Breed differences in placental development during late gestation between Chinese Meishan and White crossbred gilts in response to intrauterine crowding

Jeremy Miles (jeremy.miles@usda.gov)
USDA-ARS Roman L Hruska US Meat Animal Research Center https://orcid.org/0000-0003-4765-8400
Jeffrey L. Vallet
USDA-ARS Roman L Hruska US Meat Animal Research Center

Research

Keywords: Uterine capacity, intrauterine crowding, placental development, Meishan, swine

Posted Date: September 21st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-79405/v1

Version of Record: A version of this preprint was published at Animal Reproduction Science on March 1st, 2021. See the published version at https://doi.org/10.1016/j.anireprosci.2021.106711.

Abstract

Background: The porcine placenta plays a critical role on uterine capacity, fetal growth, and survival as well as postnatal piglet growth and survival. The objective of this study was to evaluate placental development during late gestation between Chinese Meishan (MS) and White crossbred (WC) gilts following intrauterine crowding. To induce uterine crowding, MS (n = 7) and WC (n = 5) gilts were unilaterally hysterectomized-ovariectomized, bred to sires from their same breed, and the remaining uterine tract was collected at day 100 of gestation. Gross placental morphology and areolae density as well as histological morphology (i.e., folded bilayer and placental stroma) were analyzed using computer-assisted morphometry from placentas of the smallest and largest fetuses within each litter. All data were analyzed using MIXED model procedures for ANOVA.

Results: Fetal and placental weight were not different (P > 0.10) between MS and WC pregnancies. In contrast, within-litter fetal weight variation and allometric growth relationship between fetal and placental weights were decreased ($P < 0.08$) in MS gilts compared to WC gilts indicating greater fetal sparing within MS gilts. There was a breed by fetal size interaction ($P < 0.01$) for areolae density in which placentas from large MS fetuses had greater areolae density compared to small MS fetuses, but the density of areolae was greater from MS fetuses compared to WC fetuses, irrespective of fetal size. The width of the folded bilayer was greater ($P < 0.01$) in placentas from WC gilts compared to MS gilts, irrespective of fetal size. Placentas from small fetuses had greater ($P < 0.01$) folded bilayer width compared to large fetuses, irrespective of breed. The placental stromal width was greater ($P < 0.01$) in placentas from large fetuses compared to small, irrespective of breed. However, the difference between stromal width in placentas between divergent-sized littermates was greater ($P = 0.05$) in WC gilts compared to MS gilts, suggesting limited response to intrauterine crowding in MS gilts.

Conclusions: These results demonstrate altered placental development during late gestation in MS pregnancies compared to WC pregnancies corresponding to different mechanisms for responding to intrauterine crowding between breeds.

Background

As the swine industry has selected for increased ovulation and litter size over the past 20 years, there has been a consequential increase in preweaning mortality. Increased piglet mortality has been due in part to greater within-litter birth weight variation and the production of smaller littermate piglets due to limitation in uterine capacity and fetal crowding [1, 2]. The development of the placenta has direct implications on uterine capacity, fetal growth and survival as well as postnatal piglet growth and survival [3, 4]. The porcine placenta is classified as a non-invasive, epitheliochorial placenta in which primary nutrient, gas and waste transfer between the mother and fetus occurs via hemotrophic (i.e., capillary blood) exchange [5]. During late gestation, increased hemotrophic exchange occurs via modification of the folded bilayer consisting of an intact uterine epithelium and trophectoderm embedded in loose stroma that increases in width and complexity [6, 7]. In addition, histotrophic (i.e., glandular) exchange occurs via accessory placental areolae juxtaposed to uterine glandular openings [8]. Histotrophic exchange is utilized during later stages of gestation for exchange of many different glycoproteins [8, 9], particularly uteroferrin, which transfers iron from the mother to the fetus [10]; thereby, illustrating the importance of the placental areolae as gestation progresses. Taken together, adequate hemotrophic and histotrophic exchange across the placenta is critical to provide proper growth and development of the fetus and ultimately the well-being of neonatal piglets.

Differences in placental morphology during gestation have been observed from divergent-sized littermates following intrauterine crowding induced by unilateral hysterectomy ovariectomy (**UHO**; [7]). Placental stromal width above the folded bilayer from the largest fetuses were greater than the smallest fetuses. In contrast, the folded bilayer width and complexity was greater for the smallest fetuses compared to the largest fetuses. These morphometric differences illustrate a compensatory mechanism of placentas of small fetuses in a crowded uterine environment by increasing the feto-maternal surface area in response to the overall decreased size of the placenta [7]. Unfortunately, at some point the placental stroma become limited for small littermate fetuses and the folded bilayer can no longer be modified resulting in limited growth of the small fetuses, increased probability of fetal loss and greater within-litter birth weight variation.

Chinese Meishan (**MS**) pigs have increased litter size and produce piglets that weigh significantly less than contemporary Western pig breeds [11, 12]. However, despite their small size, MS piglets have reduced preweaning mortality rates compared to Western breeds [13, 14]. Hypotheses for the mechanisms of improved reproductive prolificacy of MS pigs include increased ovulation rate [15, 16], greater uniformity of conceptus development [17], increased uterine capacity [18, 19], increased placental efficiency [20], increased placental vascularity [21, 22], increased complexity of the placental folded bilayer [23], and greater physiological maturation at birth [12, 24, 25]. Currently, it is unknown how the MS pig placenta responds to intrauterine crowding in terms of gross development as well as microscopic development of the placenta from divergent-sized littermates. Therefore, the objective of the current study was to evaluate placental development during late gestation between Chinese MS and White crossbred gilts following intrauterine crowding induced by UHO.

Methods

Animals

All animal protocols were approved by the US Meat Animal Research Center (**USMARC**) Animal Care and Use Committee and met the USDA and Federation of Animal Science Societies [26] guidelines for the care and use of animals. Purebred Chinese MS pigs were imported to the USMARC in 1989 and were maintained by inter se mating among 8 sire lines. White crossbred (**WC**) pigs from a randomly selected control population of pigs consisting of a 4-breed composite having equal contributions of Chester White, Landrace, Large White, and Yorkshire were maintained by *inter se* mating among 10 sire lines [27]. Similar-aged MS and WC gilts underwent UHO surgeries at approximately 60 days of age to induce intrauterine crowding following subsequent breeding [28]. Performing UHO surgery before puberty in MS gilts was key to success due to the limited vasculature of the reproductive tract (unpublished observation). Briefly, gilts were sedated with acepromazine (0.3 mg/kg BW; Iowa Veterinary Supply Company, Sioux City, IA, USA) followed by induction of anesthesia using thiopental sodium (Iowa Veterinary Supply Company) and maintained using isoflurane (Iowa Veterinary Supply Company). For UHO surgeries, gilts had one ovary and corresponding ipsilateral uterine horn removed. This process reduces the total intrauterine space by one-half and the remaining ovary undergoes compensatory ovarian hypertrophy such that ovulation rate remains similar; thereby, creating a crowded intrauterine environment in which litter size is no longer affected by ovulation rate and is a direct measure of each gilt's uterine capacity [28]. Following recovery from UHO surgeries (<200 days of age) and after having at least two estrous cycles, gilts (n = 7 and 5 for MS and WC, respectively) were naturally bred with single sires of the same breed at detection of estrus (day 0) and again 24 h later with the same sire.

