Embryonic and fetal development in a commercial dam-line genotype

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Abstract

During depopulation of a breeding unit within Swine Graphics Enterprises, extensive data were collected and used to examine relationships among ovulation rate, the pattern of prenatal loss, and placental and fetal development. Groups of Large White $\times$ Landrace females ($n = 447$) were slaughtered between day 20–30, 50–55 or 85–90 of gestation, with approximately equal numbers of animals representing gilts and parity 1 (G/P1), parity 2–3 (P2/3), and parity >4 (P4+). Ovulation rate and embryo number were recorded for all animals. With the exception of the G/P1 animals, embryonic and placental weight were recorded for four conceptuses per sow on day 20–30; on day 85–90 two conceptuses per sow were dissected to determine placental and fetal development. Ovulation rate (22.7 ± 0.2 overall) was higher ($P < 0.05$) in P2/3 (23.6 ± 0.4) and P4+ (24.7 ± 0.4) than in G/P1 (20.2 ± 0.5). Embryonic/fetal survival was $61.8 \pm 2.1\%$ at day 20–30, $50.2 \pm 2.2\%$ at day 50–55 and $48.7 \pm 1.9\%$ at day 85–90 and the number of surviving conceptuses was higher ($P < 0.05$) in the P2/3 sows than in other parity groups. There was no relationship between ovulation rate and number of live embryos at day 20–30 or 85–90. At day 20–30 and 85–90, embryo weight was positively correlated with placental weight, but neither placental weight nor embryonic/fetal weight was correlated with number of viable embryos. A parity by gestation day interaction existed; placental weight for P4+ (3.42 ± 0.43 g) was less than for P2/3 (7.55 ± 0.40 g) at day 20–30 ($P < 0.0001$), whereas at day 85–90, placental weight of P2/3 (209.5 ± 8.5 g) was less ($P = 0.05$) than both G/P1 (235.7 ± 7.3 g) and P4+ (235.4 ± 7.1 g). At day 85–90, fetal brain weight, relative to body weight ($R^2 = 0.61$, $P < 0.0001$), and fetal brain:liver weight ratio ($R^2 = 0.35$; $P < 0.0001$) were negatively related to mean fetal weight, and brain:liver weight ratio showed a trend towards a relationship with number of viable fetuses ($P = 0.08$). Parity also affected brain:liver weight ratio ($P = 0.01$). Clearly, high ovulation rates in the higher parity sows have the potential to cause

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excessive in utero crowding of conceptuses in the post-implantation period. Even with moderate crowding, increased brain:liver weight ratios in smaller fetuses in late gestation indicate that uterine capacity impacts fetal development as well as the number of surviving fetuses.

1. Introduction

The concept of uterine capacity as the ultimate constraint on litter size in swine has been widely studied using different animal models to examine effects of crowding in utero. Techniques including uterine ligation, oviduct resection, unilateral hysterectomy and ovariectomy (UHO; Christensen et al., 1987), superovulation, and embryo transfer have been employed and led to the conclusion that when the number of embryos exceeded 14, intrauterine crowding was a limiting factor for litter size born (Dziuk, 1968). Fenton et al. (1970) determined that uterine capacity only becomes a limiting factor for fetal survival after day 25 of gestation and Knight et al. (1977) further defined day 30–40 of gestation as the critical period when uterine capacity exerts its effects. Subsequent studies in both intact and UHO females support this conclusion (see Vallet, 2000). Wu et al. (1989) restricted the length of uterus available to each fetus and concluded that 36 cm of initial uterine length was required for fetal survival and development. Bennett and Leymaster (1989, 1990a, 1990b) developed a simulation model of litter size and highlighted the importance of interaction among its components, namely ovulation rate, embryonic viability and uterine capacity. The greatest increase in litter size was produced following combined selection for indices of ovulation rate and uterine capacity. Whilst earlier studies addressed uterine capacity in terms of number of embryos, uterine space requirement for embryo survival and time of embryonic loss, less focus has been directed towards associated effects on fetal development in utero. In addition, ovulation rates of animals used in these early studies were relatively low (10–12) compared with ovulation rates >25 reported in contemporary commercial sow populations for which data are available (Orzechowski, 1998; Vonnahme et al., 2002) and the concept of uterine capacity needs to be re-evaluated in such populations.

The extremes of intrauterine growth retardation (IUGR) or "runting" have been described in the pig (Adams, 1971; Widdowson, 1971; Cooper et al., 1978; Hegarty and Allen, 1978; Flecknell et al., 1981), and were identified within a discrete subpopulation of lighter weight fetuses (Royston et al., 1982; Wootton et al., 1983). However, conclusions based only on a consideration of fetal weight may overlook critical effects on fetal development that are established early in gestation. Indeed, Hegarty and Allen (1978) reported that within a litter, runts have a reduced muscle growth potential and, as a consequence, needed 23 days longer to reach a weight of approximately 105 kg (slaughter weight). In addition, data from a study by Aberle (1984) indicated that naturally occurring IUGR may delay myofibre differentiation, preferentially affecting secondary muscle fibre development.

High ovulation rates with only modest increases in litter size born in commercial dam-line sows may result in a dramatic change in the dynamics of prenatal loss (Foxcroft, 1997). This change is associated with crowding of embryos in utero in the immediate post-implantation

period and a peak of prenatal loss between day 30 and 50 of gestation (Vonnahme et al., 2002). The consequences of this pattern of prenatal loss for placental and fetal development, and as a factor contributing to increased variability in postnatal growth performance, need to be determined. A recent study of associations among within-litter variation in birth weight, and pre-weaning survival and weight gain, also led to the conclusion that selection for increased litter size that results in more low-birth-weight piglets may not be beneficial, unless measures are taken to improve the survival of the low-birth-weight offspring (Milligan et al., 2002). Thus, both the developmental competence of the pigs born, as well as the size of the litter, are critical factors.

