ml day⁻¹) into the unoccupied contralateral horn on days 12-19. Adsorption of steroids with charcoal completely nullified this effect. However, it is unclear whether these unilateral pregnancies would
have been maintained beyond day 20. It seems likely that the bilateral maintenance of corpora lutea to
day 19 resulted from a 3–5 day delay in luteolysis as noted above.

Glossop and Foulkes (1988) pointed out that in commercial breeding units there are two distinct
periods when sows return to oestrus after service. The first was at about day 20 (range day 17–23), and
presumably represents animals in which fertilization failed or in which embryos were lost before day 12.
The second occurs at about day 26 (range day 24–31). These animals may have been exposed to
conceptus oestrogen and exhibit the short delay in luteolysis noted above (van der Meulen, 1991).
Indeed, Geisert et al. (1987) suggested that the conceptus generates two waves of oestrogen for main-
taining the function of the corpus luteum, the first at about day 11, the second between day 14 and day
16. Again, however, pharmacological doses of oestradiol were used to test the hypothesis.

It is difficult to reconcile many of these observations with the view that conceptus-derived oestro-
gens are the only biologically active substances involved in maintenance of corpora lutea in the pig. However,
there are few clues as to what the other factors might be, particularly since conceptus secretory proteins
infused into the uterine lumen between day 12 and day 15 failed to provide oestrous cycle extension in
nonpregnant gilts given a 1 mg injection of oestradiol on day 11 (Harney and Bazer, 1989).

Localized Effects of Oestrogen on Uterine Tissue

In the previous section, we argued that one aspect of maternal recognition of pregnancy in pigs, namely
maintenance of the corpora lutea, may not be mediated by conceptus-derived oestrogens alone. How-
ever, small amounts of oestrogen comparable to those likely to be released by embryos at day 11–12 do
have marked effects that are undoubtedly important in pregnancy (Table 1). In particular, uterine blood
flow is increased eight- to ten-fold within 12 h of an infusion of small amounts of oestradiol (375 ng) into
the uterine lumen of gilts at day 11 of their oestrous cycle (Ford et al., 1982). A comparable increase
occurs during pregnancy (Ford and Christenson, 1979). These effects may, in fact, be mediated by
catechol oestrogens, short-lived oestrogen metabolites known to be synthesized by porcine trophoblast
tissue (Mondschein et al., 1985). There is also evidence that conceptus-derived oestrogen is responsible
for a rapid release of calcium and uterine secretions into the uterine lumen (Table 1), a phenomenon that
must markedly alter the environment to which conceptuses are exposed (Geisert et al., 1982a). These
uterine secretions have generally been considered to involve materials already synthesized and stored
intracellularly in secretory granules. However, as will be discussed later, oestradiol can markedly
upregulate the mRNA for at least two secretory proteins, although others are less affected.

Magness and Ford (1982) have pointed out that oestrogen content of lymph as well as of the
utero-ovarian vein draining the uterus is high at days 11, 13 and 15 of pregnancy and that this steroid
and its metabolites might well reach the postganglionic sympathetic vasoconstrictor nerves, which are
believed to control blood flow to both the uterus and ovary, by such a route. Thus, onset of production
of oestradiol at day 11 might influence ovarian as well as uterine physiology.

Embryocidal Effects of Oestrogen and Oestrogen Analogue

Zearalenone is a metabolite of the fungus Fusarium roseum and acts as a potent oestrogen. It is commonly
found in mouldy corn, which, when consumed by sows, causes a range of physiological responses,
including abortion during early pregnancy and extended inter-oestrous intervals in which the corpora
lutea are bilaterally maintained (Long and Diekman, 1984). Feeding zearalenone in a controlled manner
(1 mg day⁻¹) to sows between days 7 and 10 after mating has been observed to lead to abnormalities
in the organization of the embryonic disc by day 12 in early elongating conceptuses and obvious
degeneration of the entire litter or filamentous conceptuses by day 13 (Long et al., 1992). Few, if any,
abnormalities have been noted in the uterine endometrium or in the serum profiles of pituitary hormones,
the synthesis of which could potentially be responsive to oestrogen. Identical degenerative changes and
complete pregnancy loss result when pregnant gilts are injected with fatty acid esters of oestradiol before
day 10.5 of pregnancy (Morgan et al., 1987; Gries et al., 1989). Both treatments result in premature release
of endometrial secretions into the uterine lumen.
Although these observations do not rule out a direct toxic effect of oestrogens on conceptuses just before the time they elongate, it seems more probable that the treatments cause acute changes in the uterine milieu that the conceptus, and particularly the embryo proper, cannot tolerate. This sudden exposure to a more hostile environment leads to embryonic death, not immediately, as noted in asynchronous transfers in the day 5–7 or 6–8 periods noted earlier (Geisert et al., 1991), but rather within a few days. Oestrogen administered only slightly later, that is day 11 to 12, is without any embryotoxic effect. It is a major puzzle why uterine secretions provide such a narrowly permissive environment and what components can be lethal one day yet without apparent harm on the next. We speculate later that the embryotoxic component may be retinol.

Components of Histotrophe and Their Likely Functions

Several reviews, including Roberts and Bazer (1988) and Roberts et al. (1993), have dealt with the composition and function of the progesterone-responsive components of uterine secretions of the pig. Davis (this supplement) also discusses their production by primary cultures of uterine epithelial cells. The topic will therefore be covered only briefly here.

