

## BLASTOCYST-ENDOMETRIUM INTERACTIONS AND THEIR SIGNIFICANCE IN EMBRYONIC MORTALITY

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One of the most important economic considerations for the commercial pig breeder is the number of pigs marketed per sow per year. With adequate nutritional and environmental regimes, the realization of maximal productivity is limited by the sow's reproductive potential, which in theory can be increased by reducing the farrowing interval (for instance through early weaning) and by achieving a high litter size (by raising ovulation rate and reducing embryonic, perinatal and pre-weaning losses). For a given ovulation rate, litter size is determined by losses at fertilization, during gestation and in the perinatal period. Of these, loss during gestation is quantitatively the most relevant; the majority of this loss occurs before or during embryogenesis, and is termed *embryonic mortality*.

It has been known for some time that embryonic mortality is relatively high in the pig (Hammond, 1914; Corner, 1923; Perry, 1954; Hanly, 1961;

**Table 13.1** ESTIMATES OF PRENATAL LOSS IN PIGS: TIME OF EMBRYONIC OR FOETAL DEATH

| <i>Type of animal</i> | <i>Stage of pregnancy at slaughter (days)<sup>(a)</sup></i> | <i>Mortality (%)<sup>(b)</sup></i> | <i>References</i>                  |
|-----------------------|---|------------------------------------|------------------------------------|
| Sows                  | F   | 27                                 | Hammond (1914)                     |
| Sows                  | 14-60   | 33                                 | Hammond (1921)                     |
| Sows                  | F   | 44                                 | Casida (1956)                      |
| Sows + gilts          | 25/F  | 33/40                              | Perry (1954)                       |
| Gilts                 | 25/70   | 43/50                              | Baker <i>et al.</i> (1956)         |
| Sows                  | F   | 41                                 | Lasley (1957)                      |
| Sows                  | 28  | 39                                 | King and Young (1957)              |
| Gilts                 | 17/25   | 25/34                              | Lerner, Mayer and Lasley (1957)    |
| Gilts                 | 55  | 23                                 | Reddy, Mayer and Lasley (1958)     |
| Gilts                 | 25/70   | 30/48                              | Baker <i>et al.</i> (1958)         |
| Gilts                 | 25/40   | 33/38                              | Day <i>et al.</i> (1959)           |
| Sows                  | F   | 39                                 | Pomeroy (1960)                     |
| Gilts + sows          | 13-18/26-40   | 28/35                              | Perry and Rowlands (1962)          |
| Sows                  | 36-109  | 46                                 | Marrable and Ashdown (1967)        |
| Sows                  | 9/13  | 21/52                              | Scofield, Clegg and Lamming (1974) |
| Sows                  | 25  | 17                                 | Dyck (1974)                        |
| Gilts                 | 9-18  | 17                                 | Anderson (1978)                    |

<sup>(a)</sup>Where animals were slaughtered at two different times during gestation, both are given, together with corresponding mortality rates. F = data obtained at farrowing.

<sup>(b)</sup>Mortality rates determined from numbers of ova ovulated (taken as equal to number of corpora lutea).

Scofield, 1972; see *Table 13.1*). There is little evidence to suggest it is decreasing, and no generally accepted explanation has been put forward for it (Wrathall, 1971). Rates of embryonic mortality, calculated by counting embryos and using numbers of corpora lutea as a measure of the number of eggs ovulated, usually range from 20–45%; and although this figure may under some circumstances be raised (for instance by mating late in oestrus or by exposure to high ambient temperatures), attempts to reduce embryonic losses have almost consistently failed. The purpose of this chapter is to describe some of the physiological and endocrine events occurring at the time when most embryonic loss takes place, and thereby to attempt to identify processes whose failure may cause embryos to die. It will be seen that although the study of early embryonic development has yet to lead to the identification of a generally applicable cause of embryonic mortality, it provides information which is likely to be of great value to those involved in reducing this form of loss.

To some degree, embryonic loss can be associated with known abnormalities in the fertilized ovum and with conditions of animal husbandry. Chromosomal aberrations in the conceptus are probably a major cause of embryonic death, which Bishop (1964) has described as 'unavoidable and should be regarded as a normal way of eliminating unfit genotypes in each generation'. This is the only form of embryonic mortality associated with a demonstrable abnormality in the conceptus, and it can be induced by procedures allowing polyspermic fertilization, such as mating late during oestrus (Hunter, 1967) or progesterone treatment before mating (Polge and Dziuk, 1965; Day and Polge, 1968). Such structural abnormalities in the conceptus are, of course, distinct from genetic traits which may lead to increased embryonic death through inheritance of homozygous recessive genes coding for lethal characteristics expressed in early pregnancy (Bishop, 1964). The existence of genetic factors of this kind is suggested by the observations that high loss rates are sometimes found in offspring of the same boar (Perry and Rowlands, 1962), and that losses in inbred strains are reduced by outcrossing (Squires, Dickerson and Mayer, 1952; Rampacek, Robison and Ulberg, 1975). Conditions such as heat stress (Omtvedt *et al.*, 1971; Wildt, Riegle and Dukelow, 1975; Cameron, 1980), plane of nutrition (Robertson *et al.*, 1951; Tassell, 1967) and intrauterine infections (Schofield, Clegg and Lamming, 1974), are also associated with increased embryonic mortality, though it is not certain how these conditions cause death of the conceptuses. In addition there are seasonal variations in fecundity (Stork, 1979), such as those thought to occur in European wild pigs (Mauget, 1978), which may be due in part to increased embryonic loss. However, it seems unlikely that conditions such as these account for all the embryonic deaths observed, and because of this it is concluded that some loss may occur which is of unknown aetiology, hereafter referred to as 'basal loss'. Embryonic death due to chromosomal aberrations and environmental conditions may be presumed to be either unavoidable, or susceptible to reduction by changes in animal husbandry; therefore it would appear that basal loss is the category in which improvements may be made through the study of the physiology of early pregnancy.