At day 100 of gestation, the remaining uterine horn from each gilt was recovered via laparotomy and hysterectomy using a similar anesthesia protocol as mentioned above. Ovulation rate was recorded for each gilt. The uterine horn was opened along the anti-mesometrial side of the uterus and all fetuses were weighed to identify the smallest and largest littermate fetuses within each litter. Subsequently, a tissue section (~2 cm x 2 cm) consisting of intact uterine endometrium and placenta interface were collected immediately adjacent but external to the amnion from the smallest and largest littermates. These tissue sections were immediately placed into cassettes and immersed in 10% buffered formalin for later histological evaluation. The reproductive tract was stored at room temperature (**RT**) for approximately 2 h to ensure complete separation of the placenta from the uterus and subsequent placenta matching each fetus was separated from the endometrium and weighed. The entire placentas from the smallest and largest littermates were retained for additional gross placental morphological evaluations.

Breed response to intrauterine crowding

The overall breed response to intrauterine crowding was assessed for uterine capacity, fetal survival, fetal and placental weight variation, placental efficiency, and allometric growth. Uterine capacity was measured as the total number of surviving fetuses within each litter. Fetal survival was recorded as the proportion of live fetuses to ovulation rate. Within-litter fetal and placental weight variation was determined by the coefficient of variation of the average fetal and placental weight within each litter. Placental efficiency was measured based on the fetal weight to placental weight ratio [20]. Finally, allometric growth was determine by the slope of the linear regression of the natural log transformed fetal weight to the natural log transformed placental weight within individual litters. An allometric slope of 1 indicates that changes in fetal weight are directly proportional to changes in placental weight; whereas an allometric slope of less than 1 indicates that fetal weight is maintained disproportionally to placental weight and illustrates a fetal sparing effect of growth [7].

Gross placental morphology

Intact placentas from the smallest and largest fetal littermates were submerged in distilled H_2O within a volumetric beaker to determine volume displacement. The wet placentas were then placed on a glass plate and the placenta was completely spread out with the trophectoderm surface (i.e., placental surface directly facing the maternal endometrium) facing upward. The entire placenta was photographed using a D100 Nikon digital camera (Nikon Inc., Melville, NY) with a Tamron AF 28-300 mm lens (Tamron USA, Inc., Commack, NY) and photo stand using similar positioning and lighting for each placenta. These images were used to determine length, width, and surface area of the placentas. In addition, images were taken from random 5 cm^2 locations (n = 3) on the placentas to assess areolae density across the surface of the placenta (Figure 1). The average areolae density from the three random locations was used to estimate total areolae number based on surface area of corresponding placentas. All gross morphometric analyses were performed using the Bioquant Nova Prime (version 6.90.10) image software (Bioquant, Nashville, TN, USA).

Histological placental morphology

Intact uterine endometrium and placental tissues for histology were fixed in 10% buffered formalin with gentle rocking overnight at RT. The following day tissues were washed twice in PBS for 1 h and using a graded series of ethanol washes (1 h, 25% ethanol; 1h, 35% ethanol; 1 h, 50% ethanol; 1 h 70% ethanol) with gentle rocking at RT and stored at 4˚C until embedding in paraffin. Tissues were then washed with gentle rocking at RT through a grade series of ethanol (2 h in 95% ethanol, 2 h in absolute ethanol, and overnight in absolute ethanol), xylene (2 x 2h in xylene and overnight in xylene) and paraffin (2 x 2h in paraffin and overnight in paraffin). Tissues were trimmed and embedded in fresh paraffin such that the uterine wall was oriented with the long axis of the uterine horn and perpendicular to the placental folds. Tissues were sectioned (6 μm), placed on coated glass slides, processed through a graded series of xylene and ethanol, stained with hematoxylin and eosin (Sigma, St. Louis, MO, USA), processed through a graded series of ethanol and xylene, and cover slipped with Permount (Fisher Scientific, Pittsburgh, PA, USA). Sections were imaged using a Zeiss Axioplan 2 microscope (Carl Zeiss Microscopy, LLC, White Plains, NY, USA) fitted with a charge-coupled device camera (Qioptic Imaging Solutions, Fairport, NY, USA). The images of intact uterine endometrium and placental tissues were oriented such that the placental stroma was above, the maternal endometrium was below, and the folded bilayer was centrally located between the stroma and endometrium (Figure 2). These images were used to determine the total placental width, the folded bilayer width, and the placental stromal width using morphometric techniques previously described [7]. Histological morphometric analyses were performed using the Bioquant Nova Prime image software (Bioquant).

Statistical analysis

All data were analyzed using MIXED model procedures for ANOVA [29, 30]. When a significant *F*-statistic was generated, means were separated using a Dunnett multiple comparison test [29, 30]. Means were considered statistically different at $P \leq 0.05$ and tendencies between $P = 0.06$ and $P = 0.10$. Ovulation rate, uterine capacity, and fetal survival were analyzed with a model including the fixed effect of breed.

Fetal weight, placental weight, within-litter fetal and placental weight variation, and placental efficiency were analyzed with a model including the fixed effect of breed, the covariate of litter size, and the random effect of gilt within breed. Allometric growth rates were assessed by the slope of the regression for the natural log fetal weight to the natural log placental weight and slopes from each litter were analyzed using a model including the fixed effect of breed and the covariate of litter size. Gross and histological morphological data were analyzed using a model that included the fixed effects of breed, fetal size, the interaction of fixed effects, the covariate of litter size, and the random effect of gilt within breed by fetal size interaction. Differences in total placental, folded bilayer, and placental stromal widths between divergent-sized littermates were analyzed with a model including the fixed effect of breed, covariate of litter size, and the random effect of gilt within breed.

Results

Breed response to intrauterine crowding

Table 1 illustrates the overall breed response between MS and WC gilts following intrauterine crowding at day 100 of gestation. Ovulation rate was greater ($P = 0.0286$) in MS gilts compared to WC gilts. However, uterine capacity and fetal survival did not statistically differ between MS and WC gilts (Table 1). Although fetal weight was not different between the two breeds, within-litter fetal weight variation tended ($P = 0.0790$) to be reduced in MS pregnancies compared to WC. Placental weight, within-litter placental weight variation, and placental efficiency were not different between MS and WC pregnancies (Table 1). Figure 3 demonstrates the plot of the natural log fetal weight to the natural log placental weight for individual fetuses and corresponding linear regressions for this relationship from MS and WC pregnancies. The slopes of the linear regressions correspond to the allometric growth rate and tended to be reduced ($P = 0.0712$) in MS pregnancies compared to WC (Table 1).

Table 1
Overall breed response to intrauterine crowding between Meishan and White crossbred gilts at day 100 of gestation[1]

Variable	Breed		Pr > F
	Meishan	White crossbred	
No. of litters	7	5	
Ovulation rate (no.)	18.6 ± 0.8	15.4 ± 0.9	0.0286
Uterine capacity (no.)	8.0 ± 0.9	7.2 ± 1.1	0.5917
Fetal survival (%)	43.6 ± 5.8	46.8 ± 6.9	0.7311
Fetal weight (g)	597.9 ± 33.3	646.5 ± 41.7	0.3806
Fetal wt. variation (%)[2]	16.0 ± 2.5	24.9 ± 3.1	0.0790
Placental weight (g)	169.2 ± 8.8	164.11 ± 11.1	0.7240
Placental wt. variation (%)[2]	29.5 ± 3.4	37.9 ± 4.2	0.2315
Placental efficiency[3]	3.7 ± 0.2	4.1 ± 0.2	0.2610
Allometric growth[4]	0.40 ± 0.06	0.60 ± 0.07	0.0712

[1]Values are reported as least-squares means ± SEM as determined by MIXED model analysis (refer to Methods for specific model details).

[2]Based on the litter average weight coefficient of variation.

[3]Based on the fetal:placental weight ratio.