Histotrophe or uterine milk is so called because it is assumed to nurture the developing conceptus in utero. In species such as the pig, the trophoblast of which is noninvasive and fails to make direct contact with the maternal blood supply, a reliance on uterine secretions to provide macromolecules with a nutrient or protective role is assumed to be more complete than in species such as mice or humans in which the trophectoderm immediately invades after blastocyst hatching. As a consequence, the pig uterine endometrium begins to secrete very large quantities of several secretory proteins in response to progesterone (Table 2). The most abundant of these secretory components is the purple acid phosphatase, uteroferrin, which transports iron across the placenta (Roberts et al., 1986; Roberts and Bazer, 1988) and which may also be a potent growth factor on pig haematopoietic progenator cells (Bazer et al., 1991). Uteroferrin has been fully sequenced (Hunt et al., 1987), its cDNA (Simmen et al., 1988; Ling and Roberts 1993) and its gene (Simmen et al., 1989) cloned and it has been shown to be identical to an intracellular tartrate-resistant acid phosphatase normally sequestered in lysosomes (Ling and Roberts, 1993). Purple uteroferrin-like molecules are also secreted into the uterine lumen of the horse (McDowell et al., 1982; Zavy et al., 1982) and cow (C. Ketcham, W. Clark, F. W. Bazer and R. M. Roberts, unpublished results), but an involvement in iron transport in these species, although suspected, has not been confirmed. Uteroferrin expression is responsive not only to steroids, but possibly also to prolactin (Young et al., 1989; Fliss et al., 1991).

In addition to uteroferrin, several other major progesterone-responsive polypeptides of porcine uterine secretions have by now been purified, and their cDNA cloned. They include a trio of basic glycoproteins most probably derived from a common precursor molecule by differential post-translational modification (Murray et al., 1989; Malathy et al., 1990). They are members of the widespread serpin superfamily of proteins, many of whose members are proteinase inhibitors. No function, however, has yet been ascribed to these uterine serpins, although similar molecules are secreted by the uterus of sheep (Ing and Roberts, 1989) and cows (N. Mathialagan and R. M. Roberts, unpublished).

A group of smaller basic proteins that are known to be highly effective inhibitors of plasmin, trypsin and chymotrypsin have also been described. Recently their cDNA have been cloned (Stallings-Mann and Trout, 1993), demonstrating conclusively that they belong to the Kunitz family of inhibitors, whose best known member is bovine pancreatic trypsin inhibitor (aprotinin or Trasylo®). Such inhibitors probably serve to limit any potential damage initiated by proteolytic enzymes released from the conceptus itself and may even control trophoblast invasiveness (Mullins et al., 1980; Fazleabas et al., 1982, 1984). In addition, it is conceivable that they may play a role in decreasing local inflammatory responses to the presence of conceptus tissue by neutralizing proteinases released by immune cells.

Lysozyme, which presumably has a bacteriostatic role, is a fairly minor component of uterine flushings. Its activity increases in response to progesterone, but only in rough proportion to the increase in total protein secreted (Hansen et al., 1985). It therefore remains unclear whether it is responsive to progesterone.
### Table 3. Progesterone-stimulated uterine secretory proteins of pigs

| Protein name or description | Molecular weight and other properties | Features of mRNA | Cell type in which expressed | Presumed function |
|----------------------------|--------------------------------------|------------------|-------------------------------|------------------|
| Uteroferrin                | 35 000 glycoprotein; purple with an Fe–Fe center | about 1.5 kb; 340 codons | Uterine glandular epithelium | Iron transport to conceptus |
| Uterine serpins           | 42 000–50 000 glycoproteins (extensively processed). Feature of serine proteinase inhibitors | about 1.5 kb; 417 codons | Uterine glandular epithelium | Unknown |
| Uterine Kunitz inhibitor  | M, 14 000 glycoprotein (β); contains a 6-cysteine Kunitz domain at its NH₂-terminus | 0.8 kb; 122 codons | Surface (predominantly) and glandular epithelium | Inhibits plasmin, trypsin and possibly other serine proteinases |
| Retinol-binding protein   | 21 000 protein; identical to serum retinol-binding protein | about 1 kb; 201 codons | Surface and glandular epithelium | Vitamin A transport to conceptus |
| Lysozyme                  | about 14 000 protein; identical to porcine spleen and stomach enzyme | about 1.4 kb; 119 codons | Unknown | Bacteriostatic |
The final component of histotrophe that will be discussed in any detail is retinol-binding protein (RBP). RBP is seen after analysis of two-dimensional polyacrylamide gel electrophoresis as two to three low molecular weight (Mr about 22,000) acidic (pl about 6.0) polypeptides. Although the uterine RBPs were once considered to be a group of unique molecules with only partial sequence similarity to serum RBP (Clawitter et al., 1990), more detailed analyses and most recently the cloning of their cDNA have confirmed that they are probably identical in sequence to the form secreted by liver. The presence of isoforms probably results from deamidination or other post-translational modifications. RBP is presumed to provide vitamin A to the conceptuses, and, as expected, there is a marked increase in retinol content of uterine flushings associated with RBP secretion. Thus, the RBP in uterine flushings is at least partially loaded with retinol.