Given that it is required to reduce basal embryonic loss, then it is desirable to know what proportion of loss is represented by this category.

Unfortunately this is difficult to estimate. From the examination of karyotypes of blastocysts flushed from uteri of pigs at day 10 post coitum, McFeely (1967) and Day and Polge (1968) suggested that 8–12% of fertilized eggs bear abnormalities probably reflecting polyspermy, and which are likely to be lethal. The occurrence of identified chromosomal abnormalities leading to embryonic death before day 9 generally appears to be low; recoveries of blastocysts indicate most loss occurs after day 10 (McFeely, 1967; but see Bouters, Bonte and Vandeplassche, 1974). If loss due to environmental factors is minimized, and the embryonic death rate is 30–40%, subtracting a 10% loss due to chromosomal abnormalities leaves a basal loss rate of 20–30%. However, a major flaw in this argument is the assumption that loss due to environmental factors was low in the herds in which total loss was shown to be 30–40%; much of this may be represented by animals suffering subclinical intrauterine infections, which could occur in as many as 30–45% of sows and double the rate of embryonic death in them (Scofield, 1972; Scofield, Clegg and Lamming, 1974). Thus it seems difficult at present to assign an upper limit to the proportion of embryonic death likely to be caused by chromosomal aberrations, and this leads to the possibility that the majority of loss may be accounted for by chromosomal *plus* identified environmental factors, with no contribution due to basal loss. However, there is independent evidence in the pig, as in many other polytocous animals, that endometrial factors influence blastocyst growth and survival. Transplantation of pre-elongation porcine blastocysts to the ureter or to the outer wall of the uterus shows that in such sites trophoblast growth is invasive and abnormal (Samuel, 1971; Samuel and Perry, 1972). In fact the ectopic trophoblast appears to undergo a syncytial transformation reminiscent of the invasive trophoblast of ruminants, a situation not known to exist *in utero*. This suggests there are influences acting on developing embryos *in utero* to reduce or control their growth, and this conclusion is supported by the lack of success of embryo transfer experiments in increasing litter size by the number of embryos transferred (Webel, Peters and Anderson, 1970), and by *in vitro* evidence which suggests the existence of endometrial factors required for morula development to proceed past the 4- to 8-cell stage (see Polge, Chapter 14).

As shown in *Table 13.1*, experiments in which embryos have been counted in the uterus at varying stages of pregnancy reveal that the majority of embryonic loss occurs before day 25 of gestation. This is perhaps not surprising, in view of the complexity of the physiological events occurring at this stage of gestation; this period includes the time of the maternal recognition of pregnancy (when on day 11 post coitum, the corpora lutea of the cycle are prevented from regressing, and so become the corpora lutea of pregnancy; Dhindsa and Dziuk, 1968), the formation of mesoderm and the elongation of the blastocyst, the attachment of the blastocyst to the endometrial epithelium, and increased secretion of several specific endometrial proteins. Since increases in the number of ova by superovulation and/or superinduction have failed to result in a consistent increase in litter size it has been proposed that the uterus imposes a limit on the number of developing embryos. Although physical overcrowding may result in foetal death during the last trimester, results obtained when embryos were restricted to parts of the uterus (Dziuk, 1968; Ulberg

and Rampacek, 1974) indicate that physical overcrowding is unlikely to account for a significant proportion of the loss observed prior to day 25. Consequently emphasis has been placed on biochemical interactions between the embryo and the endometrium and it has been postulated that litter size is limited by the availability of an essential biochemical factor (Bazer, 1975). In subsequent sections we will deal with substances known to be produced by the endometrium and blastocyst which may be involved in such interactions.

## Components of blastocyst–endometrial interactions

### ENDOMETRIAL PRODUCTS

The non-invasive nature of placentation in the pig might be expected to be associated with the secretion, by the endometrial glands and epithelium, of a high proportion of the nutritional requirements of the trophoblast (Amoroso, 1952; Dantzer, Björkman and Hasselager, 1981). Therefore it would not be surprising if endometrial secretions (histiotrophe) were complex in this species.

