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Early embryonic development in prolific Meishan pigs

S. P. Ford and C. R. Youngs

Iowa State University, Ames, IA 50011-3150, USA

Prenatal mortality in European pigs is estimated at 30–40%, the majority of which occurs between days 12 and 18 after mating. Chinese Meishan pigs are prolific, averaging three to five more pigs per litter than do European breeds. Early reports into the fecundity of Meishan females suggested that their prolificacy resulted from lower embryonic mortality when compared with European females exhibiting the same ovulation rate. The preponderance of evidence suggests that there are no differences between Meishan and European breeds in either morphological embryo diversity within a litter or embryo mortality before day 12 after mating. Recent studies from our laboratory suggest that preimplantation embryos from Meishan females exhibit markedly reduced growth rates and oestrogen secretory activities through day 12 when compared with embryos from Yorkshire females. The significantly reduced conceptus sizes of Meishan versus European breeds on day 30 of gestation provide additional evidence of the reduced growth rate of Meishan embryos. Furthermore, because embryonic oestrogen production is known to alter uterine secretion of histotroph, the lower oestrogen production by Meishan embryos in the Meishan uterus may result in more gradual alterations in the uterine environment that are beneficial for conceptus survival and subsequent litter size. Recent studies using cross-transfer of Meishan and Yorkshire embryos on day 2 after mating have led to the suggestion that there is a major effect of recipient genotype on embryonic growth rate and oestrogen secretion. In these studies, both Meishan and Yorkshire embryos transferred to Meishan uteri exhibited marked decreases in morphological development and oestrogen content on day 12 when compared with embryos transferred to Yorkshire recipients. These data suggest the presence of factor(s) in endometrial secretions from Meishan females that reduce the growth rate and oestrogen secretory potential of preimplantation conceptuses.

Introduction

It is during the first 30 days of gestation in pigs that about 75% of the total prenatal embryonic loss occurs (Hammond, 1914; Corner, 1923; Squires *et al.*, 1952; Perry, 1954; King and Young, 1957; Baker *et al.*, 1958; Spies *et al.*, 1959; Hanly, 1961; Perry and Rowlands, 1962; Pope and First, 1985; Dziuk, 1987). This embryo loss appears to be independent of fertilization rate (Anderson, 1978; Polge, 1982) or limitations of uterine capacity (Dziuk, 1968). A large body of evidence exists suggesting that few embryos are lost before day 12 (Polge, 1982; Pusateri *et al.*, 1990; Anderson *et al.*, 1993) or from day 18 to day 30 (Spies *et al.*, 1959; Perry and Rowlands, 1962). Thus, the period most critical to embryo survival occurs between day 12 and day 18 of gestation.

A wide range of embryonic developmental stages has been observed both within and between litters from day 9 to day 13 of gestation (Anderson, 1978; Pusateri *et al.*, 1990; Anderson *et al.*, 1993). It is during this period that critical events occur, including equidistant spacing of embryos throughout the uterus (Dhindsa *et al.*, 1967; Dziuk, 1968, 1985), embryo elongation (Anderson, 1978), and the establishment of conceptus–uterine attachment (Dantzer, 1985).

There has been an overemphasis by researchers on litter-mate variation as the major cause of embryo mortality in pigs (see Pope *et al.*, 1990 for review). Recent studies from our laboratory (Ford *et al.*, 1988; Anderson *et al.*, 1993) and others (Ashworth *et al.*, 1990; Haley *et al.*, 1990; Wilmut *et al.*, 1990; Ashworth *et al.*, 1992) have reported that there is no association between littermate synchrony and litter size. What has emerged as a critical factor in the determination of litter size is conceptus–uterine interactions which appear to affect preimplantation embryonic growth rate differentially in prolific versus nonprolific pig breeds. The latter topic will be the major focus of this paper.

