Effects of nutrient intake and number of oestrous cycles on in vitro development of preimplantation pig embryos


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The effects of nutrient intake and insemination of gilts at first versus third oestrus on the in vitro development of preimplantation pig embryos were investigated. Standard swine management involves ad libitum feeding of gilts at first oestrus and restricted feeding of gilts at third oestrus. According to previous research, gilts inseminated at first oestrus demonstrate greater embryonic mortality than gilts inseminated at third oestrus, and it is possible that differences in nutrient intake between gilts inseminated at first versus third oestrus affect the viability of eggs or embryos. In the present study, experimental gilts were assigned to three treatments: animals designated 1A were inseminated at first oestrus and fed ad libitum; animals designated 3R were inseminated at third oestrus and were fed a restricted diet; and 3A animals were inseminated at third oestrus and fed ad libitum. Embryos collected from each treatment group were cultured in vitro, and data were evaluated according to cell stage at collection. Comparison of treatments 1A and 3R supported the contention of increased embryo mortality in gilts inseminated at first oestrus under normal management conditions. When cultures were initiated at the one- to two-cell or two- to four-cell stages, the percentage of 1A embryos developing to the morula stage (50.9%, 68.0%) was significantly lower than that of 3R embryos (88.9%, 90.9%; P < 0.05). Comparison of treatments 1A and 3A addressed effects due to the number of oestrous cycles. Significantly more two- to four-cell embryos from gilts inseminated at third oestrus and fed ad libitum reached the morula and expanded blastocyst stages of development (87.0%, 41.3%) compared with embryos from gilts inseminated at first oestrus and fed ad libitum (68.0%, 20.3%; P < 0.05). Finally, the effects of ad libitum feeding were determined by comparing treatments 3A and 3R. These data were inconclusive, as both positive and negative effects were observed. More one- to two-cell embryos from treatment 3R developed to the morula stage (88.9%) compared with 3A embryos collected at the same stage (64.7%), whereas a greater number of 3A embryos in the two- to four-cell category reached the expanded blastocyst stage (41.3%) than 3R embryos (21.2%; P < 0.05). These results support the hypothesis of lower in vitro developmental capacity for embryos collected from gilts inseminated at first oestrus. Furthermore, the findings indicate that differences in embryo viability between gilts inseminated at first versus third oestrus are related to the number of oestrous cycles and possibly to differential nutrition.

Introduction

Prenatal mortality accounts for a 35–45% loss of offspring in swine, and the largest percentage loss occurs during early embryonic development (Pope and First, 1985). Gilts mated at first oestrus show lower ovulation rates (Robertson et al., 1951; Anderson and Einarsson, 1980; Paterson and Lindsay, 1980; Archibong et al., 1987) and greater embryonic mortality (Warnick et al., 1951; MacPherson et al., 1977; Young and King, 1981; Archibong et al., 1987) than gilts inseminated at a later oestrus. Furthermore, embryos collected from gilts inseminated at first oestrus show reduced in vitro development compared with embryos collected from the same gilts at third oestrus (Menino et al., 1989). There is some evidence that uteri from gilts at first oestrus are less developed than those from gilts at third oestrus (Murray and Grifo, 1976). However, the increased mortality of embryos from gilts inseminated at first oestrus does not appear to be due to an unfavourable intrauterine environment, as demonstrated by equivalent survival rates of embryos transferred from gilts inseminated at second oestrus to oviducts of recipients at either first or third oestrus (Archibong et al., 1992). Although fertilization rates of gilts inseminated at first oestrus are similar to those of gilts inseminated at third oestrus (Young and King, 1981;
Archibong et al., 1987), it has been suggested that ova released by gilts at first oestrus are inferior in quality (Archibong et al., 1992; Koenig and Stormshak, 1993).