[4]Allometric growth was assessed by the slope of the linear regression of ln fetal weight to ln placental weight within individual litters and measures fetal sparing in which a slope of 1 illustrates fetal weight is equally correlated with placental weight and highly influenced by fetal crowding.

Gross placental morphology

Table 2 demonstrates the effect of breed (MS vs. WC) and fetal size (smallest vs largest fetal littermates) for gross placental morphology at day 100 of gestation following intrauterine crowding. As expected, both fetal and placental weight were decreased ($P < 0.0008$) in the smallest littermates compared to the largest (470.1 ± 42.4 g vs. 778.5 ± 42.4 g and 118.2 ± 17.5 g vs. 217.5 ± 17.5 g, respectively for fetal and placental weight), irrespective of breed of dam. There were no significant breed or fetal size effects on placental efficiency (Table 2). The volume displacement, surface area, and length of placentas were decreased ($P < 0.0005$) in the smallest littermates compared to the largest (108.3 ± 15.7 ml vs. 201.0 ± 15.7 ml, 1129.6 ± 100.1 cm^2 vs. 1625.3 ± 100.1 cm^2, and 43.7 ± 2.6 cm vs. 63.3 ± 2.6 cm, respectively for volume displacement, surface area, and length), irrespective of breed of dam. In addition, the placental length tended ($P = 0.0592$) to be greater for MS placentas compared to WC placentas (57.3 ± 2.4 cm vs. 49.7 ± 2.9 cm, respectively). There was a tendency ($P = 0.0591$) for a breed by fetal size interaction in placental width (Table 2), which MS littermate placentas were similar in width but WC littermate placentas differed by size (i.e., smallest decreased compared to largest) and the largest littermate WC placentas were wider than all other placentas. There was a significant ($P = 0.0097$) breed by fetal size interaction for the areolae density. The greatest areolae density was found in placenta of large MS fetuses, and this density was reduced in placentas from small MS fetuses. Areolae density was further reduced from WC fetuses compared to MS fetuses, irrespective of fetal size (Table 2). Similarly, estimated total areolae number from placentas tended ($P = 0.0855$) to have a breed by fetal size interaction in which the greatest number of areolae were observed from the largest MS littermate with the smallest MS and largest WC littermate placentas having intermediate total numbers of areolae and the smallest WC littermates having the least (Table 2). Deviations from the areolae density and the estimate total number of areolae were likely due to fetal size differences in placental surface area. Furthermore, MS placentas, irrespective of fetal size, had areolae that were consistently more pronounced and distinct compared to WC placentas as illustrated in Figure 1.

Table 2:
Effect of breed and fetal size on gross placental morphology at day 100 of gestation between Meishan (MS) and White crossbred (WC) gilts following intrauterine crowding[1]

Variable	Breed by Fetal Size				$Pr > F$[2]
	MS Small	MS Large	WC Small	WC Large	
Fetal weight (g)	481.1 ± 54.9	747.1 ± 54.9	459.1 ± 65.1	810.0 ± 65.1	S < 0.0001
Placental weight (g)	126.7 ± 22.7	221.1 ± 22.7	109.7 ± 26.9	214.0 ± 26.9	S = 0.0008
Placental efficiency[3]	4.2 ± 0.4	3.6 ± 0.4	4.4 ± 0.5	3.8 ± 0.5	N.S.
Volume displacement (ml)	118.9 ± 20.3	201.3 ± 20.3	97.7 ± 24.0	200.8 ± 24.0	S = 0.0005
Surface area (cm^2)	1,130 ± 100	1,625 ± 100	985 ± 119	1,539 ± 119	S = 0.0001
Length (cm)	47.6 ± 3.4	66.9 ± 3.4	39.8 ± 4.0	59.6 ± 4.0	B = 0.0592; S < 0.0001
Width (cm)	29.2 ± 1.1[a]	29.8 ± 1.1[a]	28.1 ± 1.4[a]	33.8 ± 1.4[b]	I = 0.0581
Areolae density (cm^2)	8.3 ± 0.3[a]	10.2 ± 0.3[b]	6.9 ± 0.3[c]	7.0 ± 0.3[c]	I = 0.0097
Estimate total areolae (no.)[4]	9,488 ± 783[a]	16,422 ± 783[b]	6,890 ± 928[c]	10,722 ± 928[a]	I = 0.0855

[1]Values are reported as least-squares means ± SEM as determined by MIXED model analysis. The model included the fixed effects of breed, fetal size, interaction of fixed effects, the random effect of gilt within breed and the covariate of litter size.
[2]B = breed effect; S = size effect; I = breed by size interaction; rows with different superscripts were different ($P < 0.05$).
[3]Based on the fetal:placental weight ratio.
[4]Based on the areolae density and corresponding placental surface area.

Histological placental morphology

Table 3 illustrates the effect of breed (MS vs. WC) and fetal size (smallest vs. largest littermate) for placental histological morphology at day 100 of gestation following intrauterine crowding. Total placental width was reduced ($P = 0.0042$) from MS pregnancies compared to WC pregnancies (402.5 ± 10.7 µm vs. 460.1 ± 14.4 µm, respectively), irrespective of fetal size. Similarly, folded bilayer width was reduced ($P = 0.0024$) in MS placentas compared to WC placentas (325.8 ± 9.5 µm vs. 377.8 ± 11.3 µm, respectively). In addition, there was a significant ($P = 0.0004$) effect of fetal size on folded bilayer width in which smallest littermate placentas had greater folded bilayer width compared to the largest littermate placentas (382.9 ± 10.3 μm vs. 320.8 μm, respectively). The width of the placental stroma was decreased (*P* = 0.0051) in the smallest littermate placentas compared to the largest littermate placentas (56.8 ± 8.4 μm vs. 94.4 ± 8.4 μm, respectively), irrespective of breed of dam. When analyzing divergent-sized fetal littermate differences between the breeds for the placental histological morphology as illustrated in Figure 4, total placental and folded bilayer widths difference between smallest and largest littermates did not differ between MS and WC gilts. However, placental stromal width difference between small and larger fetuses was reduced (*P* = 0.0466) in MS gilts (21.3 ± 13.8 μm) compared to WC gilts (70.9 ± 16.3 μm).

Table 3: Effect of breed and fetal size on histological placental morphology at day 100 of gestation between Meishan (MS) and White crossbred (WC) gilts following intrauterine crowding[1]

Variable	Breed by Fetal Size				Pr > F[2]
	MS Small	MS Large	WC Small	WC Large	
Total placental width (μm)	417.0 ± 15.1	388.0 ± 15.1	470.9 ± 20.3	449.4 ± 20.3	B = 0.0042
Folded bilayer width (μm)	349.5 ± 13.4	302.1 ± 13.4	416.2 ± 15.9	339.4 ± 15.9	B = 0.0024; S = 0.0004
Stromal width (μm)	59.1 ± 10.9	78.9 ± 10.9	54.3 ± 12.9	109.8 ± 12.9	S = 0.0051

[1]Values are reported as least-squares means ± SEM as determined by MIXED model analysis. The model included the fixed effects of breed, fetal size, interaction of fixed effects, the random effect of gilt within breed and the covariate of litter size.
[2]B = breed effect; S = size effect.