In contrast to situations in which the occurrence of IUGR is limited to a discrete subpopulation of "runt fetuses" (Royston et al., 1982; Wootton et al., 1983), a changing pattern of embryonic loss that results in an increased number of embryos during early gestation may produce a more widespread effect on placental development that will likely affect the growth of all surviving fetuses. For this reason, in the present study, it is of interest to examine effects of crowding in utero on the "average" conceptus from each litter rather than the extreme examples of IUGR.

A negative relationship between the number of conceptuses and placental volume, and a significant positive relationship between placental area and embryo size at day 28 of gestation in gilts (Almeida et al., 2000), suggested that even moderate uterine crowding in early gestation affects placental and embryonic development. If this is not fully compensated for by increased placental efficiency in surviving conceptuses later in gestation, there may be major consequences for prenatal development and postnatal growth performance, analogous to IUGR previously reported in the pig (Widdowson, 1971).

Having already established that mean ovulation rate in a similar population of sows to those used in the present study was 26.6, and that ovulation rate was positively correlated with embryo numbers, and negatively correlated with placental weight, at day 25 of gestation (Vonnahme et al., 2002), we wished to explore further possible implications for fetal development. The depopulation of a second commercial sow-breeding unit within Swine Graphics Enterprises provided this opportunity.

The first objective of this study was to substantiate relationships between ovulation rate and the pattern of prenatal loss using a rare opportunity to obtain further data from a contemporary commercial crossbred population. Secondary objectives were to extend the earlier investigation (Vonnahme et al., 2002), to determine associations between the pattern of prenatal loss and placental and fetal development, and to examine parity-dependent changes in this pattern of prenatal loss.

2. Materials and methods

2.1. Animals

In an internal, porcine reproductive and respiratory syndrome (PRRS) naïve, genetic multiplication program (Swine Graphics Enterprises, Inc., Webster City, IA, USA), Pig Improvement Company (PIC-USA, Franklin, KY, USA) Camborough sows were crossed with PIC-derived boars to produce the equivalent of PIC line 1055 F1 gilts for commercial

production. These gilts were then mated with crossbred PIC terminal-line 337 boars at production level. Bred females from one such commercial breeding unit (ADL 1 Swine Unit, Osceola, IA, USA) were made available for this study as part of a herd depopulation exercise linked to mortality in nursery pigs from the sow unit due to previous PRRS outbreaks. Productivity of these sows had previously been adversely affected by infection with two different strains of PRRS virus. However, PRRS virulence within the breeding herd was stabilised by vaccinations with autogenous PRRS vaccines in July and early December 2000, at least 60 days before any of the sows used in the present study were bred.

As part of the study design, weaned sows were bred by AI with semen from Landrace or Large White Boars (Pig Improvement Company, USA) according to normal herd practice. In addition, a group of gilts was also specifically bred to be included in the parity 0–1 data set. Females were inseminated each morning that standing oestrus was detected in the presence of a boar, using pooled semen containing approximately 4 billion sperm per AI dose. Bred females were checked daily for return to oestrus in the presence of a boar until day 50 of gestation, unless they were allocated to an earlier slaughter group, in which case they were checked for oestrus until the day they were shipped. For the purposes of this study, 454 presumed pregnant females were initially allocated to slaughter on one of three days on the basis of parity; gilts and first parity (parity 0–1), second and third parity (parity 2–3), and fourth parity and greater (parity >4). The parity distribution of animals shipped is shown in Fig. 1. Of the approximately equal numbers of sows initially allocated to be at different stages of gestation at the time of slaughter, data were obtained from the reproductive tracts of 103 of 114 animals at day 20–30, 149 of 163 animals at day 50–55 and 169 of 177 animals at day 85–90 of gestation (total of 421 pregnant tracts at slaughter). Mishandling and loss of tissue during dissection resulted in some loss of material; the numbers of tracts contributing data to different reproductive characteristics within different parity groups are shown in Table 1. Logistical constraints to measuring reproductive tract data from such a large number of animals over a period of only 3 days prevented the identification of individual sows. Given this constraint, the rationale behind the parity group allocations was that in gilts and first parity sows (parity 0–1) ovulation rate and embryonic survival rate
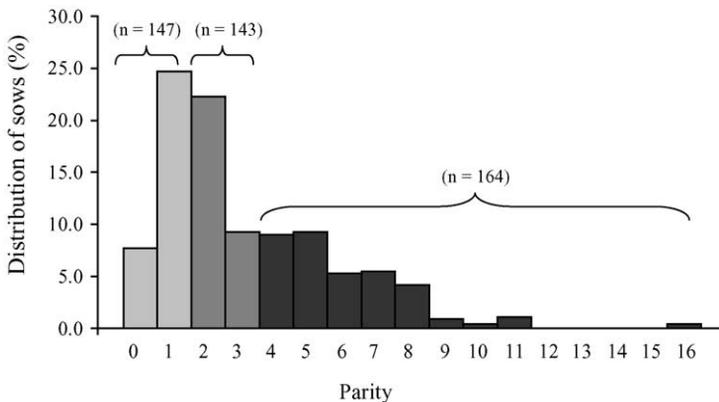
Fig. 1. Parity distribution of sows allocated for slaughter ($N = 454$).