Gilts at first oestrus differ from those at third oestrus both in terms of follicular steroid production and preovulatory follicle size. Gilts at first oestrus show lower concentrations of certain follicular steroids (testosterone, oestradiol, androstenedione) and a greater number of small follicles (4–8 mm in diameter) than gilts at a later oestrus (Smith et al., 1992). Whether the differences in follicular steroid concentration lead to production of oocytes or embryos of decreased viability is unclear. However, it has been demonstrated that plasma steroid concentrations can affect embryo survival (Anderson, 1978; Pope and First, 1985; Dziuk, 1987; Blair et al., 1994). Blair et al. (1994) reported that increased embryonic survival and decreased embryonic diversity in pigs may be associated with a closer synchrony between the onset of oestrus, the peak in oestradiol concentration and the LH surge. Furthermore, embryonic development is asynchronous within pig and sheep litters (Anderson, 1978; Wilmut and Sales, 1981), and some embryonic death may be due to secretion of oestrogens by more advanced conceptuses (Pope and First, 1985; Dziuk, 1987). However, the relationship between embryo loss in gilts inseminated at first oestrus and intra-litter asynchrony remains unclear.

The effect of energy intake on ovulation rate is well documented and has led to the practice of flushing livestock to increase ovulation rate (Hartog and van Kempen, 1980). However, nutritional flushing (Emerson and Hendricks, 1977) and high energy intake during rearing (Hartog and van Kempen, 1980; Kirkwood and Thacker, 1988; Whaley et al., 1997) increases embryonic mortality in gilts, whereas restricting feed intake during early gestation can lead to increased embryonic survival in vivo (Robertson et al., 1951) and increased fetal weight (Bryan and Hagen, 1991). Injecting well-fed gilts (2 × maintenance feed per day) with progesterone after the onset of oestrus also appears to increase embryonic survival, indicating that periovulatory plasma progesterone concentration is involved in mediating nutrition-induced effects on embryos (Jindal et al., 1997). Furthermore, Zak et al. (1997) found fewer large follicles (> 7 mm in diameter) and fewer oocytes at metaphase II in lactating feed-restricted sows, indicating that the size of pig preovulatory follicles and the maturation rate of oocytes obtained from preovulatory follicles can be influenced by feed intake during lactation. Finally, leptin, a regulatory protein associated with follicular development (Barash et al., 1996) and polarization of the preimplantation embryo (Antczak and Van Blerkom, 1997), may also be involved in regulating food intake, as mice lacking endogenous leptin showed significant decreases in food intake and body weight after receiving leptin injections (Weigle et al., 1995).

Swine management systems frequently allow ad libitum feeding of gilts at first oestrus, whereas gilts at a later oestrus receive a limited diet. This difference in nutrient intake may affect the production of viable eggs or embryos and may account, at least in part, for the difference in embryonic mortality rates between gilts inseminated at first versus third oestrus.

The objective of this study was to investigate the effects of nutrition and insemination at first versus third oestrus on in vitro development of preimplantation pig embryos. Studying the effects of excess nutrients on embryo viability may increase understanding of the origins and mechanisms of embryonic mortality.

### Materials and Methods

#### Animals

Forty-five crossbred gilts from 12 litters were represented in the study. Full siblings were distributed across treatment groups, housed in confinement at the Southern Illinois University Swine Center, and maintained under identical conditions until display of first oestrus. Gilts were penned adjacent to boars, checked twice a day for behavioural oestrus, beginning at approximately 130 days of age, and fed a 14% crude protein diet ad libitum. Gilts assigned to the first treatment (1A) were inseminated at their first oestrus and fed ad libitum. In the second treatment (3R), gilts were restricted to 1.82 kg of a 14% crude protein diet per day after their first oestrus and were inseminated at third oestrus. Gilts in the third treatment (3A) were fed ad libitum throughout the experimental period and were inseminated at third oestrus. Pre-pubertal gilts on restricted diets tend to come into oestrus at different ages, if at all. Therefore, a restricted diet treatment group was not included at first oestrus.

