Influence of sex on early growth of pig conceptuses

G. Cassar¹, W. A. King² and G. J. King^{1*}

¹Animal and Poultry Science, and ²Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Nineteen gilts were used in an experiment to examine the relationship between rate of development and embryonic sex on day 10 of pregnancy. All gilts were mated to the same boar approximately 24 h after detection of second oestrus. They were individually housed and fed similar diets until slaughter on day 10 of gestation (day 0 = day of insemination) for subsequent recovery of the conceptus. All conceptuses were photographed and their surface areas (mm²) measured by tracing outlines on a digitized tablet interfaced with a computer program. Within each litter, individuals were categorized as small, medium or large by three equal divisions of the size range between the smallest and largest member. Conceptuses were individually cultured in Medium 199 with 1% colcemid and stained with 4% Giemsa. Metaphase spreads were located and sex was determined by presence or absence of the Y chromosome in at least two spreads from each specimen. A total of 214 conceptuses were recovered but only 125 (58%) were successfully karyotyped. The overall sex ratio was not significantly different from 1:1 (57 males and 68 females; $P > 0.25$). Sex was determined in 51 of 88 small embryos, 22 of 44 medium embryos and 52 of 82 large embryos and males represented 9 (17.6%), 10 (45.5%) and 38 (73%), respectively. Logistic analysis indicated significantly more females in the small and significantly more males in the large groups ($P < 0.001$). Results demonstrate that most male conceptuses grow faster than females before commencement of attachment to the uterine lining.

Introduction

Synchronized interactions between conceptuses and the tubular genitalia are essential throughout development from pig zygote to neonatal piglet. Mortality may occur at any stage of gestation but the most critical time is the first 10 days (Lambert *et al.*, 1991). Developmental asynchrony, particularly during the period between hatching from the zona pellucida and commencement of attachment to the uterine lining, may contribute to mortality since signals from faster developing individuals could modify the uterine environment to produce conditions that might not be optimum for slower developers (Pope and First, 1985; Dziuk, 1987; Pope *et al.*, 1990). Bazer *et al.* (1988) proposed that faster and more uniform conceptus development between days 8 and 14 accounts for higher embryonic survival rates in Chinese Meishan pigs, a breed noted for its prolificacy (Bazer *et al.*, 1988). Other workers, while not disputing high prolificacy in the breed, contend that mechanisms other than uniformity of development contribute to the larger litter sizes (Ashworth *et al.*, 1992; Wilmot *et al.*, 1992).

Genetic sex influences the rate of early development in mice. At the blastocyst stage or on day 9, larger conceptuses were mostly males and smaller conceptuses mostly females (Tsunoda *et al.*, 1985; Seller and Perkins-Cole, 1987). A similar sex–size relationship occurs in cattle conceptuses derived *in vivo* and

in vitro (Avery *et al.*, 1989, 1991; Xu *et al.*, 1992). These sex differences in development rates occur before the appearance of the gonads, so cannot be a consequence of gonadal hormone action, and may be evident as early as the first cleavage (Yadav *et al.*, 1993; Zwingman *et al.*, 1993). Since the immediate preattachment phase is a critical period for conceptus survival in pigs, conceptuses were karyotyped to determine possible effects of genetic sex on development to this stage.

Materials and Methods

Yorkshire gilts were housed in groups at the Arkell Swine Research Centre and oestrus was detected by exposure to a boar between 08:00–10:00 and 16:00–18:00 h each day. At second oestrus, 19 females were mated to the same boar approximately 24 h after observation of initial signs of standing oestrus. Animals were then housed individually and fed appropriate amounts (1.5 kg) of a corn–soybean diet until slaughter 10 days later (day 0 = day of mating or second day of oestrus). Reproductive tracts were obtained immediately after exsanguination and conceptuses recovered by flushing each uterine horn with 20 ml PBS. All recovered conceptuses ($n = 214$), still maintained in the isotonic flushing fluid, were photographed within minutes of recovery through a stereomicroscope for subsequent measurement (see below). Conceptuses were then cultured individually in Medium 199 with 20% fetal calf serum and 1% colcemid (all obtained from Gibco, New York) for 4 h at 37°C in an atmosphere of 5% CO₂ in

*Correspondence.