Table 1
Reproductive characteristics of sows (least square means ± SEM)

Parameter	Parity group		
	0–1	2–3	>4
Ovulation rate ($N = 405$)	20.18 ± 0.49 a ($n = 138$)	23.59 ± 0.43 b ($n = 121$)	24.71 ± 0.38 b ($n = 146$)
Number of viable conceptuses	N/A	13.65 ± 0.39 a	11.81 ± 0.34 b
Day 20–30 sows ($N = 80$)	$n = 0$	$n = 37$	$n = 43$
Placental weight (g) at day 20–30	N/A	7.55 ± 0.43 a	3.42 ± 0.40 b
Fetal weight (g) at day 20–30	N/A	0.46 ± 0.03 a	0.40 ± 0.03 a
Placental efficiency at day 20–30	N/A	0.07 ± 0.01 a	0.12 ± 0.01 b
Day 50–55 sows ($N = 149$)	$n = 71$	$n = 31$	$n = 47$
Day 85–90 sows ($N = 166$)	$n = 57$	$n = 48$	$n = 61$
Placental weight (g) at day 85–90	235.7 ± 7.3 a	209.5 ± 8.5 b	235.4 ± 7.1 a
Fetal weight (g) at day 85–90	751.8 ± 19.4 a	755.8 ± 22.3 a	738.7 ± 18.7 a
Placental efficiency at day 85–90	3.30 ± 0.09 a	3.68 ± 0.11 b	3.23 ± 0.09 a
Brain:liver weight ratio at day 85–90	1.00 ± 0.03 a	0.85 ± 0.04 b	0.92 ± 0.03 a,b
	Gestation day		
	20–30 ($n = 80$)	50–55 ($n = 74$)	85–90 ($n = 100$)
Number of viable conceptuses (parities 2–3 and >4)	14.98 ± 0.46 a	12.17 ± 0.48 b	11.05 ± 0.41 b
Survival rate (%) parities 2–3 and >4 ($N = 254$)	61.8 ± 2.1 a	50.2 ± 2.2 b	48.7 ± 1.9 b

Least square means ± SEM within a row with different letters differ ($P \leq 0.05$). N/A denotes data not available for analysis.

would typically be limiting factors for litter size born. In contrast, in second and third parity sows (parity 2–3) reproductive performance would be optimal and uterine capacity, rather than ovulation rate and embryo survival rate would be the critical constraint on litter size. Sows of fourth parity and greater (parity >4) would represent sows in which reproductive performance would be declining, associated with increased variability in the uniformity of pigs born in terms of numbers and development.

2.2. Slaughter and dissection procedures

Reproductive tracts were dissected at cooperating commercial abattoirs immediately after slaughter (PorkKing Packing Company, Marengo, IL; Abbyland, Curtis, WI) and ovulation rate and number of viable conceptuses were recorded for all animals. A requirement of the depopulation process was to ship all animals over a 3-day period. Time and manpower constraints limited the number of conceptuses that could be dissected from each reproductive tract. Therefore, day 20–30 and 85–90 were chosen as critical time points to monitor any effects on development. Unfortunately, these logistical constraints did not allow collection of embryo data from day 20–30, parity 0–1 animals, which resulted in a missing

cell of data for some reproductive characteristics. However, ovulation rate and number of viable conceptuses were recorded for all other females (Table 1, Figs. 3 and 4). As a review of available literature (Town, 2004) suggested that extremes of in utero development are associated with the ovarian and cervical ends of the uterine horn, two conceptuses were dissected from each mid-uterine horn on day 20–30 and embryonic and placental weights recorded, thus providing a conservative measure of overall effects on embryonic development. Again, to avoid extremes of development determined by fetal position in utero, one representative fetus was selected from each mid-uterine horn on day 85–90 tract (*n* = 166), and used to record fetal and placental weights. The fetuses were then dissected to determine brain and liver weights. The brain:liver weight ratio was then used as an estimate of disproportionate changes in organ development, indicative of the occurrence of IUGR.

2.3. Statistical analysis

To determine the effects of parity group, gestation day and their interaction on ovulation rate, embryonic survival rate, number of viable embryos, placental and fetal weights, placental efficiency and fetal brain:liver weight ratio, data were analysed as appropriate for a completely randomised design. Sow was used as the experimental unit for analysis, and fetal weights, placental weights and organ weights were sub-sampled and averaged within each reproductive tract (sow) before analysis. As logistical constraints prevented collection of embryo data from day 20–30 parity 0–1 animals, different statistical models were used to analyse parameters of interest and thus extract the most information from the data available.

Ovulation rate data were complete for all nine groups of animals, and therefore, were analysed as a 3×3 factorial for parity group and gestational day using the general linear model (GLM) procedure of the statistical analysis system (SAS, 1990, SAS Inst. Inc., Cary, NC). Due to missing embryo data from the day 20–30, parity 0–1 group (see Table 1), embryo survival rate and the number of live embryos were first analysed as a 3×2 factorial (using only data from parity 2–3 and parity >4 sows, at all gestational days, to examine primarily the effect of gestational day). Number of viable fetuses and fetal survival data were then analysed as a 2×3 factorial using only gestational day 50–55 and 85–90, but all three parity groups, to examine primarily the effect of parity.

Since placental and fetal data were only obtained from day 20–30 animals and 85–90 animals, the effect of parity on conceptus development at these time points was further examined by analysing placental and embryonic/fetal weights both as a 2×2 factorial using data from gestational day 20–30 and 85–90 and parity groups 2–3 and >4, and using data for all three parities and gestational day 85–90. The effect of parity on brain:liver weight ratio, was also examined using data for all three parities and gestational day 85–90.