#### Embryo recovery

Gilts were artificially inseminated with fresh semen pooled from two boars at 0 and 12 h after detection of oestrus. At 36–48 h after detection of oestrus, gilts were anaesthetized with a combination of 10 ml acepromazine and 40 ml ketamine i.m., and their reproductive tracts were exposed. The local anaesthetic, lidocaine (25 ml), was also applied along the incision area. Ovaries were inspected to confirm the gilts were at first oestrus, and the number of ovulations was recorded. Embryos were collected by flushing the oviducts and uteri with Whitten’s medium (Whitten and Biggers, 1968) buffered with Hepes and without BSA. Each gift was flushed only once. All embryos were collected within 12 h starting at 36 h after oestrus was detected. The embryos collected were cultured in vitro and observed once a day for 8 days under an inverted stage phase-contrast microscope at × 200 to determine developmental potential. Indication of fertilization, stage of development, quality of embryos, and abnormal features were recorded. Embryos were cultured in microdrops of Whitten’s medium with 15 mg BSA ml⁻¹ under paraffin oil in a humidified atmosphere of 5% CO₂ in air at 37°C. Three to six embryos were cultured in each 30–40 μl drop.

#### Statistical analysis

Differences in the number of ovulations and ova recovered were evaluated using analysis of variance and least significant
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egg or embryo viability of gilts at first oestrus, as has been suggested for dairy cows fed a high protein diet (Jordan and Swanson, 1979; Williams et al., 1987). In a review of 24 studies, Hartog and van Kempen (1980) reported that a high energy intake during pig rearing generally increased embryonic mortality. In addition, although nutritional flushing of gilts for 5 days before insemination increased ovulation rate, it appeared to have no effect on the number of embryos remaining viable at day 25 of gestation (Emerson and Henricks, 1977). Although the mechanism by which high energy intake affects embryonic survival is unknown, it has been suggested that follicular and plasma steroid concentrations may play important roles (Matamorous et al., 1990; Jindal et al., 1997). Gilts fed a high energy diet do not show the typical changes in follicular steroid content during the oestrous cycle that are observed in feed-restricted gilts (Matamorous et al., 1990). This difference in follicular steroid content may affect the viability of the ovum, as suggested by Frachimont et al. (1989) in a study in which follicular fluid steroid content was correlated with the ability of human oocytes to be fertilized during in vitro fertilization. Furthermore, the addition of exogenous progesterone after the onset of oestrus increased embryonic survival in gilts with no feed restriction, indicating a role for periovulatory plasma progesterone in mediating nutrition-induced embryonic viability (Jindal et al., 1997).

The results of the present study are not conclusive regarding the effects of nutrition on in vitro embryo viability. Depending on initial cell stage and developmental endpoint, embryos from gilts inseminated at third oestrus and fed ad libitum demonstrated either increased or decreased development. Further studies are necessary to clarify the effects of nutrition on embryo viability.

Comparison of 1A and 3A treatments revealed significant differences in the distribution of embryonic stages at collection. A greater number of one- to two-cell embryos were collected from gilts inseminated at first oestrus and fed ad libitum, whereas gilts inseminated at third oestrus and fed ad libitum produced significantly more embryos in the one- to eight-cell category. Further investigation is required to determine whether embryos from developmentally heterogeneous flushes are at greater risk of mortality.

Although no oestrus-related effect on embryo recovery at the two- to four-cell stage was observed, data from the present study indicate that two- to four-cell embryos from gilts inseminated at first oestrus and fed ad libitum are less likely to survive to the morula stage than embryos from gilts inseminated at third oestrus and fed ad libitum. Furthermore, 1A embryos collected at the two- to four-cell stage were much less likely to become expanded blastocysts than were embryos at the same stage from the 3A treatment group.

Since nutrition is not a factor when comparing treatments

<table>
<thead>
<tr>
<th>Table 2. Number of pig embryos collected at each stage and cultured in vitro</th>
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<tr>
<td>Treatment</td>
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<td>Flashes with two- to four-cell embryos</td>
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<td>Number developed to morula</td>
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<td>Flashes with four- to eight-cell embryos</td>
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<td>Number of embryos cultured</td>
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<td>Number developed to morula</td>
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<td>Flashes with one- to eight-cell embryos</td>
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1A, animals were inseminated at first oestrus and fed ad libitum; 3R, animals were inseminated at third oestrus and fed a restricted diet; 3A, animals were inseminated at third oestrus and fed ad libitum.
Effects of oestrus and nutrition on pig embryos

Fig. 1. Percentage of pig embryos at various cell stages at collection. Animals were inseminated at first oestrus and fed ad libitum (○, group 1A); were inseminated at third oestrus and fed a restricted diet (■, group 3R); or were inseminated at third oestrus and fed ad libitum (□, group 3A). Initial cell stage 1–8 represents flushes that contained embryos at three or more cell stages. Values within an initial cell stage with different superscripts are significantly different (P < 0.05).