Discussions

Because of the critical function of the placenta for exchange of gases, nutrients, and wastes to developing fetuses, the porcine placenta plays a major role in uterine capacity, fetal growth and survival, which further influences postnatal piglet growth and survival. Uterine crowding also influences fetal growth and placental development, resulting in reduced fetal weights [27, 31] and altered placental size, structure, and function [4, 7]; thereby, providing a mechanism for increased within-litter birth weight variation and smaller piglets that result in increased preweaning piglet mortality. Unique pig breeds can serve as useful models of reproductive prolificacy and improved preweaning mortality, particularly the Chinese Meishan pig, which has decreased preweaning mortality rates compared to Western breeds despite having large litters with smaller average piglet weights [13, 14]. In the present study, we report breed differences in placental development between MS and WC gilts in response to intrauterine crowding, both on gross and histological levels. These differences may indicate that mechanisms for accommodating intrauterine crowding differ between MS and WC breeds.

In the current study, ovulation rate was greater for similar-aged MS gilts compared to WC gilts following the UHO procedure to induce intrauterine crowding. The corresponding ovulation rates from MS and WC gilts observed in this study were similar to the ovulation rates from intact MS and WC gilts [12]. This first reported use of the UHO model in MS females illustrates that the UHO procedure caused compensatory ovarian hypertrophy, resulting in similar ovulation rates to intact reproductive tracts but with only one ovary and half of the uterine space; thereby, inducing uterine crowding [28]. The breed differences in ovulation observed in the current study support previous literature from intact females illustrating that age-matched MS dams have greater ovulation rate compared with Western breed dams [12, 16, 32]. This difference in ovulation rate is likely due to the reduced age (~ 80–100 days) for MS females to undergo puberty compared to WC females [32, 33]; thereby, resulting in 3–5 more ovulations at a given age for MS females.

Despite differences in ovulation rates, there were no breed differences in uterine capacity or fetal survival in the current study following intrauterine crowding. Inconsistencies in litter size and fetal survival have been reported when comparing differences between MS and Western breed females with intact uteri. Studies evaluating litter size from purebred MS to purebred Western breeds (i.e., Large White or Yorkshire) of various ages illustrate increased litter size for MS females compared to purebred Western breeds [11, 34]. However, Haley et al. reported no differences in prenatal survival between Large White and MS females in their 1st and 2nd parities [34]. In contrast, White et al. demonstrated that 2nd parity MS females had reduced embryo survival during mid-gestation (~ day 50) compared to Yorkshire [11]. Furthermore, limited differences in litter size and fetal survival during early gestation (day 30) have been reported between purebred MS and White crossbreds from intact gilts of the same age [32]. Several investigators have hypothesized improved uterine capacity from MS females as a mechanism from improved reproductive prolificacy [3, 18, 19]. However, all studies to date evaluating litter size between MS and Western breeds have involved intact females and did not fully assess uterine capacity due to the influence of ovulation rate on litter size, particularly in the young female [28]. As a result, to fully assess uterine capacity, it is necessary to crowd the uterine environment either by superovulation, embryo transfer, or surgically using the UHO to gain a full measure of uterine capacity. Contrary to previous hypotheses, the results of the current study following induced intrauterine crowding do not seem to fully support that MS females have greater uterine capacity compared to their Western breed contemporaries. Although uterine capacity was not significantly different in this study, there was a numerical increase in uterine capacity observed in MS pregnancies with limited number of gilt observations in the current study. Therefore, a larger scale evaluation of uterine capacity may be necessary to fully evaluate this hypothesis.

Fetal and placental weights were not different at day 100 of gestation from MS or WC pregnancies following intrauterine crowding in the current study. This finding was unexpected and contradictory to the literature comparing weights of the fetus, placenta, and corresponding piglet birth weight from intact dams illustrating significant reductions in fetal [35–37], placental [17, 32], and piglet birth [11, 12, 24] weight from MS pregnancies compared to Western breeds. Intrauterine crowding induced in dams using the UHO procedure results in decreased fetal and placental weights when compared to intact dams throughout gestation [38, 39]. Given this observation, one plausible explanation for the limited differences in fetal and placental weights between MS and WC pregnancies may be reduced sensitivity to intrauterine crowding on fetal and placental growth within MS pregnancies; thereby, resulting in limited reductions in fetal and placental weights in MS pregnancies as compared to WC pregnancies in response to intrauterine crowding following UHO. Intrauterine crowding highly influences fetal growth and subsequent within-litter birth weight variation [40–42]. However, previous reports indicate that MS conceptuses elongate to a lesser extent during early pregnancy, reducing negative interactions between adjacent conceptuses [17, 43]. A reduction in the sensitivity to intrauterine crowding in MS gilts is further supported by decreased within-litter fetal weight variation from MS pregnancies compared to WC observed in the current study. Finally, when comparing the allometric growth rate between MS and WC gilts following intrauterine crowding, there was a reduction in the allometric growth rate in MS pregnancies compared to WC. Allometric growth measures the sensitivity of deviations of growth between fetus and placenta [7], where a slope (i.e., allometric growth rate) of 1 in this relationship indicates proportional growth and slopes significantly less than 1 illustrates a fetal sparing effect unaffected by changes in placental growth [44]. Taken together, these results illustrate that fetal and placental development from MS pregnancies are less sensitive to intrauterine crowding when compared to WC pregnancies. Although the exact mechanisms for limited sensitivity in fetal growth to intrauterine crowding observed in MS pregnancies are not clear, one likely mechanism involves the development and function of the placenta.

To evaluate breed difference in placental development following intrauterine crowding in the current study, an assessment of divergent-sized littermates based on fetal weight (i.e., smallest and largest littermates) was made for both gross and histological morphology of placentas between MS and WC pregnancies. As expected, there were significant differences in the weights of the fetus and placenta from the smallest and largest littermates. However, like the overall litter average, there were no differences in fetal or placental weights between MS and WC when evaluating fetal size, further supporting the hypothesis that MS fetuses are less sensitive to intrauterine crowding on a weight basis. Interestingly, placental efficiency was not different between divergent-sized littermates or between the two breeds and this lack of breed difference for placental efficiency was also observed for the litter average in the current study. This finding contradicts reports of increased placental efficiency in purebred MS compared to Yorkshire pregnancies from intact uteri [19–21], but is supportive of data from comparing divergent-sized littermates in a crowded uterine environment following UHO from WC gilts throughout pregnancy [7]. Given that the allometric growth (i.e., natural log relationship between fetal and placental weight) was significantly less than 1 from both breeds and lowest in MS pregnancies in the current study, lack of breed and fetal size differences in placental efficiency indicates that fetal growth is not proportional to placental growth and further supports the hypothesis that fetal to placental weight ratio (i.e., placental efficiency) is not indicative of the entire function of the placenta on fetal growth, particular during late gestation and in a crowded uterine environment [44]. Therefore, other aspects of placental development and function likely explain the breed differences in limiting reductions in fetal placental growth as observed in MS pregnancies.

When evaluating gross morphology of placentas from divergent-sized littermates following intrauterine crowding in MS or WC pregnancies, volume displacement, surface area, and length of placentas were decreased in the smallest littermates compared to largest contemporaries, regardless of breed. During the last few days of gestation (< day 110), Vonnahme et al. and Bienson et al. reported increased surface area from purebred Yorkshire placentas compared to purebred MS from intact or co-breed (i.e., MS and Yorkshire embryos into either MS or Yorkshire dams) reciprocal embryo transfer pregnancies, respectively [21, 45]. However, Bienson et. al. reported no breed differences in surface area of placentas at day 90 of gestation following co-breed reciprocal embryo transfer [21]. As a result, lack of breed difference in surface area in the current study could be due to timing in which placentas were evaluated (day 100) intermediate of the previous sampling time points and therefore, breed difference may not appear until the very end of pregnancy. Alternatively, the limited breed differences observed in surface area in the current study could be the influence of intrauterine crowding as the previous studies were performed with intact females with litter size less than 10 fetal pigs [21, 45]. Interestingly, the length of MS placentas, irrespective of size, were longer than WC placentas; whereas, the width of the placentas for the largest littermate from WC pregnancies had the wider placentas compared to all other groups, indicating size difference in placental width from WC pregnancies, but not for MS pregnancies following intrauterine crowding. Increased placental length in MS pregnancies further supports reduced sensitivity to intrauterine crowding for MS pregnancies given that placental length decreases following intrauterine crowding compared to intact controls [38]. Increased number of fetuses has been shown to increase uterine length particularly during later stages of gestation [46]. Although uterine length was not recorded in the current study, one likely mechanism for increased length of placentas from MS pregnancies may have resulted from increased uterine length in MS pregnancies possibly driven by the numerical increase in uterine capacity observed in the current study.