Trends analysis (Steel et al., 1997) was used to determine if there was a linear relationship between gestation day and both embryonic/fetal survival rate and the number of viable embryos/fetuses. The Tukey–Kramer test (SAS, 1990) was used for making pairwise comparisons between least squares means.

Relevant associations within gestational age between ovulation rate, number of viable embryos, fetal weight, placental weight, placental efficiency (calculated as the fetal:placental weight ratio; day 20–30 and 85–90), and fetal brain and liver weights and brain:liver weight

ratio (day 85–90), were examined using the INSIGHT procedure (SAS, 1990), and only involved animals for which complete data sets were available.

3. Results

Of the 454 presumed pregnant animals initially allocated for slaughter, seven sows died during transport, resulting in 447 presumed pregnant sows slaughtered. Of these, 421 were confirmed pregnant by the presence of viable conceptuses. Conceptuses were classified as viable using semi-objective criteria based on visual appearance of the embryo or fetus and the placenta. In general, if the placenta was well vascularised and did not look necrotic, it was classified as viable. As specified later, data from other sows were excluded from the analysis as a result of damage during tract removal and collection (missing ovaries, etc.). Ovulation rate (22.7 ± 0.2 overall), was affected by parity ($P < 0.0001$; Table 1) and was higher in parity 2–3 (23.6 ± 0.4) and parity >4 (24.7 ± 0.4) sows than in parity 0–1 sows (20.2 ± 0.5). As shown in Fig. 2, approximately 18% of the higher parity sows had ovulation rates ≥ 30. There was no effect of gestational day, nor a parity by gestational day interaction, on ovulation rate.

Analysis of number of viable embryos over all three gestation time points, but using only parity groups 2–3 and >4 (3×2 factorial design), revealed an effect of gestational day ($P < 0.0001$), and a parity by gestational day interaction ($P = 0.04$; Fig. 3). Within parity groups, there was a negative linear relationship between number of viable embryos and gestational day at slaughter in parity 2–3 (number of viable embryos = 16.30–0.045 (gestational day at slaughter); $R^2 = 0.07$; $P = 0.003$) and parity >4 (number of viable embryos = 16.38−0.078 (gestational day at slaughter); $R^2 = 0.19$; $P < 0.0001$) sows. When analysed as a 2×3 factorial using only gestational day 50–55 and 85–90 but all three parity groups, the number
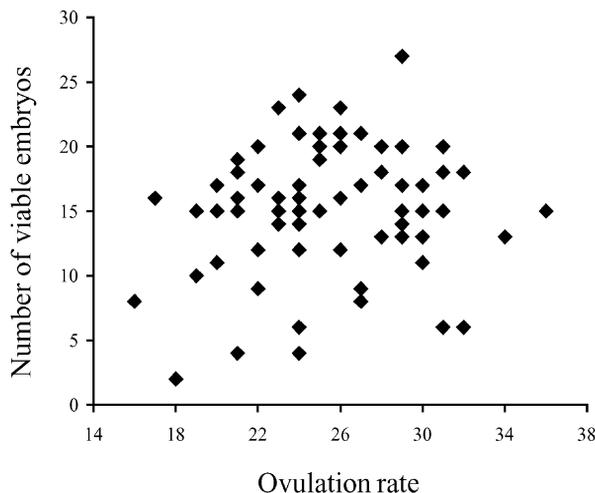
Fig. 2. Lack of a relationship between ovulation rate and number of viable embryos at day 20–30 of gestation ($P = 0.18$) in parity groups 2–3 and >4 ($n = 73$).
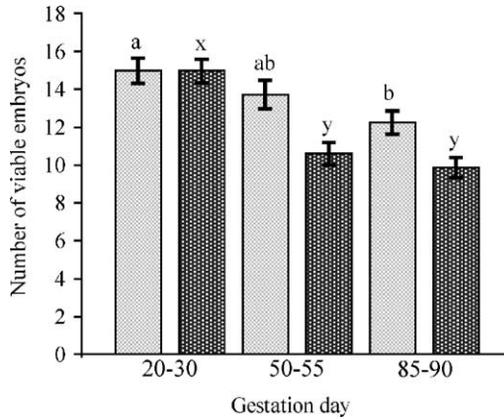
Fig. 3. 3 × 2 Factorial analysis of number of viable embryos over all three gestational days for parity groups 2–3 (light bars) and >4 (dark bars). LS means with different superscripts differ within parity group.

of viable fetuses was affected by parity ($P < 0.0001$; Fig. 4(a) and was higher ($P < 0.05$) in the parity 2–3 sows than in the other parity groups. There was no relationship between ovulation rate and number of live embryos at day 20–30 ($P = 0.18$; Fig. 2) or day 85–90 ($P = 0.72$).
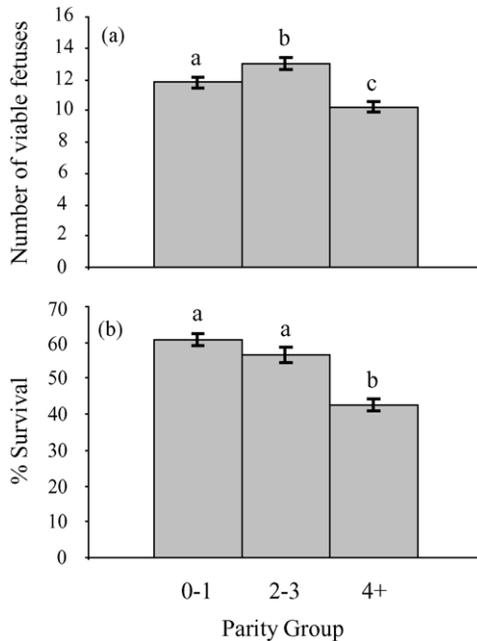
Fig. 4. Results of 2 × 3 factorial analysis of (a) the number of viable fetuses and (b) % embryonic survival on day 50–55 and 85–90 of gestation using all three parity groups. Least square means with different superscripts differ. Error bars denote S.E. of least square means. Bars that do not share a common letter indicate significantly different values ($P < 0.05$).