Received 8 October 1993.

Fig. 1. Metaphase spread from a male pig conceptus on day 10 of gestation. Arrow indicates the Y chromosome.

humidified air and then prepared for karyotyping by the method described by Romagnano *et al.* (1985) with minor modifications. In brief, conceptuses were moved from the culture medium into 1% sodium citrate for 10–12 min, fixed in a 3:1 (v:v) mixture of ethanol and acetic acid and transferred into 0.5 ml of 50% acetic acid to dissociate cells. From the resulting suspension, five slides were prepared and stained with 4% Giemsa (Fisher Scientific, Pittsburgh, PA). Metaphase spreads were located and sexes determined by the presence or absence of the Y chromosome in at least two spreads from each individual. Conceptuses whose sex could not be identified owing to lack of metaphase cells or readable metaphase spreads were classified as undetermined. After the cytogenetic study, individual images were measured by tracing outlines on a digitized tablet interfaced with a computer program designed to calculate surface areas (mm²). Within each litter, individuals were classified and grouped as small, medium or large by three equal divisions of the size range between the smallest and largest litter members. Males, females and undetermined conceptuses were then matched to the size groups.

Statistical procedure

The overall sex ratio was tested by χ^2 and logistic analysis used to compare proportions of male, female and undetermined conceptuses in the three size groups (SAS, 1988).

Results

Ovulations in the experimental animals averaged 14.05 ± 2.1 and a mean of 11.26 ± 2.9 normally developing conceptuses were recovered, giving a survival rate of 80%. Uterine flushings from 19 gilts yielded a total of 214 conceptuses but only 125 (58%) of these provided metaphase spreads suitable for successful karyotyping (Fig. 1). No metaphase spreads were obtained in any of the 12 specimens from one gilt; sex data therefore came from 18 litters. Of the 77 remaining conceptuses that could not be sexed, 56 had no metaphase spreads

Fig. 2. Numbers of (▨) male, (□) female and (■) sex-undetermined porcine conceptuses classified into small ($n = 88$), medium ($n = 44$) or large-sized ($n = 82$) groups within litter on day 10 of gestation.

and 21 had spreads that were not of sufficient quality to positively determine sex. One triploid individual was also found. The overall sex ratio of 68 females to 57 males was not significantly different from 1:1 ($\chi^2 = 0.97$; $P > 0.25$). The actual ratio of females:males for the three size groups within litters was 42:9 in small, 12:10 in medium and 14:38 in large groups (Fig. 2). Logistic analysis indicated that there were significantly more females ($P < 0.001$) among small conceptuses and more males in the large group. Sex ratio in the medium sized group was 1:1. From the 18 litters that provided data, 42% (84) of all conceptuses were small, 20% (41) medium and 38% (77) large. The distribution for 77 unsexed conceptuses was similar with 43% (33) small, 25% (19) medium and 32% (25) in the large sized groups ($\chi^2 = 1.08$; $P > 0.1$).

Discussion

Conceptuses recovered on day 10 after mating ranged in shape from small and large spheres to ovoid and tubular forms in keeping with previously reported variations (Hunter, 1974; Anderson, 1978; Pope and First, 1985; Pusateri *et al.*, 1990). The range of sizes varied between litters but it was possible to partition each litter into small, medium and large groups. Karyotyping members in each of these three size divisions indicated that male pig conceptuses are generally larger than female conceptuses on day 10 after mating. This sex-size relationship is similar to that found in preimplantation mice (Tsunoda *et al.*, 1985; Sellar and Perkins-Cole, 1987; Valdivia *et al.*, 1993) and cattle for blastocysts produced *in vivo* (Avery *et al.*, 1989) and *in vitro* (Avery *et al.*, 1991, 1992; Xu *et al.*, 1992).