In the current study, there was a breed by fetal size interaction for placental areolae density illustrating increased areolae density from MS pregnancies compared to WC pregnancies. Although largest littermate fetuses in MS pregnancies had increased areolae densities compared to their smaller littermate contemporaries, smaller MS placentas had greater areolae density compared to either sized fetus of WC placentas. Furthermore, visual evaluation of placental areolae between the breeds, irrespective of fetal size (Fig. 1), illustrated that MS placentas had more pronounced and distinct areolae compared to WC placentas. Placental areolae form over the uterine glands and play a critical role in uptake of glandular secretions (i.e., histotrophic exchange) into the placenta [47, 48]. Knight et al. has demonstrated that intrauterine crowding using the UHO model results in a reduction in both the density and total number of placental areolae throughout gestation compared to non-crowded intact controls [38]. Although histotrophic exchange is primarily thought to provide primary nutrient exchange during pre- and peri-implantation stages of gestation [9], histological and biochemical analysis of glandular secretions at the maternal gland and placental areolae interface illustrate the importance of these glandular secretions throughout gestation, particularly during late gestation when fetal growth is maximal [8, 49]. Given the importance of the placental areolae for the uptake of glandular secretion, the increased density and development of the areolae in MS pregnancies compared to WC pregnancies following intrauterine crowding provides a mechanism by which MS pregnancies have reduced effect of intrauterine crowding on fetal and placental growth observed in the current study.

On a histological level, the maternal-placental interface of the pig placenta consists of a folded bilayer of intact uterine epithelium and trophectoderm embedded in loose placental stroma (Fig. 2; [6, 7]). In the current study, the widths of the total placental interface and folded bilayer were reduced in MS placentas compared to WC placentas, irrespective of fetal size. Hong et. al. also reported decreased folded bilayer width from MS and Yorkshire placentas during late gestation [23]. Conversely, these authors reported an assessment of fold complexity based on the fold length per unit area of placenta illustrating greater complexity in folded bilayer from MS gilts compared to Yorkshire and suggested that this complexity was due to greater expression and levels of heparanase in MS placentas resulting in modification of the folded bilayer [23]. Although these results suggest compensatory mechanisms within the MS placentas to improve surface area at the maternal-placental interface, it is not clear as to what type of influence litter size and fetal size plays in this model as no information of fetal size nor litter size of sampled placentas were reported in this study [23].

The folded bilayer width was also greater from the smallest littermate fetus within both breeds in the current study. Conversely, placental stroma weight was reduced from placentas of the smallest littermate fetus, again regardless of breed. Vallet and Freking previously demonstrated that smallest fetal littermates have deeper folded bilayer, but decreased placental stromal depth compared to the larger contemporaries following intrauterine crowding in gilts [7]. These investigators hypothesized that differences in placental histology between divergent-sized littermates following intrauterine crowding result in a compensatory response due to reductions in uterine surface access from smallest littermates to increase maternal-fetal interface by increasing the surface area; thereby, resulting in deeper folded bilayer at the expense of the placental stroma. However, at a certain point the placental stroma becomes limited and thus provides a mechanism that limits growth and development of smaller littermates resulting in greater susceptibility for pre- and post-natal mortality [7]. Although the current study findings support the compensatory hypothesis that small littermates have increased folded bilayer width but decreased stromal width, regardless of breed, the breed difference in divergent-sized littermates for placental stromal width was significantly reduced from MS pregnancies compared to WC (Fig. 4). This illustrates that MS placentas might be less sensitive to limitations in placental stroma development between divergent-sized littermates, or alternatively they may have mechanisms for improved maintenance of placental stroma between littermates. The placental stroma is composed of many glycoproteins and glycosaminoglycans like hyaluronan and heparan sulfate [50]. Biochemical analysis of the composition within the placental areolae illustrate many glycoproteins, proteoglycans and glycosaminoglycans are present in these structures [51]. Furthermore, uterine glands juxtaposed to placental areolae produce and secrete many growth factors, proteases, enzymes, transport proteins, and adhesion proteins, which likely play a role in the development and modification of placenta [52]. Therefore, maintenance of placental stroma between littermates maybe improved in MS placentas due to enhanced development and function of the placental areolae.

Conclusions

In conclusion, the results of this study demonstrate altered placental development for both histotrophic and hemotrophic exchange components of placenta from MS compared to WC pregnancies following intrauterine crowding. Meishan placentas had greater density and morphologically different development of placental areolae compared to WC placentas; thereby, demonstrating greater glandular exchange potential in MS pigs. In contrast, MS pregnancies had reduced influence of altered hemotrophic exchange (i.e., placental stroma width) between divergent-sized littermates compared to WC pregnancies indicating greater uniformity of placental development on a histological level. These alterations in placental development of MS pregnancies provide potential mechanisms for reduced sensitivity of fetal growth, decreased within-litter fetal weight variation and decreased allometric growth following intrauterine crowding.

Abbreviations

Meishan (MS), room temperature (RT), unilateral hysterectomy ovariectomy (UHO), US Meat Animal Research Center (USMARC), White crossbred (WC)

Declarations

Availability of data and materials

All data and analyses used for this study are available from the corresponding author upon reasonable request.

Ethics approval

All animal protocols were approved by the USMARC Animal Care and Use Committee and met the USDA and Federation of Animal Science Societies guidelines for the care and use of animals.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Funding

The study was supported by USDA-Agricultural Research Service, Current Research Information System Project No. 5438-31000-084-00D.

Authors' Contribution

Jeremy R. Miles (JRM) and Jeffrey L. Vallet (JLV) conceived, designed, and performed the UHO surgeries; JRM collected all the data, performed all statistical analyses, and wrote the manuscript; JLV reviewed and modified the manuscript. The authors have read and approved the final manuscript.

Acknowledgements

The authors would like to acknowledge Mr. David Sypherd and Dr. Elane Wright-Johnson for technical support with UHO surgeries and data collection, Drs. Gary Rohrer and Clay Lents for critical review of the manuscript, Mrs. Amber Moody for secretarial assistance, and the USMARC swine crew for animal husbandry.