Survival rate, calculated by dividing the number of viable embryos/fetuses per sow by ovulation rate, was analysed over all three gestation time points using parity groups 2–3 and >4 (3 × 2 factorial). Survival was affected by day of gestation ($P < 0.0001$; Table 1) and there was a negative linear relationship between survival rate and gestational day (survival rate = 0.62−0.0017 (gestational day at slaughter); $R^2 = 0.05$; $P < 0.0001$). There was no day of gestation by parity interaction for survival rate. The 2 × 3 factorial analysis of embryo survival rate (gestational day 50–55 and 85–90 only, analysed over all three parities) indicated an effect of parity ($P < 0.0001$; Fig. 4(b)) and there was also a negative linear relationship between survival rate and parity (survival rate = 0.70−0.0865 (parity group); $R^2 = 0.16$; $P < 0.0001$). Again, no gestational day by parity interaction was present.

Mean embryonic and placental weights from the four day 20–30 conceptuses dissected per sow were available for 80 of the 87 sows slaughtered. The analysis of placental and embryonic/fetal weights, as either a 2 × 2 factorial using data from gestation day 20–30 and 85–90 and parity groups 2–3 and >4, or using data for all three parities and gestation day 85–90, revealed effects of gestation day, and a parity by gestational day interaction, for placental weight (Table 1). When the parity by gestational day interaction was examined, the average placental weight for parity >4 (3.42 ± 0.43 g) was less than half that of parity 2–3 (7.55 ± 0.40 g) at day 20–30 ($P < 0.0001$). However, at day 85–90, the average placental weight of parity group 2–3 (209.5 ± 8.5 g) was lower than both parity 0–1 (235.7 ± 7.3 g) and parity >4 (235.4 ± 7.1 g) ($P = 0.05$) which were not different from each other. As expected, there was a significant effect of gestational day on fetal weight, but there was no main effect of parity, or a parity by gestational day interaction.

Average embryonic weight was positively related to average placental weight at day 20–30 of gestation ($R^2 = 0.20$; $P < 0.0001$; Fig. 5(a), but neither average placental weight ($P = 0.75$), nor average embryonic weight ($P = 0.88$) was correlated with number of viable embryos. Data from the two conceptuses dissected per sow were available from 166 of the 175 pregnant sows slaughtered at day 85–90. Average fetal weight was positively correlated with average placental weight ($R^2 = 0.29$; $P < 0.0001$; Fig. 5(b) and there was a tendency
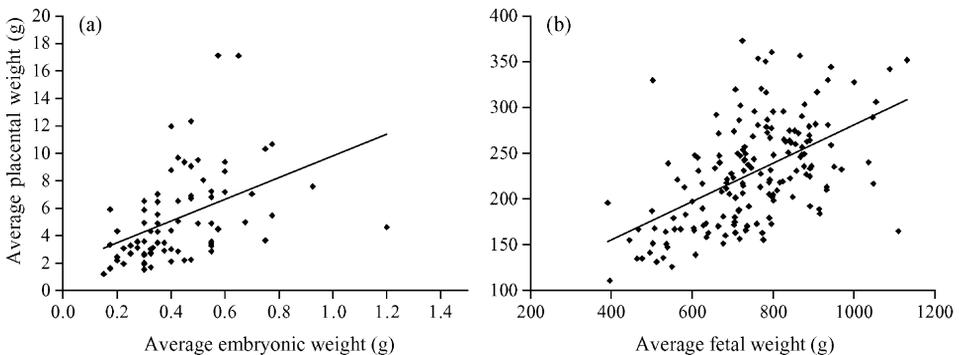
Fig. 5. Relationship between average placental weight and (a) average embryo weight at day 20–30 of gestation (average placental weight = 1.90 + 7.94 (average embryonic weight); $R^2 = 0.20$; $P < 0.0001$; $n = 79$) and (b) average fetal weight at day 85–90 of gestation (average placental weight = 71.89 + 0.21 (average fetal weight); $R^2 = 0.29$; $P < 0.0001$; $n = 166$).

towards a relationship between average placental weight and number of viable fetuses (P = 0.057). However, average fetal weight (P = 0.32), was not correlated with number of viable fetuses.

Placental efficiency was calculated as the embryonic weight:placental weight ratio. The analysis of placental efficiency (2×2 factorial for gestational day 20–30 and 85–90 and parity groups 2–3 and >4), revealed effects of gestational day, parity, and a parity by gestational day interaction (Table 1). When the parity by gestational day interaction was examined, placental efficiency for parity >4 (0.12 ± 0.01) was seen to be almost double that of parity 2–3 (0.07 ± 0.01) at day 20–30 ($P < 0.0001$), consistent with the placental weight results. The analysis of placental efficiency for all three parities and gestation day 85–90 revealed an effect of parity. At day 85–90, the average placental efficiency of parity groups 2–3 (3.68 ± 0.11) was higher ($P < 0.03$) than both parity 0–1 (3.30 ± 0.09) and parity >4 (3.23 ± 0.09), which were not different ($P > 0.05$).