Chronology of Early Conceptus Development

Four-cell embryos enter the uterus approximately 48 h after ovulation, develop to the blastocyst stage by day 5, and finally hatch from the zona pellucida on day 6 or 7 (Oxenreider and Day 1965; Hunter, 1974; Papaioannou and Ebert, 1986). Over the next few days, the blastocyst expands from a spherical structure a fraction of a millimetre in diameter to a structure 10 mm in diameter. The growth of pig blastocysts beyond 4 mm in diameter occurs from day 11 to day 12 of gestation and represents the beginning of a particularly critical stage of conceptus development. It is during these days that the placenta is established from the trophectoderm, entoderm and the mesoderm, and that the embryonic disc forms the embryo proper. It is also on day 12–13 when a dramatic metamorphosis takes place during which the spherical blastocyst is transformed into a long, thin filament up to 1 m in length (Patten, 1948; Anderson, 1978; Geisert *et al.*, 1982a). Elongation, which proceeds at a rate of approximately 30–45 mm h^{-1} (Geisert *et al.*, 1982b) results primarily from a remodelling of the cells of the trophoblast (Geisert *et al.*, 1982b; Mattson *et al.*, 1990) rather than from hyperplasia. This lack of hyperplasia was confirmed by Pusateri *et al.* (1990), who found no significant increase in the DNA content of embryos undergoing the transformation from spherical to elongated forms.

Conceptus development is often defined by blastocyst diameter (measured *in vitro*), and by this criterion it is characteristically diverse among littermate pig embryos (Heuser and Streeter, 1929; Perry and Rowlands, 1962; Geisert *et al.*, 1982b; Wright *et al.*, 1983; Pusateri *et al.*, 1990). On days 11 and 12 of gestation, the diversity is particularly obvious, for at this time embryos within a litter can vary in size from spherical blastocysts 1 mm in diameter to filamentous forms almost 500 mm in length (Anderson, 1978; Pusateri *et al.*, 1990). Furthermore, it has been proposed (Pope, 1988; Pope *et al.*, 1990) that the less developed embryos within a litter are more likely to be lost than are their more developed littermates (i.e. embryonic diversity may affect embryo survival). There are many possible causes of this developmental diversity (reviewed by Pope *et al.*, 1990), some of which remain controversial (Soede *et al.*, 1992).

Role of the Embryonic Genome in Controlling Embryo Developmental Rate

There are many potential mechanisms whereby the rate of development of the pig conceptus could be controlled. Until the time just before the third cleavage division, embryonic growth and development depend on cytoplasmic factors that are products of the maternal genome inherited during oocyte maturation (Schoenbeck *et al.*, 1992). After this point, the genome of the embryo has a major influence on its rate of development. We previously demonstrated an effect of embryonic genotype on conceptus development in studies using three highly inbred strains of miniature swine which were defined relative to their swine leucocyte antigen (SLA) haplotype. In particular, we demonstrated that a specific SLA haplotype, the 'd' haplotype, is associated with a reduced embryonic growth rate from day 6 to day 11 (Ford *et al.*, 1988) and an increased litter size (Conley *et al.*, 1988) when compared with the 'a' or 'c' haplotypes.

Meishan pigs are known to be more prolific than are European pigs, averaging three to five more pigs per litter (Cheng, 1983; Bolet *et al.*, 1986; Haley *et al.*, 1990). We have observed a reduced developmental rate of preimplantation conceptuses of the prolific Meishan pigs versus our European pigs both *in vivo* (Anderson *et al.*, 1993) and *in vitro* (Youngs *et al.*, 1993). Furthermore, we observed that, whereas embryos from prolific Meishan sows and gilts developed more slowly than do their Yorkshire contemporaries, they were similar with respect to the developmental variability within a litter. Thus, the positive association between a reduced preimplantation embryonic growth rate and an increased litter size is seen for both a prolific strain of miniature pig and for the prolific Meishan pig.