References

1. Roehe R, Kalm E. Estimation of genetic and environmental risk factors associated with pre-weaning mortality in piglets using generalized linear mixed models. Animal Science. 2000;70:227-240.
2. Mesa H, Safranski TJ, Cammack KM, Weaber RL, Lamberson WR. Genetic and phenotypic relationships of farrowing and weaning survival to birth and placental weights in pigs. J Anim Sci. 2006;84(1):32-40.
3. Vallet JL, Leymaster KA, Christenson RK. The influence of uterine function on embryonic and fetal survival J Anim Sci. 2002;80(E. Suppl. 2):E115-E125.
4. Vallet JL, McNeel AK, Miles JR, Freking BA. Placental accommodations for transport and metabolism during intra-uterine crowding in pigs. J Anim Sci Biotechnol. 2014;5(1):55.
5. Friess AE, Sinowatz F, Skolek-Winnisch R, Traautner W. The placenta of the pig. I. Finestructural changes of the placental barrier during pregnancy. Anat Embryol (Berl). 1980;158(2):179-91.
6. Leiser R, Kaufmann P. Placental structure: in a comparative aspect. Exp Clin Endocrinol. 1994;102(3):122-34.
7. Vallet JL, Freking BA. Differences in placental structure during gestation associated with large and small pig fetuses. J Anim Sci. 2007;85(12):3267-75.
8. Friess AE, Sinowatz F, Skolek-Winnisch R, Trautner W. The placenta of the pig. II. The ultrastructure of the areolae. Anat Embryol (Berl). 1981;163(1):43-53.
9. Bazer FW, Song G, Kim J, Dunlap KA, Satterfield MC, Johnson GA, et al. Uterine biology in pigs and sheep. J Anim Sci Biotechnol. 2012;3(1):23.
10. Roberts RM, Raub TJ, Bazer FW. Role of uteroferrin in transplacental iron transport in the pig. Fed Proc. 1986;45(10):2513-8.
11. White BR, McLaren DG, Dziuk PJ, Wheeler MB. Age at puberty, ovulation rate, uterine length, prenatal survival and litter size in Chinese Meishan and Yorkshire females. Theriogenlogy. 1993;40:85-97.
12. Miles JR, Vallet JL, Ford JJ, Freking BA, Cushman RA, Oliver WT, et al. Contributions of the maternal uterine environment and piglet genotype on weaning survivability potential: I. Development of neonatal piglets after reciprocal embryo transfers between Meishan and White crossbred gilts. J Anim Sci. 2012;90(7):2181-92.
13. Legault C. Selection of breeds, strains and individual pigs for prolificacy. J Reprod Fertil Suppl. 1985;33:151-66.

14. Lee G, Haley C. Comparative farrowing to weaning performance in meishan and large white pigs and their crosses. Anim Sci. 1995;60:269-280.
15. Haley CS, Lee GJ. Genetic basis of prolificacy in Meishan pigs. J Reprod Fertil Suppl. 1993;48:247-59.
16. Ashworth CJ, Haley CS, Aitken RP, Wilmut I. Embryo survival and conceptus growth after reciprocal embryo transfer between Chinese Meishan and Landrace x Large White gilts. J Reprod Fertil. 1990;90(2):595-603.
17. Bazer FW, Thatcher WW, Martinat-Botte F, Terqui M. Conceptus development in large white and prolific Chinese Meishan pigs. J Reprod Fertil. 1988;84(1):37-42.
18. Christenson RK, Vallet JL, Leymaster KA, Young LD. Uterine function in Meishan pigs. J Reprod Fertil Suppl. 1993;48:279-89.
19. Ford SP, Vonnahme KA, Wilson ME. Uterine capacity in the pig reflects a combination of uterine environment and conceptus genotype effects. J Anim Sci. 2002;80 (E. Suppl. 1):E66-E73.
20. Wilson ME, Ford SP. Comparative aspects of placental efficiency. Reprod Suppl. 2001;58:223-32.
21. Biensen NJ, Wilson ME, Ford SP. The impact of either a Meishan or Yorkshire uterus on Meishan or Yorkshire fetal and placental development to days 70, 90, and 110 of gestation. J Anim Sci. 1998;76(8):2169-76.
22. Wilson ME, Biensen NJ, Youngs CR, Ford SP. Development of Meishan and Yorkshire littermate conceptuses in either a Meishan or Yorkshire uterine environment to day 90 of gestation and to term. Biol Reprod. 1998;58(4):905-10.
23. Hong L, Hou C, Li X, Li C, Zhao S, Yu M. Expression of heparanase is associated with breed-specific morphological characters of placental folded bilayer between Yorkshire and Meishan pigs. Biol Reprod. 2014;90(3):56.
24. Le Dividich J, Mormede P, Catheline M, Caritez JC. Body composition and cold resistance of the neonatal pig from European (Large White) and Chinese (Meishan) breeds. Biol Neonate. 1991;59(5):268-77.
25. Herpin P, Le Dividich J, Amaral N. Effect of selection for lean tissue growth on body composition and physiological state of the pig at birth. J Anim Sci. 1993;71(10):2645-53.
26. FASS. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. 2010, Champaign, IL: Federation of Animal Science Societies 177.
27. Freking BA, Leymaster KA, Vallet JL, Christenson RK. Number of fetuses and conceptus growth throughout gestation in lines of pigs selected for ovulation rate or uterine capacity. J Anim Sci. 2007;85(9):2093-103.
28. Christenson RK, Leymaster KA, Young LD. Justification of unilateral hysterectomy-ovariectomy as a model to evaluate uterine capacity in swine. J Anim Sci. 1987;65(3):738-44.
29. Steel RGD, Torrie JH, Dickey DA. Principles and Procedures of Statistics: A Biometrical Approach. 3rd ed. 1997, New York: McGraw-Hill Inc. 666.

30. SAS. The SAS System for Windows, release 9.1. SAS 2003.
31. Vallet JL, Freking BA. Changes in fetal organ weights during gestation after selection for ovulation rate and uterine capacity in swine. J Anim Sci. 2006;84(9):2338-45.
32. Christenson RK. Ovulation rate and embryonic survival in Chinese Meishan and white crossbred pigs. J Anim Sci. 1993;71(11):3060-6.
33. Kuehn LA, Nonneman DJ, Klindt JM, Wise TH. Genetic relationships of body composition, serum leptin, and age at puberty in gilts. J Anim Sci. 2009;87(2):477-83.
34. Haley CS, Lee GJ, Ritchie M. Comparative reproductive performance in Meishan and Large White pigs and their crosses. Animal Science. 1995;60:259-267.
35. Finch AM, Antipatis C, Pickard AR, Ashworth CJ. Patterns of fetal growth within Large White x Landrace and Chinese Meishan gilt litters at three stages of gestation. Reprod Fertil Dev. 2002;14(7-8):419-25.
36. Vallet JL, Klemcke HG, Christenson RK, Pearson PL. The effect of breed and intrauterine crowding on fetal erythropoiesis on day 35 of gestation in swine. J Anim Sci. 2003;81(9):2352-6.
37. Ashworth CJ, Nwagwu MO, McArdle HJ. Genotype and fetal size affect maternal-fetal amino acid status and fetal endocrinology in Large White x Landrace and Meishan pigs. Reprod Fertil Dev. 2013;25(2):439-45.
38. Knight JW, Bazer FW, Thatcher WW, Franke DE, Wallace HD. Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: interrelations among hormonal status, placental development, fetal fluids and fetal growth. J Anim Sci. 1977;44(4):620-37.
39. Tarraf CG, Knight JW. Effect of uterine space and fetal sex on conceptus development and in vitro release of progesterone and estrone from regions of the porcine placenta throughout gestation. Domest Anim Endocrinol. 1995;12(1):63-71.
40. Foxcroft GR, Dixon WT, Novak S, Putman CT, Town SC, Vinsky MD. The biological basis for prenatal programming of postnatal performance in pigs. J Anim Sci. 2006;84 Suppl:E105-12.
41. Yuan TL, Zhu YH, Shi M, Li TT, Li N, Wu GY, et al. Within-litter variation in birth weight: impact of nutritional status in the sow. J Zhejiang Univ Sci B. 2015;16(6):417-35.
42. Wang J, Feng C, Liu T, Shi M, Wu G, Bazer FW. Physiological alterations associated with intrauterine growth restriction in fetal pigs: Causes and insights for nutritional optimization. Mol Reprod Dev. 2017;84(9):897-904.
43. Ford SP. Embryonic and fetal development in different genotypes in pigs. J Reprod Fertil Suppl. 1997;52:165-76.
44. Vallet JL, McNeel AK, Johnson G, Bazer FW. Triennial Reproduction Symposium: limitations in uterine and conceptus physiology that lead to fetal losses. J Anim Sci. 2013;91(7):3030-40.
45. Vonnahme KA, Wilson ME, Ford SP. Conceptus competition for uterine space: different strategies exhibited by the Meishan and Yorkshire pig. J Anim Sci. 2002;80(5):1311-6.