At day 20–30, placental efficiency was weakly correlated with average fetal weight (R^2 = 0.09; P = 0.009) but was not related to number of viable embryos (P = 0.34). However, placental efficiency showed a stronger negative correlation with average placental weight
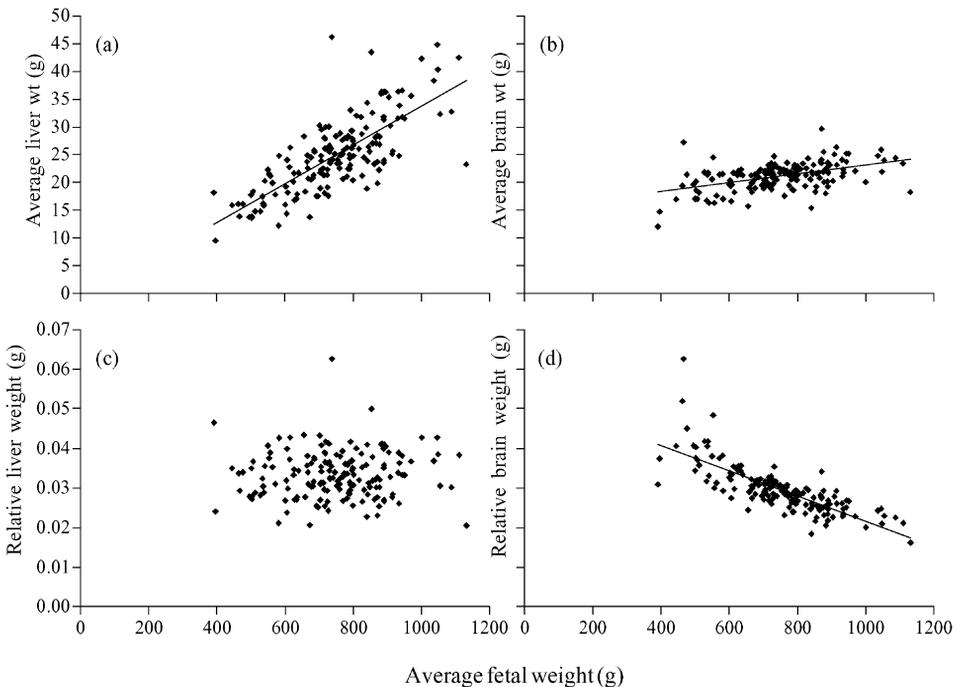
Fig. 6. Positive relationship between (a) average liver weight (liver weight = $-1.19 + 0.035$ (fetal weight); R^2 = 0.56; P <0.0001) and (b) average brain weight (brain weight = $15.23 + 0.008$ (fetal weight); R^2 = 0.23; P < 0.0001), and average fetal weight at day 85–90 of gestation. Nonsignificant relationship (P = 0.45) between mean relative liver weight (c) and a negative correlation (relative brain weight = 0.054 to 3.2×10^{-5} (fetal weight); R^2 = 0.61; P < 0.0001) between mean relative brain weight (d) and average fetal weight at day 85–90 of gestation (n = 166).
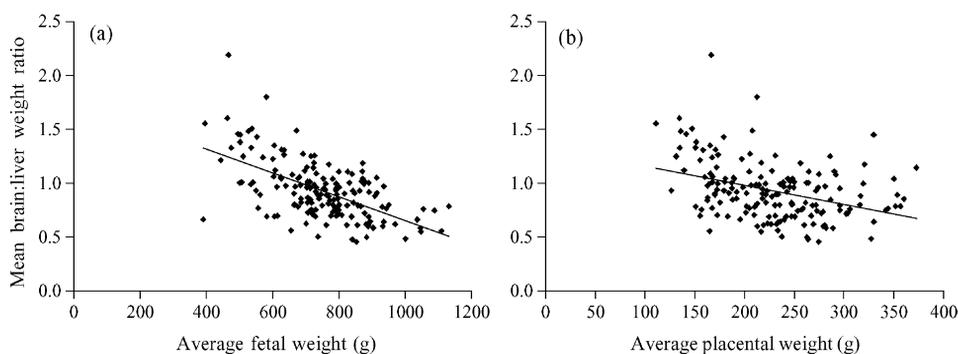
Fig. 7. Relationship between mean brain:liver weight ratio and (a) average fetal weight at day 85–90 of gestation (brain:liver weight ratio $= 1.75 - 0.0011$ (fetal weight); $R^2 = 0.35$; $P < 0.0001$) and (b) average placental weight at day 85–90 of gestation (brain:liver weight ratio $= 1.33 - 0.0018$ (placental weight); $R^2 = 0.14$; $P < 0.0001$; $n = 166$).

($R^2 = 0.37$; $P < 0.0001$). Average placental weight at day 85–90 was negatively correlated to placental efficiency ($R^2 = 0.38$; $P < 0.0001$), whilst average fetal weight showed a very weak positive correlation with placental efficiency ($R^2 = 0.08$; $P = 0.0002$). Placental efficiency was not related to the number of viable fetuses at day 85–90 of gestation ($P = 0.18$).

Parity affected brain:liver weight ratio ($P = 0.01$; Table 1), such that parity 0–1 had a higher brain:liver weight ratio than parity 2–3. Brain:liver weight ratio was not different between parity 0–1 and parity >4, nor between parity 2–3 and parity >4. Both mean absolute liver weight ($R^2 = 0.56$; $P < 0.0001$; Fig. 6(a) and brain weight ($R^2 = 0.23$; $P < 0.0001$; Fig. 6(b)) were positively correlated to average fetal weight. However, when relative organ weights were calculated as the absolute organ weight:body weight ratio, mean relative liver weight was not related to average fetal weight ($P = 0.45$; Fig. 6(c), whilst mean relative brain weight showed a strong negative correlation to mean fetal weight ($R^2 = 0.61$; $P < 0.0001$; Fig. 6(d)). The mean brain:liver weight ratio was negatively correlated with mean fetal weight ($R^2 = 0.35$; $P < 0.0001$; Fig. 7(a) and mean placental weight ($R^2 = 0.14$; $P < 0.0001$; Fig. 7(b)). However, the mean brain:liver weight ratio was not related to number of viable fetuses ($P = 0.08$).