It has been proposed that at about day 10, the more developed conceptuses, through earlier synthesis of oestrogen,

advance the uterine environment (Geisert *et al.*, 1982) to allow preferential support for their survival and loss of smaller litter members (Morgan *et al.*, 1987). Pope *et al.* (1990) and Xie *et al.* (1990) proposed that size variability at this stage of gestation results from the protracted period over which ovulation takes place. However, a recent study using transrectal ultrasonography to study ovulation in naturally ovulating sows indicated a relatively short range (1.8 h) for duration of ovulation and no relationship between ovulation time and conceptus diversity at approximately 100 h after the follicular rupture commenced (Soede *et al.*, 1992). This relatively short duration of ovulation is similar to that reported for Meishan pigs (Martinat-Botte *et al.*, 1989) and casts doubt on the suggestion that ovulations occurring over an extended period account for developmental diversity observed between day 11 and day 12.

Various mechanisms might contribute towards the occurrence of phenotypic differences between the sexes before development of the gonads. Sex-determining region genes, *Sry* and *Zfy* are transcribed at the two-cell stage in mouse zygotes, suggesting that sex determination starts before gonadal differentiation (Zwingman *et al.*, 1993). Thornhill and Burgoyne (1993) contend that the paternally derived X chromosome has a retarding effect on development in mice, while Tsunoda *et al.* (1985) and Burgoyne (1993) presented evidence that pre-implantation sex differences in mice may be due to an accelerating effect of the Y chromosome. Sex-related metabolic differences also appear early in gestation since total glucose metabolism in male bovine blastocysts collected directly from donor cattle 7 days after oestrus is greater than in females (Tiffin *et al.*, 1991). Some or all of these mechanisms may be involved in pig conceptuses.

Lambert *et al.* (1991) proposed that most embryonic loss occurs within the first 10 days of gestation and, therefore, the sex ratio at the end of this period should be a reasonable representation of the ratio at birth. For the period January 1990 to December 1992, the secondary sex ratio of 14 021 piglets from 1595 litters born in the research centre where the current experimental gilts originated was 1.07:1 (52% males:48% females). While sex ratio at fertilization is unknown, Pineda and Faulkner (1980) indicated that the secondary sex ratio in pigs approximates 1:1 with a range of 52.8–48.8% for males. In the present study, the ratio of sexed conceptuses was 0.84:1, 57 males (46%) and 68 females (54%). This is not significantly different from the expected 1:1 ratio ($P > 0.25$), and with a larger sample size might be even closer to 1:1. It is also within the range expected from previous results recorded in this herd.

In pigs, the theory of early gestational loss resulting from asynchrony of some conceptuses and the uterus implies that smaller or slower developing individuals die because the advancing uterine environment favours larger or more differentiated ones. The finding of sex-related developmental diversity indicates that females are smaller at the critical preattachment stage. If the above hypothesis is correct, more females would be lost and more males survive. It is generally expected that the sex ratio at birth is 1:1. If females are lost preferentially by virtue of their smaller size, then they must have been represented in greater numbers initially. There is, however, no evidence suggesting differential fertilization by X- or Y-bearing

spermatozoa. In the present study, there was 20% embryo loss by day 10. While the cause of this loss is uncertain, greatest developmental diversity occurs 11–12 days after oestrus (Pusateri *et al.*, 1990).

The numbers and sex distribution of conceptuses karyotyped indicated no bias towards identification of either males or females. Unsexed specimens ($n = 77$) were distributed among all size groups in a reasonably uniform manner and it is unlikely that there was any predominance of one sex among these. Even if the sex ratio was approximately equal in small and large specimens not successfully karyotyped, there would still be substantially more males among large and substantially more females among small conceptuses. Recovery rate was 80% on day 10, implying that the majority of losses had already taken place, so the sex ratio at that time should closely approximate to the sex ratio at birth (approximately 1:1). Karyotyping showed that the overall sex ratio was not significantly different from 1:1, but there were significantly more males in the large and more females in the small groups ($P < 0.001$). Both sexes were equally represented in the medium-sized group. These results demonstrate that genetic sex influences the rate of development of pig conceptus before attachment commences and indicate that early survival–mortality is neither size nor sex-dependent.

The technical assistance of D. Sheppard and financial support of the Natural Science and Engineering Research Council of Canada and the Canadian Commonwealth Scholarship Plan is gratefully acknowledged.

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