Finally, examination of two-dimensional gels on which uterine secretions have been analysed reveals a variety of more minor proteins that have yet to be identified. These probably include growth factors, hormones and hormone-binding proteins that are themselves hormonally responsive (Simmen et al., 1992, 1993). Serum proteins, including albumin and immunoglobulins, are invariably present in various amounts, presumably as a transudate from serum as they do not appear to be synthesized by the endometrium (Basha et al., 1980a,b). In part, their presence may have arisen as an artefact of the flushing process, for unlike uteroferrin, the uterine serpins and RBP, there is no evidence that serum proteins, including transferrin, albumin or immunoglobulins, are taken up by the conceptus at any time during pregnancy (Buhi et al., 1983).

Oestrogen and Control of Uterine Secretory Activity

All of the major components of uterine histotrophe, with the possible exception of lysozyme, the localization and steroid responsiveness of which has not been examined closely, are synthesized by the surface and glandular epithelial cells of the endometrium of ovariectomized gilts in response to prolonged daily treatment with progesterone (Table 3). Oestrogens clearly have a modulatory role. At low daily doses, for example, total protein in uterine flushes increases markedly. At higher amounts, however, secretion of progesterone-induced proteins is markedly depressed (Roberts and Bazer, 1980).

During the oestrous cycle, the progesterone induced components are not secreted in significant quantities until the late luteal phase (day 14–16) (Geisert, 1982a). As with progesterone treatment of ovariectomized gilts, this delay probably represents the time required for the uterine epithelium to develop a fully functional secretory capacity. In pregnancy, however, the pattern of secretion is somewhat different in that there is an abrupt release of secretions into the lumen at the time the conceptuses begin to elongate and to produce oestrogen, i.e. on or about day 12. This release of histotrophe can be mimicked in nonpregnant gilts by a single injection of oestrogen at day 11 (Geisert et al., 1982c). Until recently it was believed that the action of oestrogen was solely on the secretory rather than the biosynthetic limb of the pathway of histotrophe production. This view may have to be modified as it appears that injections of oestradiol markedly upregulate the content of mRNA for some of the secretory proteins, for example RBP and uterine serpin (Trout et al., 1992) but not others, for example uteroferrin (W. E. Trout and M. Stallings-Mann, unpublished; Simmen et al., 1991). Concentrations of RBP and uterine serpin mRNA are markedly higher in uterine horns of day 12 gilts carrying filamentous conceptuses than in horns in which none of the blastocysts has elongated (Fig. 1; Trout et al., 1992).

Protein Composition of Conceptus Secretions at the Time of Maternal Recognition of Pregnancy

LaBonnardière (this supplement) has reviewed the evidence that pig conceptuses secreted both a type I, i.e. interferon α (IFN-α)-like and type II, i.e. IFN-γ-like interferon, during the early elongation phase of development. However, these cytokines have not been implicated in protecting the corpus luteum from the luteolytic effects of uterine-derived PGF\(_2\)\(_a\) as has been demonstrated for the IFN-τ (also a type I IFN) in cattle and sheep. For example, neither Harney and Bazer (1989) nor ourselves (K. Kramer, J. C. Cross and R. M. Roberts, unpublished) have been able to extend luteal lifespan by intrauterine infusion of
Fig. 1. Concentrations (means ± SEM) of vitamin A (retinol) in uterine flushings obtained from gilts on days 11–12 of the oestrous cycle (NP), on day 6 of pregnancy (P) and on days 10–13 of pregnancy. Flushings obtained on days 10–13 of pregnancy were subclassified depending upon whether all of the embryos in the litter were spherical (P–S) or whether some embryos had reached the filamentous stage (P–F) of development. The number of gilts within each group is indicated within each bar. *Significantly different from all other groups ($P < 0.001$).

conceptus secretory proteins presumably containing the two types of IFN. We have also found that recombinant bovine IFN-α was also ineffective in this regard. Nevertheless, the local production of such potent cytokines within the uterine lumen is unlikely to proceed unnoticed by the mother, and it would be surprising if they did not initiate some form of physiological response.

The major proteins released by the conceptuses during the critical day 10–12 period are not, however, IFN but retinol-binding proteins apparently identical in sequence to those produced by the liver and endometrium (Harney et al., 1990; Trout et al., 1991). Presumably, the RBP is secreted as the apo-form, that is carrying no retinol, as mammalian embryos do not store vitamin A. Production of RBP by the conceptus appears to precede uterine RBP mRNA expression in the uterus by at least a day (Trout et al., 1991, 1992). As development progresses, several other proteins, the majority of which have not been characterized, can be identified on polyacrylamide gels (Godkin et al., 1982). Their contributions to maternal–conceptus interactions remain completely unknown.

Vitamin A and its Effects on Reproduction and Embryonic Development

A requirement for vitamin A in reproduction has long been known. Both males and females on a vitamin A-deficient diet become sterile, and lack of vitamin A during pregnancy often causes abortion. In addition, various malformations have been noted in progeny of female rats on a low vitamin A diet (Thompson et al., 1964). Retinoic acid is not an adequate substitute for retinol in preventing these disorders of pregnancy and is most likely not transported across the placenta. Dietary vitamin A, provided in the form of retinol, its esters or precursors such as β-carotene, seems to be required to allow normal rates of cell division, proper organ development, and for growth of the placenta in rats and most probably all mammals (Thompson et al., 1964).