Similar results suggesting that there are no differences in embryonic littermate synchrony between Meishan and European breeds were observed through day 30 of gestation by researchers in the United Kingdom (Ashworth *et al.*, 1990; Haley *et al.*, 1990; Wilmut *et al.*, 1990; Ashworth *et al.*, 1992). In contrast to our observations, however, these researchers reported that there were no breed differences in embryonic developmental rate between Meishan and European breeds through day 10 of gestation. These researchers corrected for the supposed difference in the time of ovulation (34.3 and 49 h after oestrus for European and Meishan breeds, respectively; Wilmut *et al.*, 1990) which may account for the difference. This 'correction' resulted in collection of embryos from Meishan females later (about 24 h) with respect to the initiation of oestrus than from European females, possibly confounding any breed differences in developmental rate with experimentally induced asynchrony. In support of embryonic developmental rate differences, however, Ashworth *et al.* (1990, 1992) observed that Meishan conceptuses were smaller than Landrace × Large White fetuses on day 30 of gestation. In contrast to these observations, Bazer *et al.* (1988a, b) reported that Meishan embryos developed at a faster and more uniform rate than did embryos from Large White gilts. Bazer *et al.* (1988a) reported that embryos from Meishan pigs were smaller on day 8 of gestation than embryos from Large White pigs, a result which is consistent with our findings. However, on days 10–12 of gestation Meishan embryos were larger than embryos from Large White gilts. The reason for these apparent differences in the growth rate of Meishan embryos is not clear but may have resulted from a greater embryonic loss (about 50%) seen in their Large White gilts during this period.

Role of the Uterine Environment in Modulating Conceptus Development

The uterine environment is also known to affect embryonic growth (Roberts and Bazer, 1988). Development can be experimentally altered by the restriction of pig embryos to the oviduct (Pope and Day, 1972; Broerman *et al.*, 1990), embryo–uterine asynchrony (Pope, 1988) or steroid treatment (Pope *et al.*, 1986; Morgan *et al.*, 1987a, b). In addition, endometrial secretions may affect embryo development as evidenced by Simmen *et al.* (1989) and Bazer *et al.* (1991), who reported temporal differences in uterine histotroph composition between Meishan and Large White gilts.

Haley and Lee (1990) reported that a higher level of embryo survival at a given ovulation rate seemed to account for the increased litter size in Meishan pigs; however, the role of the embryo versus the uterine environment was not elucidated. To investigate the specific effects of the embryonic genome versus those of the uterine environment on embryo development, we conducted studies similar to those attempted by Ashworth *et al.* (1990) who cross-transferred embryos between day 5 Meishan and day 4 Landrace × Large White gilts. We used Meishan and Yorkshire gilts, transferred day 2 embryos, and transferred to synchronous recipient gilts (i.e. no 'correction' for Meishan recipients). Previous studies from our laboratory (Youngs *et al.*, 1993) suggested that there are no breed differences in the time of ovulation with respect to the initiation of oestrus, as evidenced by the number of gilts of each breed (Meishan or Yorkshire) that had completed ovulation or were in the process of ovulation from 48 to 54 h after first detection of oestrus. Furthermore, the embryos from gilts of both breeds in the study of Youngs *et al.* (1993) had the same range in number of cells (1–8 cells) at the time of collection. These data were confirmed in our recent reciprocal embryo transfer experiment in which we found a similar range of developmental stages of embryos (1–6 cells) at the time of collection from Meishan and Yorkshire females on day 2 after mating. In addition, we used naturally cycling gilts, in contrast to Ashworth *et al.* (1990) who synchronized donors and recipients with a synthetic progestin, altrenogest. Unlike Ashworth *et al.* (1990), who observed poor survival of Meishan embryos following transfer, our results demonstrated similar survival of Meishan and Yorkshire embryos to day 12 when developed in Meishan (70%) or Yorkshire (66%) uteri. This difference may result from the apparent detrimental effects of altrenogest on Meishan embryo viability (Ashworth *et al.*, 1990, 1992), different techniques of collecting, handling and transferring of embryos, or differences due to day of collection and transfer.