#### *Proteins*

A number of characteristic proteins appear in uterine flushings during the luteal phase of the oestrous cycle, when progesterone levels are high (Murray *et al.*, 1972; Squire, Bazer and Murray, 1972) and the same endometrial secretory proteins can be induced in ovariectomized pigs by treatment with progesterone (Knight, Bazer and Wallace, 1973; Roberts *et al.*, 1976). They are also produced in early pregnancy (Zavy *et al.*, 1977). Three of these proteins (an iron-containing purple acid phosphatase, uteroferrin, which is also known as purple protein, lysozyme and leucine aminopeptidase) are enzymes, and have been shown to accumulate in allantoic fluid after day 30 of pregnancy (Bazer *et al.*, 1975; Roberts *et al.*, 1976). Results of immunofluorescence studies support the concept that components of histiotrophe, such as uteroferrin, are secreted by the endometrial glands and pass into the foetus via the chorio-allantoic areolae (Chan *et al.*, 1975).

#### *Prostaglandins*

In common with other species the pig endometrium produces prostaglandin  $F_{2\alpha}$  *in vitro* (Patek and Watson, 1976; Guthrie and Rexroad, 1980; 1981), and the raised levels of prostaglandin  $F_{2\alpha}$  reported in the uterine venous effluent towards the end of the oestrous cycle (Gleeson and Thorburn, 1973; Moeljono *et al.*, 1977) are likely to be endometrial in origin. Prostaglandin  $F_{2\alpha}$  and its analogues are luteolytic on administration to pigs after day 12 post coitum and it is postulated that prostaglandin  $F_{2\alpha}$  is the uterine luteolysin in this species (Gleeson, 1974; Hallford *et al.*, 1975; Guthrie and Polge, 1976; Moeljono, Bazer and Thatcher, 1976; Guthrie and Polge, 1978).

### Steroids

Porcine endometrium contains enzymes catalyzing the reductive metabolism of progesterone to pregnanones and pregnanediols (Henricks and Tindall, 1971). In addition it has recently been shown that the endometrium may synthesize  $C_{21}$  and  $C_{19}$  steroids, and that this may be physiologically significant. In particular  $3\beta$ -hydroxysteroid dehydrogenase is present in the endometrium on day 17 post coitum in pregnant animals, and at every stage of pregnancy examined thereafter (V.A. Craig, unpublished observations). Small amounts of androgens and oestrogen have also been shown to be synthesized in pregnant endometrium (Dueben *et al.*, 1979), and this may provide aromatizable steroid to the blastocyst, thereby contributing towards oestrogen synthesis in early pregnancy.

Oestrogens circulate in early pregnancy predominantly in the form of oestrone sulphate (Robertson and King, 1974) which is thought to be formed as a result of sulphation in the endometrium of unconjugated oestrogens produced by the blastocyst (and also, possibly, the endometrium itself). The sulphokinase responsible for this process is a progesterone-dependent enzyme, the activity of which alters during the oestrous cycle in parallel with circulating progesterone concentrations (Pack and Brooks, 1974). Measurement of oestrone sulphate in peripheral plasma between days 26–29 post coitum has been proposed as a pregnancy test (Saba and Hattersley, 1981); furthermore, recent studies of Stoner *et al.* (1980) have revealed that circulating oestrone sulphate levels at day 30 of pregnancy may be correlated with litter size and total litter weight at birth, suggesting that measurement of oestrone sulphate in blood in early pregnancy may provide a useful indication of placental functions affecting foetal development and/or survival. However it is not established whether high oestrone sulphate levels simply reflect increased placental weight in view of the uncertain contribution from the endometrium. Furthermore, other authors have failed to confirm the relationship between oestrone sulphate levels and litter size (Hattersley *et al.*, 1980).

### Unidentified endometrial products

In an attempt to evaluate effects of endometrial products on blastocyst metabolism, co-culture techniques have been applied, utilizing blastocyst and endometrium explants and determining blastocyst protein or DNA synthesis. In the technique used by Wyatt (1976) and by Rice, Ackland and Heap (1981) blastocysts dissected from the endometrium on day 16 post coitum were cultured (5 mm lengths on lens tissue at a fluid/gas interface) with or without explants ( $2\text{ mm}^3$ ) of maternal tissues. Protein synthesis was monitored by incorporation of [ $^3\text{H}$ ]leucine. Leucine incorporation into blastocyst tissue proteins and proteins recovered from the medium was significantly raised if embryonic tissue was cultured together with endometrium, when blastocyst protein synthesis was low; however this effect was absent when the basal rate of protein synthesis was higher. Other maternal tissues were without effect. Disc gel electrophoresis of blastocyst extracts after culture revealed that stimulated leucine incorporation reflected increased synthesis of specific pre-albumins (with molecular

weights 25 000–30 000). It is not certain what product of the endometrium is responsible for increasing leucine incorporation into blastocyst prealbumins; experiments with a variety of purified proteins and serum have failed to identify stimulants, with the possible exception of uteroferrin (Rice, Ackland and Heap, 1981).