However, the diffuse epitheliochorial nature of the pig placenta is thought to place considerable limitations on the amount of water-insoluble nutrients, such as retinol, that can reach the conceptuses, since such compounds normally require protein chaperones in order to circulate in maternal blood. The
nutrient carrier protein complex must cross several maternal cell layers, including an intact uterine epithelium to reach the pig conceptus from uterine capillaries. In the case of iron, the endometrium synthesizes uteroferrin as an intermediary and not the iron transport protein of serum, transferrin (Roberts and Bazer, 1988). Possibly as a consequence of this indirect mechanism of transport, piglets are almost devoid of iron stores at birth. With retinol, however, the transport protein synthesized by the endometrium appears identical to the serum retinol-binding protein released by the liver (Stallings-Mann et al., 1993). Nevertheless, it seems likely that some of the same limitations exercised over iron transport may still be operative, particularly during a period of vitamin A need. At about day 12 the amount of retinol in uterine flushings during a normal pregnancy rises 10- to 50-fold within hours (Fig. 1) and large amounts of RBP mRNA are detected in the endometrium (Fig. 2; Trout et al., 1992). The actual concentration of retinol to which the embryos are exposed is difficult to estimate because the secretions are diluted by a large excess of saline during uterine flushing, but they must exceed 10 μmol l⁻¹. Thus, the conceptuses themselves appear to signal uterine secretion of retinol and RBP, suggesting that high amounts of retinol may be required at this stage of development.
Paradoxically, such high amounts of retinol may also pose a threat to embryonic survival because in excess, the compound is embryotoxic (Thompson et al., 1964). Moreover, vitamin A derivatives (Isotretinoin or 11-cis-retinoic acid and etretinate) cause abortion and severe teratogenesis in humans and various experimental animals (Lammer et al., 1985). The basis of the teratogenesis possibly lies in the ability of the synthetic retinoids and various byproducts of retinol metabolism to occupy both retinoic acid receptors and the more recently discovered retinoid X receptors (RXR, β and γ). The latter bind 9-cis-retinoic acid, but their affinities for trans-retinoic acid are low (Levin et al., 1992; Heyman et al., 1992). Both types of receptor function somewhat similarly to steroid receptors (Evans, 1988; Beato, 1989). Once occupied, they act as transactivators in a dimeric association to specific nucleotide sequences (cis-enhancer elements), which are usually placed upstream of the transcription start sites of responsive genes, and either increase or decrease transcriptional rates. The situation is, however, quite complex, since there are many kinds of receptor with different tissue distributions. Moreover, the RXR form heterodimeric associations with both the various trans-retinoic acid receptors and the receptors for thyroid hormone and vitamin D (Green, 1993). These complex interactions probably provide for graded responsiveness towards retinoic acid and its derivatives in a very large group of genes. Excess retinoic acid or the presence of unusual retinoic acid homologues probably alters such a balanced response or distorts the normal function of the receptors.

At this stage, it is worth speculating why retinoic acid might be so potent in pigs at the time of blastocyst elongation. First, the considerable change in the general morphology of the conceptus requires extensive tissue remodelling and growth of the extra-embryonic membranes (Geisert et al., 1982b). Retinoic acid is likely to be important in this regard because it is known to influence production of several components of the extracellular matrix (Vasios et al., 1989; Schüle et al., 1990), cell surface adhesive molecules (Agura et al., 1992) and at least one proteinase (urokinase-type plasminogen activator) and its associated inhibitor that are key players in control of matrix breakdown (Tienari et al., 1991). Interestingly, plasminogen activator is released in large amounts as pig blastocysts elongate (Mullins et al., 1980; Fazleabas et al., 1983). A second reason why retinoic acid may be important at day 11 is that porcine embryos are at a critical developmental stage. Formation of the primitive streak and embryo plate are initiated at about this time (Patten, 1948; Jainudeen and Hafez, 1987). Since retinoic acid is known to control genes involved in embryonic organization in chickens (Brickell and Tickle, 1989; Yokouchi et al., 1991), amphibians (López and Carrasco, 1992) and mice (Simeone et al., 1990), it would be surprising if it were not also involved in pigs.

**Retinol-binding Protein, Oestrogen and Embryonic Loss in the Pig: a Hypothesis**

Here we wish to speculate on the interrelated functions of oestrogen and retinol-binding protein during early pregnancy in pigs. First, as discussed in the previous section, we propose that retinol concentrations within the uterine lumen may be limiting at the time of blastocyst elongation. Indeed, provision of retinol and other components released from the endometrium at about day 12 may be required for conceptus elongation. Competent conceptuses probably signal their need for these secretions by synthesizing oestradiol. However, because no retinol storage capacity has been described for the pig uterus, the uterine epithelium must presumably acquire the retinol it needs from the blood circulation and thus may not be able to deliver all of the retinol required for optimal conceptus survival, particularly in the case of large litters. Competition for retinol may therefore be one cause of embryonic loss.

We also propose that advanced conceptuses, which are the first to synthesize oestrogen, are also the first to secrete large quantities of apo-retinol-binding protein, possibly as a result of an autocrine effect of oestradiol. Advanced conceptuses may therefore be able to protect themselves from exposure to the high concentrations of retinol in the uterus at that time. The least advanced conceptuses, which may not secrete sufficient apo-RBP to protect themselves, then die owing to premature and inappropriate gene expression resulting from intracellular conversion of retinol to retinoic acid and its derivatives. The observations pertaining to embryonic death caused by injections of oestradiol at days 9–10.5 of pregnancy or by exposure to zearalenone during the period preceding elongation are entirely consistent with the above hypothesis, although other explanations are clearly possible.