46. Wu MC, Hentzel MD, Dziuk PJ. Relationships between uterine length and number of fetuses and prenatal mortality in pigs. J Anim Sci. 1987;65(3):762-70.
47. Sinowatz F, Friess AE. Uterine glands of the pig during pregnancy. An ultrastructural and cytochemical study. Anat Embryol (Berl). 1983;166(1):121-34.
48. Dantzer V, Leiser R. Microvasculature of regular and irregular areolae of the areola-gland subunit of the porcine placenta: structural and functional aspects. Anat Embryol (Berl). 1993;188(3):257-67.
49. Perry JS, Crombie PR. Ultrastructure of the uterine glands of the pig. J Anat. 1982;134(Pt 2):339-50.
50. Vallet JL, Miles JR, Freking BA. Development of the pig placenta. Soc Reprod Fertil Suppl. 2009;66:265-79.
51. Siraziev RZ. Histologycal and histochemical characteristics of areolae in the pig placenta. Tsitologiia. 2015;57(5):379-86.
52. Spencer TE, Kelleher AM, Bartol FF. Development and Function of Uterine Glands in Domestic Animals. Annu Rev Anim Biosci. 2019;7:125-147.

Figures

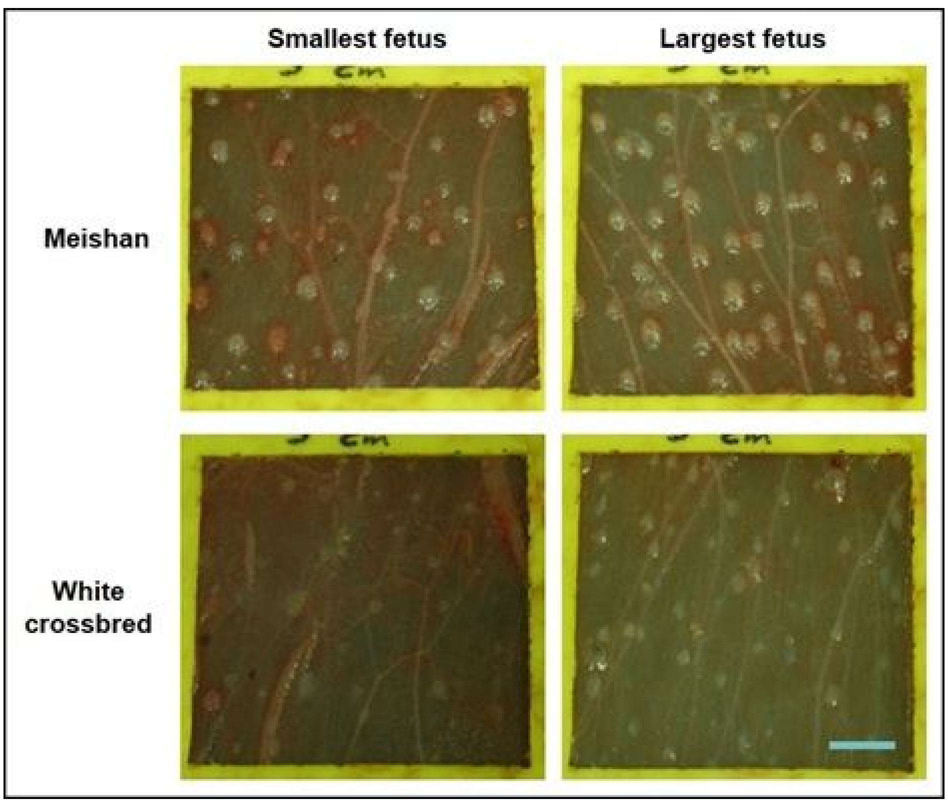

Figure 1

Representative images of placental areolae at day 100 of gestation from the smallest and largest fetal littermate placentas following intrauterine crowding in Meishan or White crossbred gilts. Scale bar equals 1 cm.

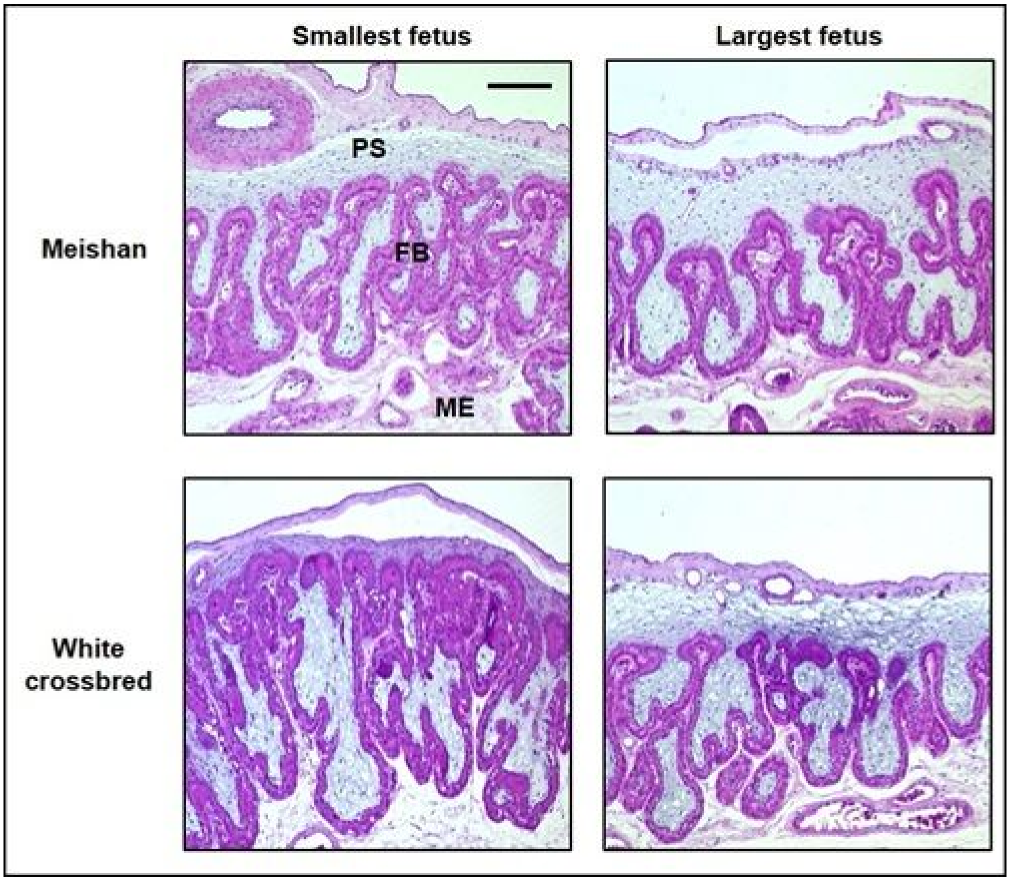

Figure 2

Representative images of the feto-maternal interface at day 100 of gestation from the smallest and largest fetal littermate placentas following intrauterine crowding in Meishan or White crossbred gilts. PS = placental stroma; FB = folded bilayer; ME = maternal endometrium. Scale bar equals 200 μm.