Similar explant culture techniques have been used to investigate effects of endometrial products on blastocyst DNA synthesis, using [<sup>3</sup>H]thymidine as precursor (Flint, 1981). A component of uterine flushings obtained from ovariectomized gilts treated chronically with progestagen has been shown to stimulate incorporation of [<sup>3</sup>H]thymidine into DNA; the active component is present in a small molecular weight, non-basic fraction, and is unstable during storage lyophilized at 4°C. Preparations in which the stimulatory activity has decomposed are inhibitory when tested in the same culture system, suggesting either that the stimulatory materials break down to yield inhibitors of thymidine incorporation, or that stimulators mask the effects of inhibitors, the latter being present throughout. Further work will be required to decide between these two possibilities.

An attempt has also been made to isolate proteins which may play a role in the maintenance of the preimplantation blastocyst by employing a number of immunological techniques (P.T.K. Saunders, unpublished observations). Antisera were raised in rabbits against endometrial tissue cytosols and maternal plasmas taken from pregnant and non-pregnant sows 12 and 16 days after the onset of oestrus. Proteins not unique to the endometrial tissue were selectively absorbed by chromatography on Affigel blue (Bio-Rad Laboratory Ltd) followed by absorption with an immobilized antiserum immunoabsorbent (Arrameas and Ternynck, 1969). Unabsorbed proteins were visualized by crossed immunoelectrophoresis into gels containing immunoglobulins raised against tissue and fluids from pregnant and non-pregnant sows. Whilst preliminary studies resulted in the isolation of protein(s) with  $\alpha$  electrophoretic mobility at pH 8.6 unique to gravid endometrial tissue (day 16), contradictory results were obtained from a more detailed examination of the proteins present in both gravid and non-gravid uteri. Therefore this work and that of Zavy *et al.* (1977) and Basha, Bazer and Roberts (1979) provides no clear evidence that the endometrium synthesizes any unique proteins during pregnancy.

#### *Other components of endometrial secretion*

In addition to the components described above, endometrial secretions may be assumed to contain other compounds required for the growth of the conceptus. Those isolated from uterine flushings to date include the vitamins riboflavin (Murray, Moffatt and Grifo, 1980; Moffatt *et al.*, 1980) and retinol (vitamin A) (Adams, Bazer and Roberts, 1981).

#### BLASTOCYST PRODUCTS

##### *Steroids*

X One of the best known properties of the pig blastocyst is its ability to synthesize oestrogens. Since Perry, Heap and Amoroso (1973) first demonstrated aromatase activity in preimplantation pig blastocysts, the

initial findings have been both confirmed and extended (Perry *et al.*, 1976; Flint *et al.*, 1979; Gadsby, Heap and Burton, 1980). Aromatase activity becomes measurable between days 12 and 14 after mating and has been found as early as day 10 (i.e. before blastocyst elongation) in ovariectomized gilts treated with medroxyprogesterone acetate (Heap *et al.*, 1981). Other enzymes of oestrogen synthesis ( $3\beta$ -hydroxysteroid dehydrogenase,  $17\alpha$ -hydroxylase and C-17,20-lyase) are also present in the blastocyst on days 16 and 20, and blastocysts in culture produce progesterone, androstenedione and oestrogens (Heap, Flint and Gadsby, 1981). They also contain high concentrations of these steroids (Gadsby and Heap, 1978).

Since all the enzymes of oestrogen synthesis from pregnenolone are present in the elongated blastocyst, it seems likely that the conceptus may be capable of sufficient *de novo* steroid synthesis to account for the rise in oestrogens in the peripheral plasma on days 25 to 30 post coitum. This has been tested in ovariectomized gilts in which pregnancy was maintained by administering a synthetic progestin (medroxyprogesterone acetate); the results show that peripheral circulating oestrone sulphate concentrations are normal in ovariectomized animals, rising to levels identical to those in intact controls (Heap *et al.*, 1981). However in view of recent evidence that the endometrium has the potential to contribute to oestrogen synthesis, it is not certain to what extent this represents steroidogenesis confined to the blastocyst.

### Proteins

Among the trophoblast-specific proteins identified in other domestic animals are hormones such as chorionic gonadotrophins (found in the horse) and placental lactogens (which are present in the blastocysts of the sheep and cow). Although no placental lactogen has been demonstrated in the Suidae (the pig and the hippopotamus have been investigated; Kelly *et al.*, 1976; A.P.F. Flint, unpublished observations; W.B. Currie, personal communication), there is some evidence for a chorionic gonadotrophin. An LH-like material, which cross reacts in a radioreceptor assay but not in a radioimmunoassay has been demonstrated in the pig blastocyst (Saunders, Ziecik and Flint, 1980): this activity is neither due to the presence of a protease, nor to a protein binding, the [ $^{125}$ I]-labelled porcine LH used as tracer, and is present in blastocyst extracts (but not in liver or skeletal muscle) as early as day 10 post coitum. A similar compound has been partially purified by ion exchange chromatography from porcine placentas; however it has not proved consistently possible to obtain a preparation of sufficiently high potency to demonstrate biological activity (one of five preparations were active by rat ovarian ascorbic acid depletion test). At present, therefore, it is not certain whether this material represents a biologically active gonadotrophin.