This paper is a contribution from the Missouri Agricultural Experiment Station, Journal Series No. 11,909 and was supported by grants from USDA (89-37240-4586 to R. M. Roberts and 92-37203-7995 to W. E. Trout.)
References

Agura ED, Howard M and Collins SJ (1992) Identification and sequence analysis of the promoter for the leukocyte integrin β-subunit (CD18): a retinoic acid-inducible gene Blood 79 562-569

Ainsworth L, Tsang BK, Bowney BR, Marcus GJ and Armstrong ET (1980) Interrelationship between follicular fluid steroid levels, gonadotropic stimuli and oocyte maturation during preovulatory development of porcine follicles Biology of Reproduction 23 621-627

Anderson LL (1978) Growth, protein content and distribution of early pig embryos Anatomical Record 190 143-153

Archibong AE, England DC and Stormshak F (1987) Ovulation and embryonic survival in pubertal gilts treated with gonadotropin releasing hormone Journal of Animal Science 65 752-755

Ball GD and Day BN (1982) Bilateral luteal maintenance in unilaterally pregnant pigs with infusions of embryonic extracts Journal of Animal Science 54 142-149

Basha SMM, Bazer FW and Roberts RM (1980a) Effect of the conceptus on quantitative and qualitative aspects of uterine secretion in pigs Journal of Reproduction and Fertility 60 41-48

Basha SMM, Bazer FW, Geisert RD and Roberts RM (1980b) Progesterone-induced uterine secretions in pigs: recovery from pseudopregnant and unilaterally pregnant gilts Journal of Animal Science 50 113-123

Bazer FW and Thatcher WW (1977) Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin F<sub>2α</sub> by the uterine endometrium Prostaglandins 14 397-400

Bazer FW, Geisert RD, Thatcher WW and Roberts RM (1982) The establishment and maintenance of pregnancy Control of Pig Reproduction, pp 227-252 Eds DA Cole and GR Foxcroft, Butterworth Scientific, London

Bazer FW, Vallet JL, Roberts RM, Sharp DC and Thatcher WW (1986) Role of conceptus secretory products in establishment of pregnancy Journal of Reproduction and Fertility 76 641-650

Bazer FW, Vallet JL, Harney JP, Gross TS and Thatcher WW (1989) Comparative aspects of maternal recognition of pregnancy between sheep and pigs Journal of Reproduction and Fertility Supplement 37 85-89

Bazer FW, Worthington-White D, Fliss M and Gross S (1991) Uteroferrin: a progesterone-induced hematopoietic growth factor of uterine origin. International Society of Experimental Hematology 19 910-915

Beato M (1989) Review: gene regulation by steroid hormones Cell 56 335-344

Biggs C, Tilton JE, Craigon J, Foxcroft GR, Ashworth CJ and Hunter MG (1993) Comparison of follicular heterogeneity and ovarian characteristics in Meishan and Large-White hybrid pigs Journal of Reproduction and Fertility 97 263-269

Brickell PM and Tickle C (1989) Morphogenesis in chick limb development BioEssays 11 145-149

Buhi WC, Ducasay CA, Bartol FF, Bazer FW and Roberts RM (1983) A function of the allantoic sac in the metabolism of uteroferrin and maternal iron by the fetal pig Placenta 4 455-470

Clawetter J, Trout WE, Burke MG, Araghi S and Roberts RM (1990) A novel family of progesterone-induced, retinol-binding proteins from uterine secretions of the pig Journal of Biological Chemistry 265 3248-3255

Conley AJ, Christenson RK, Ford SD, Geisert RD and Mason JJ (1992) Stereoidogenic enzyme expression in porcine conceptuses during and after elongation Endocrinology 131 896-902

Dhindsa DS and Dziuk PJ (1968) Effect on pregnancy in the pig after killing embryos or fetuses in one uterine horn in early gestation Journal of Animal Science 27 122-126

Dziuk P (1985) Effect of migration, distribution and spacing of pig embryos on pregnancy and fetal survival Journal of Reproduction and Fertility Supplement 33 57-63

Evans RM (1988) The steroid and thyroid hormone receptor family Science 240 889-895

Fazleabas AT, Bazer FW and Roberts RM (1982) Purification and properties of progesterone-induced plasmin/trypsin inhibitor from uterine secretions of pigs and its immuno- cytochemical localization in the pregnant uterus Journal of Biological Chemistry 257 6886-6897

Fazleabas AT, Geisert RD, Bazer FW and Roberts RM (1983) The relationship between release of plasminogen activator and oestrogen by blastocysts and secretion of plasmin inhibitor by uterine endometrium in the pregnant pig Biology of Reproduction 29 225-238

Fazleabas AT, Bazer FW, Hansen PJ, Geisert RD and Roberts RM (1984) Differential patterns of secretory protein localization within the pig uterine endometrium Endocrinology 116 240-245

Fischer HE, Bazer FW and Fields MJ (1985) Steroid metabolism by endometrial and conceptus tissues during early pregnancy and pseudopregnancy in swine Journal of Reproduction and Fertility 75 69-78