Fig. 2. Development of pig embryos to the compacted morula stage. Animals were inseminated at first oestrus and fed ad libitum (○, group 1A); were inseminated at third oestrus and fed a restricted diet (■, group 3R); or were inseminated at third oestrus and fed ad libitum (□, group 3A). Initial cell stage 1–8 represents flushes that contained embryos at three or more cell stages. Values within an initial cell stage with different superscripts are significantly different (P < 0.05).

Fig. 3. Development of pig embryos to the blastocyst stage. Animals were inseminated at first oestrus and fed ad libitum (○, group 1A); were inseminated at third oestrus and fed a restricted diet (■, group 3R); or were inseminated at third oestrus and fed ad libitum (□, group 3A). Initial cell stage 1–8 represents flushes that contained embryos at three or more cell stages.

Fig. 4. Development of pig embryos to the expanded blastocyst stage. Animals were inseminated at first oestrus and fed ad libitum (○, group 1A); were inseminated at third oestrus and fed a restricted diet (■, group 3R); or were inseminated at third oestrus and fed ad libitum (□, group 3A). Initial cell stage 1–8 represents flushes that contained embryos at three or more cell stages. Values within an initial cell stage with different superscripts differ (P < 0.05).

1A and 3A, there must be a factor inherent in the production of oocytes or early embryos at first oestrus that affects viability. Several studies have implicated plasma steroid concentration in embryo survival (Anderson, 1978; Pope and First, 1985; Dziuk, 1987; Blair et al., 1994). Embryonic growth is asynchronous in pig litters (Anderson, 1978), and some mortality may be attributed to oestrogen secretion by developmentally advanced embryos (Pope and First, 1985; Dziuk, 1987). Finally, although it has been demonstrated that gilts at first oestrus have lower concentrations of follicular steroids than do gilts at second or later oestrus (Smith et al., 1992), the significance of this differential steroid concentration remains unclear.

In conclusion, the results from the present study demonstrate that inseminating gilts at first oestrus results in decreased in vitro embryo development compared with insemination at a later oestrus. Although this effect is not removed by changing the nutrition of the gilts, ad libitum feeding may have an effect on in vitro embryo viability and may account for some of the reported difference in embryo survival between gilts inseminated at first and third oestrus. By clarifying the relationship between nutrition and the number of oestrous cycles, it may be possible to achieve a better understanding of the origins and mechanisms of embryonic mortality.

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References

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

Andersson AM and Einarsson S (1980) Studies in the estrus and ovarian
activity during five successive estrous cycles in gilts Acta Veterinaria
Scandinavica 21 677–688

Antczak M and Van Blaricom J (1997) Oocyte influences on early development:
The regulatory proteins leptin and STAT3 are polarized in mouse and human
oocytes and differentially distributed within the cells of the preimplantation
stage embryo Molecular Human Reproduction 3 1067–1086

early embryonic development in gilts bred at first estrus Journal of Animal
Science 64 474–478

Archibong AE, Maurer RR, England DC and Stormshak F (1992) Influence of
sexual maturity of donor on in vivo survival of transferred porcine embryos
Biological Reproduction 47 1026–1030

Barash IA, Cheung CC, Weigel DS, Ren H, Kabigting EB, Kuijper JL, Clifton
DK and Steiner RA (1996) Leptin is a metabolic signal to the reproductive
system Endocrinology 137 3144–3147

Blair RM, Coughlin CM, Minton JE and Davis DL (1994) Peri-ovestrous
hormone profiles, embryonic development in gilts and primiparous sows

Bryan KA and Hagen DR (1991) Effects of nutrient intake and sexual age of
the dam at mating on fetal development in swine Growth, Development
and Aging 55 27–33

Dziuk PJ (1987) Embryonic Loss in the Pig: An Enigma Manipulating Pig
Production p. 28 Eds APSA Committee, Australasian Pig Science Association,
Werribee