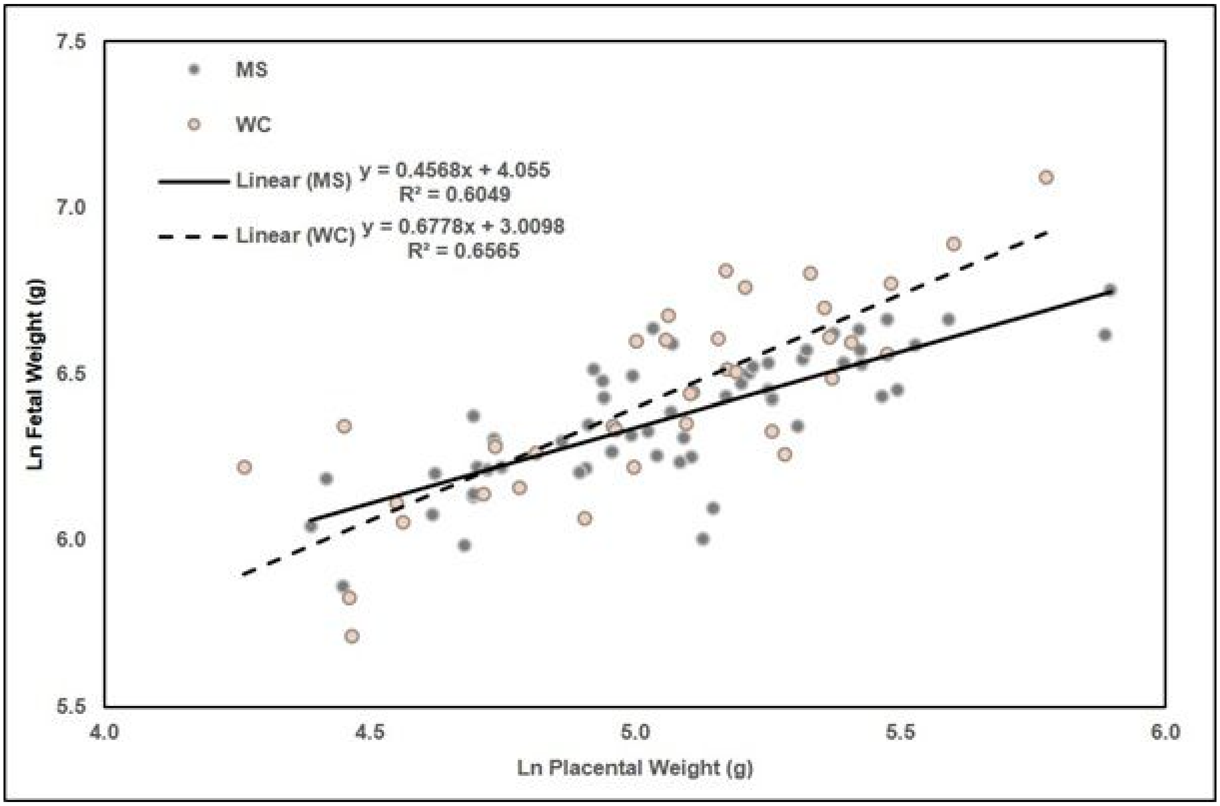

Figure 3

Scatterplots of the natural log fetal weight to natural log placental at day 100 of gestation following intrauterine crowding in Meishan (grey dots) or White crossbred (beige dots) gilts. The linear regression for these relationships (Meishan = solid line; White crossbred = dotted line) demonstrates that the allometric growth rate (corresponding slopes) was less ($P = 0.0103$) from Meishan gilts compared to White crossbred; thereby, illustrating a fetal sparing affect for Meishan pregnancies following intrauterine crowding. Differences in total placental (dark grey), folded bilayer (mid grey) and placental stromal (light grey) width in placentas between the divergent-sized littermate fetuses following intrauterine crowding in Meishan or White crossed gilts. Values are reported as least-squares means ± SEM as determined by MIXED model analysis. Columns with different superscripts (placental stroma) were different ($P = 0.0466$) between Meishan pregnancies compared to White crossbred.

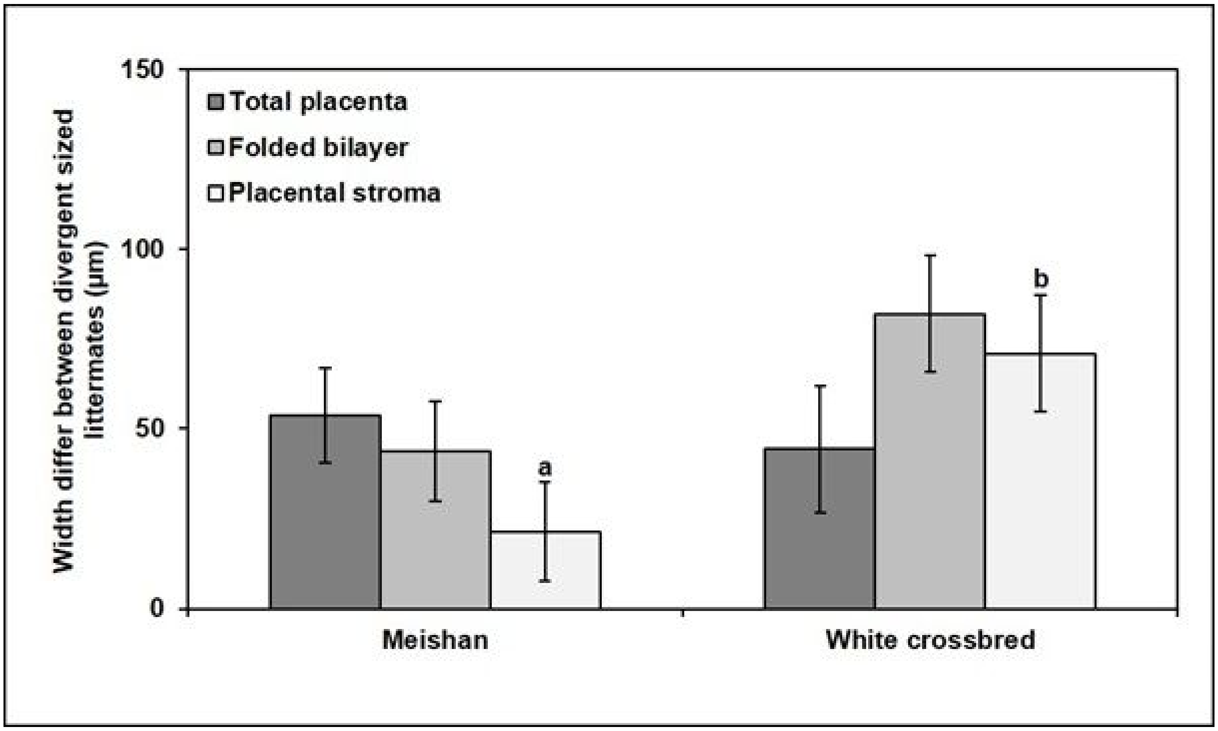

Figure 4

Differences in total placental (dark grey), folded bilayer (mid grey) and placental stromal (light grey) width in placentas between the divergent-sized littermate fetuses following intrauterine crowding in Meishan or White crossed gilts. Values are reported as least-squares means ± SEM as determined by MIXED model analysis. Columns with different superscripts (placental stroma) were different (P = 0.0466) between Meishan pregnancies compared to White crossbred.