Fliss AE, Michel FJ, Chen CL, Hofig A, Bazer FW, Chou JY and Simmen RCM (1991) Regulation of the uteroferrin gene promoter in endometrial cells: interactions among estrogen, progesterone, and prolactin Endocrinology 129 697-704

Ford SP and Christenson RK (1979) Blood flow to uteri of sows during the estrous cycle and early pregnancy: local effect of the conceptus on the uterine blood supply Biology of Reproduction 21 617-624

Ford SP, Christenson RK and Ford JJ (1982) Uterine blood flow and uterine arterial, venous and laminal concentrations of oestrogens on days 11, 13 and 15 after oestrus in pregnant and nonpregnant sows Journal of Reproduction and Fertility 64 185-190

Frank M, Bazer FW, Thatcher WW and Wilcox CJ (1978) A study of prostaglandin F<sub>2α</sub> as the luteolytic in swine: IV. An explanation of the luteotropic effect of estradiol Prostaglandins 15 131-160

Gardner ML, First NL and Casida LE (1963) Effect of exogenous estrogens on corpus luteum maintenance in gilts Journal of Animal Science 22 132-134

Geisert RD, Renegar RH, Thatcher WW and Wilcox CI (1978a) Establishment of pregnancy in the pig. I. Interrelationships between preimplantation development of the pig blastocyst and uterine endometrial secretions Biology of Reproduction 27 925-939

Geisert RD, Brookbank JW, Roberts RM and Bazer FW (1982a) Establishment of pregnancy in the pig. II. Cellular remodeling of the porcine blastocyst during elongation on day 12 of pregnancy Biology of Reproduction 27 941-955

Geisert RD, Thatcher WW, Roberts RM and Bazer FW (1982c) Establishment of pregnancy in the pig. III. Endometrial secretory response to estradiol valerate administered on day 11 of the estrous cycle Biology of Reproduction 27 957-965
Geisert RD, Zavy MT, Wettmann RP and Biggers BG (1987) Length of pseudopregnancy and pattern of uterine protein release as influenced by time and duration of oestrogen administration in the pig. *Journal of Reproduction and Fertility* 79:163–172

Geisert RD, Morgan GL, Zavy MT, Blair RM, Gries DK, Cox A and Yellin T (1991) Effect of asynchronous transfer and estrogen administration on survival and development of porcine embryos. *Journal of Reproduction and Fertility* 93:475–481

Glossop CE and Foulkes JA (1988) Occurrence of two phases of nuclear hormone receptors: promiscuous liaison. *Nature* 331:590–591

Gries DK, Geisert RD, Zavy MT, Garrett JE and Morgan GL (1989) Uterine secretory alterations coincident with embryonic mortality in the gilt after exogenous estrogen administration. *Journal of Animal Science* 67:276–284

Hansen PJ, Bazer FW and Roberts RM (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. *Journal of Reproduction and Fertility* 73:411–424

Harney JP and Bazer FW (1989) Effect of porcine conceptus secretory proteins on interoestrous interval and uterine secretion of proglandins. *Biography of Reproduction* 41:277–284

Harney JP, Miranda MA, Smith LC and Bazer FW (1990) Retinol-binding protein: a major secretory product of the pig conceptus. *Biography of Reproduction* 42:523–532.

Heap RB, Flint APF, Gadsby JE and Rite C (1979) Hormones, the early embryo and the uterine environment. *Journal of Reproduction and Fertility* 55:267–275

Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eidehe C, Evans RM and Thaller C (1992) 9-Cis retinoic acid is a high affinity ligand for the retinoid X receptor X receptor Cell 68:397–406

Hunt DF, Yates JR, Ill, Shabanowitz J, Zhu N-Z, Zirino T, Averill BA, Daurat-Laroque ST, Shewale JC, Roberts RM and Brew K (1987) Sequence homology in the metalloproteins: purple acid phosphatase from beef spleen and uteroferrin from porcine uterus. *Biochemical and Biophysical Research Communications* 144:1154–1160

Ing NH and Roberts RM (1989) The major progesterone-modulated proteins secreted into the sheep uterus are members of the serpin superfamily of serine protease inhibitors. *Journal of Biological Chemistry* 264:3372–3379

Jainudeen MR and Hafez ESE (1987) Gestation, prenatal physiology and parturition. *Reproduction in Farm Animals* (5th Edn) pp. 229–259. Ed. ESE Hafez. Lea & Febiger, Philadelphia

Jarrell VL, Beckmann LS, Cantley TC, Rieke AR and Day BN (1990) The effect of exposure to an asynchronous uterus on development of day 4 swine embryos. *Journal of Animal Science* 68 (Supplement 1) 429

Keys JL and King GJ (1990) Microscopic examination of porcine conceptus maternal interface between days 10 and 19 of pregnancy. *American Journal of Anatomy* 188:221–238

LaBonnardière C, Martinat-Botte F, Terqui M, Lefèvre F, Zouari K, Martal J and Bazer FW (1991) Production of two species of interferon by Large White and Meischan pig conceptuses during the peri-attachment period. *Journal of Reproduction and Fertility* 91:469–478

Laforest JP and King GJ (1992) Effect of intrauterine application of oestriadiol-17β and proglandin E-2 on the porcine oestrous cycle and uterine endocrinology. *Journal of Reproduction and Fertility* 94:381–394