4. Discussion

A previous depopulation exercise provided an initial opportunity to establish reproductive characteristics in a large number of sows representing a contemporary commercial crossbred population (Vonnahme et al., 2002). In that study, results were obtained from a total of 244 sows of parities 2–14, slaughtered on day 25, 36 or 44 of gestation. The present study provided a second opportunity to examine reproductive characteristics in sows of the same genetic lines over a greater range of parities (gilts up to parity 16) and over a longer duration of gestation (day 25–90). We were then able to interpret the collective data from the two

studies against our working hypothesis that high ovulation rates in higher parity sows in contemporary dam-lines could be the driver of critical changes in the dynamics of prenatal loss and hence fetal development. If high ovulation rates are combined with high embryonic survival, this results in uterine crowding at day 30 of gestation, and a negative relationship between placental size and embryo number around day 25–30 of gestation. Crowding of embryos in utero around day 30 of gestation, in turn, drives a peak of post-implantation loss between day 30 and 50 of gestation (Vonnahme et al., 2002). Unless placental compensation occurs during and after this time, reduced placental size will have negative consequences for fetal development of the remaining conceptuses and for postnatal outcomes. In many respects, we consider that these effects of in utero crowding in swine may be analogous to the effects of IUGR reported both in swine (Bauer et al., 1998; Flecknell et al., 1981; Widdowson, 1971) and in other species (McMillen et al., 2001; Gluckman and Harding, 1997).

The justification for dissecting fetuses for measurement from the mid-uterine horn was to ensure a representative and conservative measure of overall effects on fetal development. Wise et al. (1997) studied the relationships of light and heavy fetuses to uterine position and found no differences between fetal weight and uterine position at day 30. However, at day 70 and 104, heavier fetuses were found to be located at the tubal ends whilst lighter fetuses were found at the cervical ends of the uterus. Since our aim was to be able to evaluate the overall effects of fetal number on the development of the whole litter, we chose to examine the average fetuses, rather than the extremes, within a litter.

The overall ovulation rate for the present study of 22.7 ± 0.2 is a little lower than the ovulation rate of 26.6 ± 0.4 observed by Vonnahme et al. (2002). In part, this resulted from the inclusion of gilts and parity 1 sows that had lower ovulation rates (20.2) than parity 2 and 3 (23.6) or parity >4 (24.7) sows. Nevertheless, the upper range of ovulations rates recorded, with approximately 50% of higher parity sows having ovulation rates of 25 or higher, and 18% of sows having ovulation rates of 30 or higher, confirms the great disparity between ovulation rate and litter size born in this commercial dam-line. The results of the present study confirm that high ovulation rates in commercial sow populations have the potential to dramatically affect the pattern of prenatal loss and hence fetal development.

The extent to which the number of conceptuses at day 30 exceeds uterine capacity to support subsequent fetal development is critically dependent on the interaction between ovulation rate and early embryonic survival. The significant positive relationship between ovulation rate and numbers of viable conceptuses in utero at day 25 of gestation established by Vonnahme et al. (2002) was not evident in the present study, and the reason for this is not clear. Embryonic survival to day 20–30 for the parity groups 2–3 and >4 was only 61.8% in the present study, but is comparable to the 60.2% survival to day 25 observed by Vonnahme et al. (2002). In both studies, this probably reflects the relatively poor health status of the herds at the time of depopulation. Clearly, however, the high embryonic loss to day 20–30 of gestation meant that excessive crowding of embryos in utero at day 20–30 was not universally present in the sows included in the present study. Notwithstanding the absence of extreme levels of crowding in this group of animals, conclusions on placental function and fetal development were still possible.

The second factor driving a dynamic change in the pattern of prenatal loss would be a level of uterine crowding at day 30 that substantially exceeds uterine capacity later in gestation,

such that the peak of prenatal loss would now occur in the immediate post-implantation, rather than in the pre-implantation period. Although in utero crowding of embryos was not recorded in the immediate post-implantation period, a significant linear decrease was observed in the number of viable conceptuses in both parity groups examined, decreasing from 15.0 embryos present at day 20–30 in both parity groups to 12.2 fetuses in parity 2–3, and 9.9 fetuses in parity >4, sows at day 85–90 of gestation. Although no parity effect was observed by Vonnahme et al. (2002), a significant decrease in conceptus number was observed between day 25 and 36 of gestation in that study. Consistent with the effect on number of viable conceptuses, day of gestation affected embryonic/fetal survival, which decreased from 61.8% at day 20–30 to 50.2% at day 50–55 and to 48.7% by day 85–90. Together with estimates of survival from the study by Vonnahme et al. (2002) of 60.2% on day 25, 50.1% on day 36 and 46.3% on day 44, these results support the suggestion that the majority of post-implantation loss already occurs by day 50. In both studies, therefore, there was evidence that the number of viable conceptuses at day 25–30 of gestation still exceeded uterine capacity. If the high ovulation rates in these sows were associated with the higher (85–100%) levels of pre-implantation survival reported in studies with high health status commercial genotypes (Zak et al., 1997), excessive in utero crowding of embryos would be present around day 30 of gestation, and would drive a major peak of prenatal loss before day 50 of gestation. The consequences of this changing dynamic of prenatal loss then becomes a critical question.