Emerson DD and Hendricks DM (1977) The effects of low levels of PMSG and
flush feeding upon embryonic survival in gilts Theriogenology 8 281–291

Franchimont F, Hazee-Hagelstein A, Haazout A, Friedman R, Schatz B and
Demeer F (1989) Correlation between follicular fluid content and the
results of in vitro fertilization and embryo transfer 1. Sex steroids Fertility
and Sterility 53 1006–1011

Hartog LA and van Kempen GJM (1980) Relation between nutrition and
fertility in pigs Netherlands Journal of Agricultural Science 28 211–227

Sciences 2nd Edn. Houghton Mifflin Company, Boston

Jindal R, Cosgrove JR and Foxcroft GR (1997) Progesterone mediates
nutritionally induced effects on embryonic survival in gilts Journal of Animal
Science 75 1063–1070

Jordan ER and Swanson LV (1979) Effect of crude protein on reproductive
efficiency, serum total protein, and albumin in the high-producing dairy

survival in pigs (Results and Speculations) Pig News Speculations 9 1–15

Koenig JLF and Stormshak F (1993) Cyrogentic evaluation of ova from
puberal and third estrous gilts Biology of Reproduction 49 1159–1162

MacPherson RM, Howell FDD and Jones AS (1977) Performance of sows first
mated at puberty or second or third estrus, and carcass assessment of once-
bred gilts Animal Production 24 333–342

Matamov R, Cox NM and Moore AB (1990) Exogenous insulin and additional
energy affect follicular distribution, follicular steroid concentrations,
granulosa cell human chorionic binding in swine Biology of Reproduction 43 1–7

Comparison of in vitro development of embryos collected from the same
gilts at first and third estrus Journal of Animal Science 67 1387–1393

Murray EA and Grifo AP, Jr (1976) Development of capacity to secrete
progesterone-induced protein by the porcine uterus Biology of Reproduction
15 620–625

Paterson AM and Lindsay DR (1980) Induction of puberty in gilts Animal
Production 31 291–298

Pope WF and First NL (1985) Factors affecting the survival of embryos
Theriogenology 25 91–105

Robertson GL, Casida LE, Grummer RH and Chapman AB (1951) Some
feeding and management factors affecting age at puberty and related
phenomena in Chester White and Poland China gilts Journal of Animal
Science 10 841–866

Smith GD, Menino AR, Jr, Rowe KE and Stormshak F (1992) Steroids and
plasminogen activator concentrations in follicular fluid of gilts at first and
third estrus Journal of Animal Science 67 1387–1393


Warnick AC, Wiggins EL, Casida LE, Grummer RH and Chapman AB (1951)
Variation in puberty phenomena in inbred gilts Journal of Animal Science
10 479–493

Weigle DS, Bukowski TR, Foster DC, Holderman S, Kramer JM, Lasser G,
Recombinant ob protein reduces feeding and body weight in the ob/ob
mouse Journal of Clinical Investigations 96 2065–2070

Whaley SL, Hagedorn VS and Britt HH (1997) Evidence that injection of
vitamin A before mating may improve embryo survival in gilts fed normal
or high energy diets Journal of Animal Science 75 1071–1077

Whitten WK and Biggers JD (1968) Complete development in vitro of the
preimplantation stages of the mouse in a simple chemically defined
medium Journal of Reproduction and Fertility 17 399–401

Williams JS, Gardiner CS, Schuller LS, Swanson LV and Menino AR, Jr
(1987) Evaluation of uterine flushings collected from dairy cows fed two
levels of crude protein Proceedings of the Western Section of the American
Society of Animal Scientists 38 253–258

Wilmut I and Sales DI (1981) Effect of an asynchronic environment on
embryonic development in sheep Journal of Reproduction and Fertility 61
179–184

Young LG and King GJ (1981) Reproductive performance of gilts bred on first
versus third estrus Journal of Animal Science 53 19–25

Zak LJ, Xu X, Hardin RT and Foxcroft GR (1997) Impact of different patterns of
feed intake during lactation in the primiparous sow on follicular development
and oocyte maturation Journal of Reproduction and Fertility 110 95–106