Lambert E, Williams DII, Lynch PB, Hansen TJ, McGeady TA, Austin FH, Boland MP and Roche JF (1991) The extent and timing of prenatal loss in gilts. *Theriogenology* 36:653–665

Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Gris AW, Lott JT, Richard JM and Sun SC (1985) Retinoic acid embryopathy. *New England Journal of Medicine* 313:837–841

Levin AA, Sturzenbecker LJ, Kazmer S, Bosakowski H, Haselton C, Allenby G, Speck J, Kratzesice LN, Rosenberger M, Lovey A and Grippo J (1992) 9-Cis retinoic acid stereosensor binds and activates the nuclear receptor RXR alpha. *Nature* 355:359–361

Ling P and Roberts RM (1993) Uteroferrin and intracellular trastate-resistant acid phosphatases are products of the same gene. *Journal of Biological Chemistry* (in press)

Long GG and Dickman MA (1984) Effect of purified zearalenone on early gestation in gilts. *Journal of Animal Science* 59:1662–1670

Long GC, Turek J, Dickman MA and Scheidt AB (1992) Effect of zearalenone on Days 7 to 10 post mating on blastocyst development and endometrial morphology in sows. *Veterinary Pathology* 29:60–67

Lopez SL and Carrasco AE (1992) Retinoic acid induces changes in the localization of homeobox proteins in the anterior–posterior axis of Xenopus laevis embryos. *Mechanisms of Development* 36:153–164

McDowell KJ, Sharp DC, Fazleabas A, Roberts RM and Bazer FW (1982) Partial characterization of the equine uteroferrin-like protein. *Journal of Reproduction and Fertility Supplement* 32:329–334

Magnes RR and Ford SP (1982) Steroid concentrations in uterine lymph and uterine arterial plasma of gilts during the estrous cycle and early pregnancy. *Biography of Reproduction* 27:871–877

Malathy P-V, Imakawa K, Simmen RCM and Roberts RM (1990) Molecular cloning of the uteroferrin-associated protein, a major progesterone-induced serum secreted by the porcine uterus, and the expression of its mRNA during pregnancy. *Molecular Endocrinology* 4:428–440

Mandschein JS, Hersey RM, Dey SK, Davis DL and Weisz J (1985) Catechol estrogen formation by pig blastocysts during the preimplantation period: biochemical characterization of estrogen-2/4-hydroxylase and correlation with aromafase activity. *Endocrinology* 117:2339–2346

Morgan GL, Geisert RD, Zavy MT, Shawley RV and Fazleabas AT (1987) Development of pig blastocysts in a uterine environment advanced by exogenous oestrogen. *Journal of Reproduction and Fertility* 80:125–131

Mullins DE, Bazer FW and Roberts RM (1980) Secretion of a progesterone-associated inhibitor of plasminogen activator by the porcine uterus. *Cell* 20:865–872

Murray MK, Malanthy PV, Bazer FW and Roberts RM (1989) Structural relationship, biosynthesis and immunocytochemical localization of uteroferrin-associated basic glycoproteins. *Journal of Biological Chemistry* 264:4143–4150

Pack BA and Brooks SC (1974) Cyclic activity of estrogen sulfotransferase in the gilt uterus. *Endocrinology* 95:1690–1690