## Blastocyst-endometrium interactions

### MAINTENANCE OF LUTEAL FUNCTION

One of the better known relationships between the blastocyst and the endometrium is that leading to maintenance of luteal function; in the pig

the blastocyst must exert an antiluteolytic, or luteotrophic, effect before day 11 in order for this to occur, and since the endometrium is the source of the uterine luteolysin, this signal is most probably directed at the endometrium. The maintenance of luteal function ensures a continued supply of progesterone, which in turn is necessary for the secretion of specific endometrial products such as uteroferrin and the constituents of histiotrophe required for blastocyst growth.

In order for luteal function to be maintained in early pregnancy it is important that the effects of the uterine luteolysin are prevented, and there has been much discussion as to how this is brought about. The utero-ovarian venous concentration of prostaglandin F is reduced in early pregnancy relative to the levels found at luteal regression (Moeljono *et al.*, 1977; Zavy *et al.*, 1980) and this reduction is reflected in peripheral levels of the prostaglandin F metabolite, 13,14-dihydro-15-ketoprostaglandin  $F_{2\alpha}$  (Zavy *et al.*, 1980; Guthrie and Rexroad, 1981). Therefore it seems likely that uterine secretion of prostaglandin  $F_{2\alpha}$  is reduced in the presence of embryos on days 16–22 post oestrus, and this is consistent with the reduction in endometrial prostaglandin F production observed *in vitro*, in pregnant animals (Watson and Patek, 1979; Guthrie and Rexroad, 1981). However a reduction in endometrial synthesis of prostaglandin  $F_{2\alpha}$  at this time may not be the only explanation for the decline in utero-ovarian venous concentrations in early gestation, since Bazer and Thatcher (1977) and Zavy *et al.* (1980) have provided compelling evidence for a redirection of release of prostaglandin  $F_{2\alpha}$  away from the vasculature and into the lumen of the uterus in pregnant animals (see Bazer, Chapter 12). Whatever the mechanism underlying the reduction in blood prostaglandin levels, it should be noted that this effect is unlikely simply to reflect increased utero-ovarian blood flow during pregnancy (which Ford and Christensen, 1979, have demonstrated on days 11–13 post coitum), since peripheral prostaglandin metabolite levels are also reduced (Zavy *et al.*, 1980).

Decreased endometrial synthesis of prostaglandin  $F_{2\alpha}$ , or a redirection of its secretion, with a resulting reduction in utero-ovarian venous prostaglandin concentrations, provides a satisfactory explanation for the lack of a uterine luteolytic effect during pregnancy. However, it is difficult to rule out the possibility that the decline in prostaglandin F production is a result, rather than a cause, of luteal maintenance, as has been suggested previously in the sheep (Heap, Flint and Jenkin, 1978). As in the sheep, uterine prostaglandin release is stimulated by inducing premature luteal regression with a synthetic prostaglandin, and can be reduced by treatment with chorionic gonadotrophin on day 12 of the cycle, which causes luteal maintenance (Guthrie and Rexroad, 1981). Therefore it seems likely that a large proportion of the prostaglandin F released at luteal regression may result from stimulation by declining levels of progesterone, and it is not certain whether release of the rest is inhibited in pregnancy. If the early luteolytic surges are relatively small and transient during the cycle, it will be difficult to show they are inhibited during early gestation.

Whatever the nature of the antiluteolytic signal from the embryo (see below), it appears to act locally on the endometrium underlying the expanded blastocyst. Pregnancy failure (i.e. 100% embryonic loss) occurs in pigs bearing fewer than five embryos (Polge, Rowson and Chang, 1966)



presumably because when conceptus numbers are low, blastocyst tissue fails to influence an adequate proportion of the endometrium to prevent the luteolytic signal. Fewer than five embryos is consistent with pregnancy only when that part of the uterus not bearing blastocysts is removed by subtotal hysterectomy (du Mesnil du Buisson and Rombauts, 1963); the presence of a sterile uterine horn always leads to pregnancy failure (du Mesnil du Buisson, 1961; Anderson, Rathmacher and Melampy, 1966; Niswender *et al.*, 1970). In sheep the embryo produces a proteinaceous antiluteolysin (trophoblastin) which is responsible for prolonging luteal function when embryonic extracts are infused into the uterus, and similar findings have been made in unilaterally pregnant pigs in which fertilized ova were flushed from one uterine horn two days after mating (Longenecker and Day, 1972). In the pig, the active material has been shown to be heat-stable and absorbed by charcoal (Ball and Day, 1979) and may therefore be a steroid (such as an oestrogen) rather than a protein. These experiments suggest the signal for luteal maintenance arises in the embryo, and that its action depends on a close association between the embryo and the uterus.