Logistical constraints to measuring reproductive tract data from such a large number of animals over a period of only 3 days prevented the identification of individual sows. Given this constraint, the rationale behind the parity group allocations was that in gilts and first parity sows (parity 0–1) ovulation rate and embryonic survival rate would typically be limiting factors for litter size born. In contrast, in second and third parity sows (parity 2–3) reproductive performance would be optimal and uterine capacity, rather than ovulation rate and embryo survival rate would be the critical constraint on litter size. Sows of fourth parity and greater (parity >4) would represent sows in which reproductive performance would be declining, associated with increased variability in the uniformity of pigs born in terms of numbers and developmental potential.

Given the rationale described above, significant effects of parity on the number of conceptuses in utero, and the interaction between parity and gestational day on prenatal loss established in the present study, are of interest. The number of fetuses at day 85–90 increased from 11.81 in parity 0–1 animals to 12.24 in parity 2–3 sows and then decreased to 9.86 fetuses in the parity group >4. The increased number of viable embryos in parity groups 2–3 may be due to the improved uterine environment and improved placental efficiency in these more mature sows, particularly given the equivalent fetal weights observed in this group but a significantly lower placental weight. In contrast, despite maximal ovulation rates and comparable levels of pre-implantation survival in the parity >4 sows, it appears that the quality of the uterine environment and placental efficiency declines and hence reduces the functional "capacity" of the uterus. In all parities, however, ovulation rate is never a limiting factor for potential litter size born.

In the present study, placental weight was positively correlated with embryonic weight at day 20–30 and with fetal weight at day 85–90 of gestation, suggesting a functional

relationship between these two variables. Larger piglets are also attached to larger placental at term (Biensen et al., 1999; Wilson and Ford, 2000; Town et al., 2002). However, neither placental nor fetal weights were significantly associated with number of viable embryos at day 20–30 in the present study. Given the lack of a negative relationship between ovulation rate and the number of conceptuses at day 20–30 discussed above, this is probably expected, and the lack of an effect may again be related to the particular population of sows studied. As expected, neither placental nor fetal weights were associated with number of viable fetuses at day 85–90, reflecting a pattern of prenatal loss that limits the number of surviving fetuses to available uterine capacity by the later stages of gestation.

The observed positive associations between absolute liver and brain weights and average fetal weight were expected, although the weaker relationship seen with the brain compared to the liver, suggests that brain weight is less dependent on fetal weight and that a "brain-sparing" effect was present. Further investigation of the effects of IUGR on the pattern of organ development over the wide range of fetal body weights observed was carried out by analysis of relative organ weights (organ:body weight ratio) as used previously (McMillen et al., 2001). This method allows the identification of disproportionate changes of relative organ size with change of absolute body size, which occurs to the greatest extent in the brain.

When relative organ weights are related to fetal weight, the brain sparing effect is more apparent. Relative liver weight showed no relationship to fetal weight, whilst relative brain weight showed a strong negative relationship with fetal weight. These results confirm data from other studies (Town et al., 2002) in which an increase in relative brain mass with decreasing fetal weight is assumed to reflect a brain-sparing effect. In the context of the present study, it is important to recognise that the maintenance of disproportionate brain growth in growth-restricted fetuses is occurring even in situations where excessive in utero crowding of developing fetuses is not present.

The brain:liver weight ratio was calculated for each fetus as another indicator of growth retardation (Bauer et al., 1998). The mean brain:liver weight ratio was negatively associated with mean fetal weight and mean placental weight as previously observed in neonatal piglets (Town et al., 2002). These relationships demonstrate the detrimental effects of lower fetal and placental weights on organ development. This raises the question of negative consequences for commercially important aspects of development, such as myogenensis. Although brain:liver weight ratio was not related to number of viable fetuses, a positive trend was observed ($P = 0.08$). Furthermore, a significant positive correlation between brain:liver weight ratio in 1-day old piglets and litter size at term was recently observed in a study of the relationship between embryo survival to day 30 of gestation and subsequent fetal development in gilts (Town et al., 2002). The demonstration of brain sparing effects in situations in which the level of crowding in utero in the early post-implantation period is relatively low, compared to the level of crowding that is possible in higher parity sows with ovulation rates of 25 and greater and improved embryonic survival, supports our contention that the changing dynamics of prenatal loss in commercial dam-line sows may have important consequences for the developmental potential of the offspring born. The same mechanisms may also underlie reported detrimental effects of low birth weight in gilts born in large litters (Deligeorgis et al., 1985; Jorgensen, 1989). Data collected from our studies of commercial culled sows has, therefore, encouraged us to develop appropriate

experimental paradigms for studying direct effects of the pattern of prenatal loss on muscle fibre development.

5. Conclusions

Our results confirm that high ovulation rates are a characteristic of some contemporary dam-line sows. In the absence of the high embryonic loss seen in the present study, high ovulation rates provide the basis for excessive in utero crowding of conceptuses in the early post-implantation period, and a radical shift in the dynamics of prenatal loss. Negative effects of increased numbers of conceptuses in utero on placental development around day 30 of gestation reported in our earlier studies, may then have lasting effects on fetal development. Current evidence of measurable brain-sparing effects in smaller fetuses, even in the absence of serious uterine crowding in early gestation, indicates such developmental effects are likely. Possible long-term consequences for postnatal growth performance suggest that the reproductive characteristics of commercial dam-line sows merit further study.

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