Patten BM (1948) *Embryology of the Pig* (3rd Edn) The Blakiston Co., Philadelphia
Ferry JS (1960) The incidence of embryonic mortality as a characteristic of the individual sow journal of Reproduction and Fertility 17:1-83
Ferry JS and Rowlands IW (1962) Early pregnancy in the pig Journal of Reproduction and Fertility 6: 175-188
Ferry JS, Heap RB, Burton RD and Gadsby JE (1976) Endocrinology of the blastocyst and its role in the establishment of pregnancy Journal of Reproduction and Fertility Supplement 25 85-104
Polge C (1982) Embryo transplantation and preservation. Control of Pig Reproduction, pp 277-291 Eds DJA Cole and GR Foxcroft. Butterworth Scientific, London
Pope WF (1988) Uterine asynchrony: a cause of embryonic loss Biology of Reproduction 39 999-1003
Pope WF (1992) Embryogenesis recapitulates oogenesis in swine (minireview) Proceedings of the Society of Experimental Biology and Medicine 199 273-281
Pope WF and First NL (1985) Factors affecting the survival of pig embryos Theriogenology 23 91-103
Pope WF, Maurer RR and Stormshak F (1982) Intraperitoneal injection of estradiol-17B and histamine Biology of Reproduction 27 575-579
Pope WF, Wilde MH and Xie S (1985) Effect of electrocautery of nonovulated day 1 follicles on subsequent morphological variation among day 11 porcine embryos Biology of Reproduction 39 882-887
Pope WF, Xie S, Broermann DM and Nephew KP (1990) Causes and consequences of early embryonic diversity in the pig Journal of Reproduction and Fertility Supplement 40 251-260
Roberts RM and Bazer FW (1980) The properties, hormonal control and synthesis of uteroferrin, the purple protein of the pig uterus Steroid-Induced Uterine Proteins, pp 133-149 Ed. M Beatol Elsevier/North-Holland, Amsterdam
Roberts RM and Bazer FW (1988) The functions of uterine secretions and their role in the establishment of pregnancy Biology of Reproduction 39 882-887
Roberts RM, Raub TJ and Bazer FW (1986) The role of uteroferrin in transplacental iron transport in the pig Federation Proceedings 45 2513-2518
Roberts RM, Trout WE, Mathialagan N, Stallings-Mann M and Ling P (1993) Uterine secretory activity and embryo development. Preimplantation Embryo Development pp. 229-243 Ed. BD Bavister. Springer-Verlag, New York
Saunders MJ, Edgerton LA, Kagan JM, Stahly TS and Cromwell GL (1983) Comparison of intraperitoneal and subcutaneous sites of estrogen injection for luteal maintenance in swine Journal of Animal Science 57 146-149
Schüle R, Umesano K, Mangelsdorf DJ, Bolado J, Pilk JW and Evans RM (1990) Jun-fos and receptors for vitamin A and D recognize a common response element in the human osteocacin gene Cell 61 497-504
Simeone A, Acampora D, Ariconi L, Andrews PW, Boncini E and Mavilo F (1990) Sequential activation of Hox 2 homeobox genes by retinoic acid in human embryonal carcinoma cells Nature 346 763-766
Simmen RCA, Simmen FA, Geisert RD, Martinat-Botte F, Bazer FW and Terqui M (1992) Differential expression, during the oestrous cycle and pre- and post-implantation conceptus development, of messenger ribonucleic acids encoding components of the pig uterine insulin-like growth factor system Endocrinology 130 1547-1555
Simmen RCA, Ko Y and Simmen FA (1993) Insulin-like growth factors and blastocyst development Theriogenology 39 163-175
Soede NM, Noordhuizen JPTM and Kemp B (1992) The duration of ovulation in pigs, studied by transrectal ultrasonography, is not related to early embryonic diversity Theriogenology 38 653-668
Stallings-Mann ML and Trout WE (1993) Molecular cloning and expression of a Kunitz-type protease inhibitor secreted by the porcine uterus Biology of Reproduction (Abstract) (in press)
Stallings-Mann ML, Trout WE and Roberts RM (1993) Porcine retinol-binding proteins are identical gene products to the serum retinol-binding proteins Biology of Reproduction (in press)
Stone BA and Sezai MF (1985) Steroid hormones in uterine washings and in plasma of gilts between Days 9 and 15 after coitus Journal of Reproduction and Fertility 75 209-221
Stoner CS, Geisert RD, Bazer FW and Thatcher WW (1981) Characterization of estrogen patterns in early pregnancy and cyclic gilts Journal of Animal Science 53 (Supplement 1) 308 (Abstract)
Thompson JN, Howell J and Pitt GAG (1964) Vitamin A and reproduction in rats Proceedings of the Royal Society of London Series B159 510-535
Tienari J, Alanko T, Lehtonen E and Saksela O (1991) Expression and localization of a urokinase-type plasminogen activator and its type 1 inhibitor are regulated by retinoic acid and fibroblast growth factor in human teratocarcinoma cells Cell Regulation 2 285-297
Trout WE, McDonnell JJ, Kramer KK, Baumbach CA and Roberts RM (1991) The retinol-binding protein of the expanding pig blastocyst: molecular cloning and expression in trophoetoderm and embryonic disc Molecular Endocrinology 5 1533-1540
Trout WE, Hall JA, Stallings-Mann ML, Galvin JA, Anthony RV and Roberts RM (1992) Steroid regulation of the synthesis and secretion of retinol-binding protein by the uterus of the pig Endocrinology 130 2577-2584
van der Meulen J, Elseaer F, Oudenaarden CP and Helmond FA (1991) Effect of intra-uterine oestriol-17B administration on mid-oestrous interval in the pig Animal Reproduction Science 24 305-313
Vasiis GW, Gold JD, Petrovich M, Chambon P and Gudas LJ (1989) A retinoic acid-responsive element is present in the 5' flanking region of the lamin B1 gene Proceedings of the National Academy of Sciences USA 86 9099-9103
Wild M and Pope WF (1987) Stage-dependent synthesis of estradiol by porcine blastocysts Journal of Animal Science 65 (Supplement 1) 87 (Abstract)
Wild M, Xie S, Day ML and Pope WF (1988) Survival of small and large littermate blastocysts in swine after synchronous and asynchronous transfer procedures Theriogenology 30 1069-1074
Xie S, Broermann DM, Nephew KP, Geisert RD and Pope WF (1990a) Ovulation and early embryogenesis in swine Biology of Reproduction 43 236-240
Xie S, Broermann DM, Nephew KP, Ottobre JS, Day ML and Pope WF (1990b) Changes in follicular endocrinology during final maturation of porcine oocytes. *Domestic Animal Endocrinology* 7 75–82.

Yokouchi Y, Ohsugi K, Sasaki H and Kuroiwa A (1991) Chicken homeobox gene Msx-1: structure, expression in limb buds and effect of retinoic acid. *Development* 113 431–444.

Young KH, Kraeling RR and Bazer FW (1989) Effects of prolactin on conceptus survival and uterine secretory activity in pigs. *Journal of Reproduction and Fertility* 86 713–722.

Zavy MT, Sharp DC, Bazer FW, Fazleabas A, Sessions F and Roberts RM (1982) Identification of stage-specific and hormonally induced polypeptides in the uterine protein secretions of the mare during the oestrous cycle and pregnancy. *Journal of Reproduction and Fertility* 64 199–207.