In the pig the uterine luteolysin is thought to act systemically as well as unilaterally; the unilateral effect, which occurs in the pig as in other species, precedes the systemic one. Luteal function is prolonged after either complete or partial hysterectomy (du Mesnil du Buisson and Dazier, 1959; Spies *et al.*, 1960; Anderson, Butcher and Melampy, 1961); however if embryos are confined to one horn the unilateral pregnancy so established is not maintained unless the non-pregnant (contralateral) horn is removed before the 14th day after mating (see above). If left *in situ* the non-pregnant horn exerts a luteolytic influence firstly over the ipsilateral ovary, and shortly thereafter causes regression of corpora lutea in the contralateral ovary. Evidence for a systemic action of the uterus has been found in cyclic sows bearing an autotransplanted ovary (Harrison, 1979), and in sows with autotransplanted ovaries at parturition (Martin, Bevier and Dziuk, 1978). It was at first thought this systemic action reflected production of a luteolysin other than prostaglandin  $F_{2\alpha}$  since in many animals prostaglandin  $F_{2\alpha}$  is unable to act systemically by virtue of its rapid metabolism in the lung; in fact evidence has been obtained in superfusion studies for a luteolysin distinct from prostaglandin  $F_{2\alpha}$  (Watson and Maule Walker, 1977). However more recent work has explained this discrepancy in terms of the rate of metabolism of prostaglandin  $F_{2\alpha}$ ; pulmonary clearance of prostaglandin  $F_{2\alpha}$  is much less rapid in the pig than the sheep (Davis *et al.*, 1979).

The most important, and earliest, secretion of the embryo yet shown to be involved in the maintenance of luteal function is an oestrogen. Oestrogens are luteotrophic in the pig (Kidder, Casida and Grummer, 1955; Gardner, First and Casida, 1963) and are produced sufficiently early by the blastocyst (by day 12, see above) to be candidates for the antiluteolytic signal. Administration of oestradiol-17 $\beta$  to cyclic animals reduces utero-ovarian venous concentrations of prostaglandin  $F_{2\alpha}$  and raises prostaglandin  $F_{2\alpha}$  levels in the uterine lumen, changes which are suggested to reflect the redirection of prostaglandin  $F_{2\alpha}$  secretion (Frank *et al.*, 1977; 1978; Zavy *et al.*, 1980). If oestrogens produced by the blastocyst

act directly on the endometrium, there would be no requirement to postulate a systemic effect; therefore the fact that peripheral plasma oestrogen levels have not been shown to be raised before day 15 post coitum is not an important objection to this hypothesis (Robertson, King and Dyck, 1978). An alternative mechanism, which would likewise obviate the need for a systemic effect, might arise from local counter current transfer of oestradiol from the utero-ovarian vein to the ovarian artery. Such a transfer has recently been reported for testosterone (Krymowski, Kotwica and Stefanóczyk, 1981), and would be consistent with the relatively high levels of oestradiol present in the utero-ovarian vein from day 12 post coitum (Moeljono *et al.*, 1977). Oestradiol acts synergistically with human chorionic gonadotrophin in stimulating progesterone synthesis by incubated porcine granulosa cells (Goldenberg, Bridson and Kohler, 1972) and stilboestrol has been reported to increase binding of LH to these cells (Nakano *et al.*, 1977); if similar effects are exerted on luteal cells, then raised levels of oestrogens in ovarian arterial blood might be expected to have a direct effect on luteal function. However there is evidence against a blood-borne signal being involved in luteal maintenance, since Robertson, Dwyer and King (1980) were unable to prevent luteal maintenance by passive immunization of sows against both oestrone and oestradiol between days 10 and 21 after mating; the best explanation for this lack of effect is that the antisera administered were ineffective in interfering with events inside the uterus.

In addition to the changes brought about by oestrogen in the endometrium, important changes are occurring between days 12–14 in the factors controlling luteal activity. The corpus luteum functions autonomously before day 14, so that neither hypophysectomy nor lowering LH levels by progesterone administration cause immediate luteal regression (Anderson, 1966; du Mesnil du Buisson, 1961; Woody, First and Pope, 1967). Furthermore, corpora lutea are refractory to the luteolytic effect of prostaglandin  $F_{2\alpha}$  before day 12, whereas prostaglandin administration after day 8 does cause a transient decline in progesterone secretion (Guthrie and Polge, 1976); an irreversible effect is not found until some four days later. However, after day 14 of pregnancy luteal maintenance becomes dependent on LH, as indicated by the effects of hypophysectomy and administration of progesterone or antisera to LH (Sammelwitz, Aldred and Nalbandov, 1961; Brinkley, Norton and Nalbandov, 1964; Spies, Slyter and Quadri, 1967; Short *et al.*, 1968). LH dependency then continues from 14 to approximately 70 days gestation, during which time unilateral ovariectomy results in hypertrophy of the contralateral corpora lutea (Staigmiller, First and Casida, 1972); later in pregnancy, luteal function can be maintained after hypophysectomy with prolactin alone (du Mesnil du Buisson and Denamur, 1968). It is doubtful, however, whether the influence of LH is ever lost during gestation, since temporal interrelationships have been demonstrated between surges in circulating LH level and progesterone secretion as late as day 90 (Parvizi *et al.*, 1976). The onset of prolactin dependency appears to be associated with increased concentrations of prolactin receptors in the corpora lutea (Rolland, Gunsalus and Hammond, 1976). The appearance of LH dependency in the corpora lutea after day 14 implies that plasma LH concentrations must be

maintained after this time and, in fact, circulating LH levels are raised in pregnant compared with non-pregnant pigs (Guthrie, Henricks and Handlin, 1972; Ziecik, Tilton and Williams, 1981). In contrast there appears to be no influence of pregnancy on prolactin concentrations until shortly before term, when there is a dramatic surge, presumably involved in lactogenesis (Dusza and Krzymowska, 1981).