Our results demonstrated a significant effect of breed of donor ($P < 0.001$) and breed of recipient ($P < 0.001$) on size (diameter) and DNA content of embryos recovered on day 12 of gestation. Specifically, Meishan embryos were smaller and contained less DNA than did Yorkshire embryos; however, both Meishan and Yorkshire embryos were found to be larger when recovered from Yorkshire versus

Meishan uteri. Interestingly, when Meishan embryos were placed in Yorkshire females, three of seven recipient females possessed filamentous embryos on day 12. No other transfer combination (i.e. Meishan to Meishan; Yorkshire to Yorkshire; Yorkshire to Meishan) resulted in the recovery of filamentous embryos on day 12. These data suggest that Meishan embryos may have the ability to elongate from a smaller size and cell number than do Yorkshire embryos. The apparent negative effect of the Meishan uterus on embryonic growth rate confirms the previous report by Ashworth *et al.* (1990), who reported that day 30 Meishan and Landrace × Large White embryos were shorter, lighter and had smaller allantoic sacs when they occupied Meishan rather than Landrace × Large White uteri.

Our data clearly demonstrate that Meishan embryos grew faster in the uterus of Yorkshire gilts, and that litter size, based on recovery of embryos, was maintained through day 12. These data also demonstrate that Meishan embryos have the capacity for accelerated developmental rates when placed in a less limiting uterine environment (Yorkshire versus Meishan). There are potentially many uterine luminal growth factors or morphogens (Simmen *et al.*, 1988; Simmen and Simmen, 1990) which may be involved in what is undoubtedly a multifaceted embryonic response. For example, insulin-like growth factor I (IGF-I) promotes protein accretion in porcine embryonic discs (Estrada *et al.*, 1991; Hofig *et al.*, 1991). Activins, fibroblast growth factor (FGF) and transforming growth factor β (TGF-β) can affect the growth and differentiation of mesoderm (Stroband and Van der Lende, 1990; Stern, 1992), and retinoids can induce embryonic entoderm formation (De Luca, 1991).

In contrast to embryo size and DNA content, the oestradiol content of embryos was only affected by recipient genotype ($P < 0.001$) and was markedly lower for embryos (Meishan and Yorkshire) collected on day 12 from Meishan (352 ± 29 pg) versus Yorkshire (1643 ± 92 pg) recipient females. Oestradiol content appeared to be a good index of embryonic oestradiol secretion as evidenced by the highly significant correlation between the embryonic content of oestradiol and the concentration of this steroid in uterine flushings ($r = 0.72$; $P < 0.0001$).

Oestrogen Modulation of Uterine Histotroph

Perhaps the first recognizable changes in the uterine environment that occur during the establishment of pregnancy in pigs are those induced by the transient synthesis of oestrogen by conceptus tissue (Gadsby *et al.*, 1980), which occurs on or about day 12 after mating (Geisert *et al.*, 1990). Of the many steroids or other components present in pig uterine fluid at this time (Zavy *et al.*, 1980; Stone and Seamark, 1985), oestrogen alone has been shown to mimic in nonpregnant pigs (Geisert *et al.*, 1982c) the changes seen in uterine secretions during early pregnancy (Geisert *et al.*, 1982b, 1990). Among these oestrogen-induced changes are increases in uterine luminal calcium (Geisert *et al.*, 1982a) and retinol-binding protein (Trout *et al.*, 1992). In addition, oestrogen administration to nonpregnant pigs decreases uterine venous $PGF_{2\alpha}$ concentrations (Bazer and Thatcher, 1977; Ford *et al.*, 1982; Conley *et al.*, 1989) and increases uterine blood flow and uterine vascular permeability (Ford, 1989).

We examined embryonic oestrogen content, as well as oestrogen, calcium, total protein and retinol-binding protein concentrations in the uterine flushings obtained on day 12 of gestation in the embryo transfer experiment described above. Embryos transferred into Meishan uteri, regardless of embryonic genotype (Meishan or Yorkshire), contained five- to tenfold less oestrogen per μg DNA on day 12 than did embryos transferred into Yorkshire uteri. As previously stated, this marked reduction in embryonic oestrogen content was positively correlated with the concentration of oestradiol in uterine flushings from Meishan recipients (Table 1). This reduction in embryonic oestrogen synthesis resulted in reduced secretion of calcium, total protein and retinol-binding protein by the endometrium of Meishan recipients (Table 1). Palpable uterine tone was also lower at the time of embryo recovery on day 12 in Meishan versus Yorkshire recipients which is again consistent with lower oestrogen exposure and altered uterine function. Differences in oestrogen secretion by the porcine conceptus, which are known to have dramatic effects on uterine function (Pope *et al.*, 1986; Morgan *et al.*, 1987a, b; Stroband and Van der Lende, 1990; Geisert *et al.*, 1990), are therefore likely to be a critical component in the regulation of pig conceptus development and survival.

Control of Conceptus Oestrogen Secretion

Despite the potential importance of embryonic oestrogen as a regulator of uterine function, and, thereby, embryonic growth and survival, relatively little is understood about the control of oestrogen synthesis by

Table 1. Uterine histotroph components in Meishan and Yorkshire recipients on day 12 of gestation

Recipient breed	Number of animals	Oestradiol (pg ml^{-1})	Ca^{2+} (nmol l^{-1})	Total protein (mg ml^{-1})	Retinol-binding protein (relative densitometry units)
Meishan	10	286 ± 96^a	0.49 ± 0.15^a	0.67 ± 0.09^a	0.103 ± 0.024^a
Yorkshire	10	1337 ± 148^b	2.03 ± 0.18^b	1.91 ± 0.20^b	0.715 ± 0.125^b

Values are means ± SEM.
[a,b]Values within a column with different superscripts are significantly different ($P < 0.01$).

the conceptus. In general, steroidogenesis can be regulated at any one of several points including substrate supply, the level of steroidogenic enzyme expression, or the rate of steroid metabolism (Conley and Mason, 1990). Two enzymes of the steroidogenic pathway, namely cytochromes P450 17α-hydroxylase/17,20-lyase ($P450_{17\alpha}$) and aromatase ($P450_{arom}$), are positively correlated with porcine oestrogen content on day 12 of pregnancy (Conley *et al.*, 1992), whereas the amount of other steroidogenic enzymes in the conceptus is not.

Oestrogen synthesis by pig conceptus tissues also depends on embryo size or maturity and, consequently, it represents an important differentiative event. Oestrogen synthesis by cultured pig blastocysts is undetectable in the presence or absence of aromatizable substrate when blastocysts are less than 4 mm in diameter (Fischer *et al.*, 1985; Van der Meulen *et al.*, 1989; Hofig *et al.*, 1991). In addition, $P450_{arom}$ and $P450_{17\alpha}$ are first detectable by western analysis in 4–6 mm diameter blastocysts (Conley *et al.*, 1992). However, aromatase activity in littermate spherical blastocysts collected from pigs on day 11 of pregnancy is subject to considerable individual variation (Van der Meulen *et al.*, 1989). The within litter variation in oestrogen synthesis may be due to the differential growth of particular conceptus tissues or the overall developmental stage of each blastocyst. For instance, the results of incubations of tissue fragments from day 14 to day 18 conceptuses suggest that tissues nearest the embryonic disc have the greatest capacity to synthesize oestrogen (Bate and King, 1988), and, as previously discussed, increased oestrogen synthesis by the conceptus coincides with the rapid phase of blastocyst elongation (Geisert *et al.*, 1982a; Pusateri *et al.*, 1990). Furthermore, the transition from tubular to filamentous form by porcine conceptuses is associated with a reduction in oestrogen synthesis (Pusateri *et al.*, 1990; Fig. 1) and an equally rapid decline in $P450_{17\alpha}$ expression (Conley *et al.*, 1992). The coincidence of these dramatic events suggests that both may be regulated by a similar mechanism or at least that the decrease in $P450_{17\alpha}$ expression by tissues of elongating pig conceptuses may be an excellent indicator of the appearance of important regulators of differentiation and development.