Measurements of the luteal LH receptor in the pig have revealed dramatic changes in its concentration during the cycle and in early pregnancy (Ziecik, Shaw and Flint, 1980). Although the corpus luteum is independent of control by LH before day 14 of the cycle, the LH receptor is present as early as day 8; its concentration peaks on days 10 and 12 before declining as the corpora lutea regress. LH receptor concentrations in early pregnancy are lower than those during the cycle, possibly as a result of down regulation by the raised LH levels in pregnancy relative to the cycle. Subsequently luteal LH receptor concentrations rise dramatically between days 20 and 30 of gestation, at which time there is a decline in receptor occupancy by LH; this may reflect blastocyst production of a chorionic gonadotrophin which displaces LH from the receptor but does not interfere in the radioimmunoassay used to determine receptor occupancy. The demonstration, reviewed above, that the early blastocyst contains material which cross reacts in an LH radioreceptor assay is consistent with this view. Alternatively, the LH receptor may be controlled by oestrogen, or by a combination of oestrogen and prolactin. The rise in LH receptor between days 20 and 30 coincides with the rise in circulating oestrogen concentrations, and unpublished observations (H.A. Garverick, C. Polge and A.P.F. Flint, 1981) suggest that administration of oestrogen may raise luteal LH receptor concentrations in non-pregnant animals.

#### ENDOMETRIAL STEROID RECEPTORS

Another potentially important interaction between the blastocyst and endometrium is that involving endometrial enzymes catalysing the deactivation of progesterone and oestrogens, the synthesis of unconjugated oestrogen by the blastocyst, and the occupancy and nuclear translocation of endometrial progesterone and oestrogen receptors. Measurements of endometrial oestrogen receptors show that cytoplasmic concentrations are similar in non-pregnant and pregnant pigs, with levels reaching maximum values during the early or mid-luteal phase (Pack *et al.*, 1978; Deaver and Guthrie, 1980). Experiments with ovariectomized pigs have shown that oestradiol increases both the synthesis of its receptor and receptor translocation to the nucleus (Jungblut *et al.*, 1976). These effects are similar to those found in the rat and sheep, in which progesterone has also been shown to modulate oestrogen receptor concentrations, by reducing receptor synthesis; therefore it seems probable (though it has not been tested) that progesterone has a comparable action in the pig. However it is not certain that receptor concentrations are controlled by these hormones alone, since endometrial levels of progesterone and oestradiol have not been found to alter during either the cycle or in early pregnancy in a manner consistent with the observed changes in receptor levels. Concentrations of oestradiol in endometrial tissue are low during the cycle except

for a rise on day 20; levels in pregnancy are also low until day 15 or 16 (Deaver and Guthrie, 1980). Endometrial progesterone levels, as expected on the basis of concentrations in blood, are similar in non-pregnant and pregnant pigs with the exception of a fall in non-pregnant animals towards the end of the cycle (Deaver and Guthrie, 1980). Endometrial oestradiol and progesterone concentrations are presumably influenced by activities of oestrogen sulphokinase (Pack and Brooks, 1974) and enzymes metabolizing progesterone to less active pregnanediols (Henricks and Tindall, 1971); the sulphokinase is a progesterone-dependent enzyme (Pack and Brooks, 1974). Thus rising titres of progesterone after ovulation are associated with increased levels of endometrial sulphokinase which might be expected to result in a decreased intracellular oestrogen concentration; this would tend to reduce translocation of cytoplasmic receptors to the nucleus, and may contribute to the rise in endometrial cytoplasmic oestrogen receptors at this time. It has been suggested that these cytoplasmic receptors may interact with blastocyst oestrogen on days 10-12, if the animal is pregnant, and thereby mediate the antiluteolytic effect (Deaver and Guthrie, 1980). On the other hand such a hypothesis does not appear to be consistent with the lack of rise in endometrial oestrogen concentrations until day 15 or 16, although raised levels of both oestradiol and oestrone have been found in uterine flushings between days 10 and 12 post coitum (Zavy *et al.*, 1980).