Conclusion

Data from our laboratory have demonstrated that preimplantation Meishan embryos develop more slowly and produce less oestrogen than do embryos of less prolific European breeds, while exhibiting the same littermate diversity. Recently, using reciprocal embryo transfer techniques (Meishan ↔ Yorkshire), a marked effect of recipient genotype on embryonic growth rate and oestrogen synthesis was shown. Specifically, these data suggest the presence of a factor(s) in endometrial secretions from Meishan females which reduces the growth rate and oestrogen secretion of preimplantation conceptuses. Because conceptus oestrogen is known to alter uterine histotroph, the lower oestrogen production by Meishan embryos in a Meishan uterus may result in more gradual alterations in the uterine luminal environment that are beneficial for conceptus survival and subsequent litter size. Studies are continuing in our laboratory to define factors that regulate the rate of development of preimplantation pig conceptuses. It is anticipated that a greater understanding of conceptus–uterine interactions during this crucial period of early pregnancy will provide the necessary information required to optimize litter size in this species.

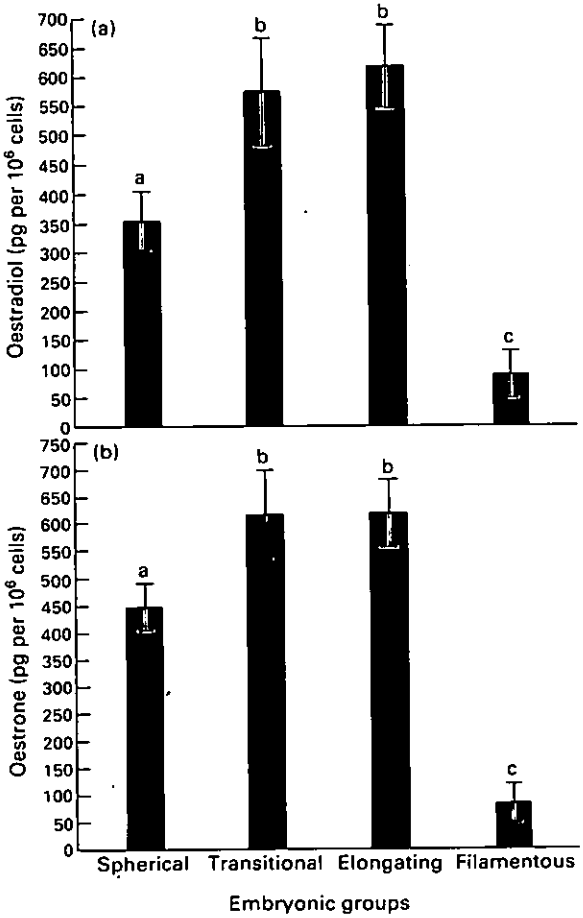

Fig. 1. (a) Oestradiol and (b) oestrone content per 10^6 cells in pig embryos in different developmental groups. The four groups are defined as follows: (1) ≥ 1 mm and ≤ 7.5 mm, spherical ($n = 218$); (2) ≥ 8 mm and ≤ 12 mm, transitional ($n = 17$); (3) ≥ 12.5 mm and ≤ 100 mm, elongating ($n = 29$); and (4) > 100 mm, filamentous ($n = 124$). Least squares means $\pm$ SEM; bars with different superscripts are significantly different ($P < 0.05$). (Reproduced with permission from Pusateri *et al.*, 1990.)

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