#### GROWTH FACTORS

Although the existence of endometrial products that influence blastocyst growth may be postulated on the basis of culture experiments, these have not been identified or measured, and there is no conclusive immunoelectrophoretic (see above) or chromatographic (Basha, Bazer and Roberts, 1979) evidence for pregnancy-specific endometrial proteins which might be involved in stimulating blastocyst growth. The proteins produced by the endometrium in early pregnancy appear to be identical to those secreted late in the cycle by the non-pregnant endometrium, though the time course of secretion may be altered by the presence of conceptuses (Basha, Bazer and Roberts, 1980). Nonetheless, a possible involvement of blastocyst oestrogen in controlling the secretion of endometrial embryotrophic factors should not be overlooked, particularly in view of their apparent importance in some other species (Flint, 1981). One possible hypothesis is that blastocyst oestrogen acts on the endometrium to increase secretion of substances that stimulate blastocyst growth (or to inhibit production of blastocyst growth inhibitors), as well as exerting an antiluteolytic effect. Evidence that some such interaction may exist is provided by the findings of Vandeplassche (1969), who postulated that the occurrence of superfetation in pigs reflects a temporary cessation of growth on the part of some of the blastocysts in a large litter. The growth promoting (and possibly inhibitory) substances demonstrated in culture experiments represent components of endometrial secretions that may be involved in this process, and competition for growth factors of this kind may be involved in deciding which embryos are lost. Such a mechanism might be envisaged if the conceptuses which elongate earliest obtain a disproportionately high share of available growth-supporting substances, and therefore deprive those

elongating later; this is consistent with the variation between blastocyst sizes which occurs during the time of elongation (Anderson, 1978). If this kind of competition for growth factors were to operate, it might lead to considerable advantages to the species if the last blastocysts to elongate arose from the last ova fertilized, as fertilization late during oestrus leads to an increased incidence of polyploidy. It should be noted however that no evidence for such a mechanism has been found (Anderson, 1978).

### Attempts to reduce embryonic mortality

Because of the evident requirement for them in early pregnancy, many attempts have been made to reduce or prevent embryonic death by administration of steroid hormones, particularly progesterone. Glasgow, Mayer and Dickerson (1951) reported a significant correlation between production of excretory metabolites of progesterone and embryonic survival, but attempts to correlate luteal function with embryonic survival have not been so promising (Mayer, Glasgow and Gawienowski, 1961; Erb *et al.*, 1962; Phillippo, 1968). Furthermore, efforts to reduce embryonic loss by progesterone administration have generally been unsuccessful or inconsistent (Sammelwitz, Dziuk and Nalbandov, 1956; Haines, Warnick and Wallace, 1958; Spies *et al.*, 1959; Day, Romack and Lasley, 1963), although there have been reports of success, particularly using potent synthetic progestagens (Schultz *et al.*, 1966) or progesterone administered with oestrogens (Reddy, Mayer and Lasley, 1958; Day *et al.*, 1959; Gentry, Anderson and Melampy, 1973; Wildt *et al.*, 1976). As pointed out by Scofield (1975), there is a poor correlation between ovulation rate and embryonic mortality within the normal range of ovulation rates (Perry, 1954), and since progesterone concentrations are closely associated with numbers of corpora lutea (Brinkley and Young, 1970) progesterone deficiency as a cause of embryonic death in the majority of normal animals would seem unlikely. This is supported by the survival to day 25 of large numbers of embryos in embryo transfer experiments in animals bearing more embryos than corpora lutea (Pope *et al.*, 1972). High doses of progesterone are in fact likely to be detrimental to pregnancy by reducing circulating LH levels at a time (from the start of the third week of gestation) when the corpora lutea are becoming dependent on this hormone. Despite such negative results, it seems possible that severe progesterone deficiency may occur in some cases, possibly as a result of incomplete inhibition of secretion of uterine luteolysin, and that these may benefit from administered progesterone (see Day, Romack and Lasley, 1963).

In contrast to the lack of effect of progesterone before attachment, subsequent placental development may be enhanced by progesterone administration. Bazer (1975) showed that treatment with progesterone increased placental length and weight and allantoic fluid volume, and this has been confirmed recently by McGovern *et al.* (1981). These authors obtained a 15% increase in mean chorionic area and allantoic fluid volume, as determined between days 30 and 35, when 25 mg progesterone *plus*

12.5 µg oestrone were administered daily between days 14 and 23. This effect is presumably mediated through increased secretion of proteins, including uteroferrin, by the endometrium, since passive immunization of gilts against uteroferrin reduces placental development (Bazer, 1975). Placental growth in early pregnancy may be important in determining embryo survival rates later in gestation, since Fenton *et al.* (1970) and Knight *et al.* (1977) have shown in unilaterally ovariectomized gilts in which one uterine horn was also removed, that artificial overcrowding of embryos (which reduced placental size) leads to increased embryonic loss after day 25 of gestation. It should be appreciated therefore that even if early embryonic death were reduced as a result of some treatment administered during the first two weeks of pregnancy, it is by no means certain that this would lead to increases in litter size unless it also raised placental size.

### Comment

It may one day be possible to reduce embryonic mortality by administering, during early pregnancy, a specific substance, perhaps an 'embryo growth factor', developed from a knowledge of the chemical dialogue between the blastocyst and the endometrium. As will be recognized from the foregoing discussion, however, our understanding of the complex, two-way, interaction between these tissues is still too rudimentary at present to allow such treatment.

Nevertheless, whilst we await a more physiological solution, considerable improvements in fecundity, including reduced embryonic mortality, can be achieved by attending to relevant aspects of husbandry (such as breeding programmes involving out-crossing, time of mating, flush feeding, etc.).

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