

Differences in placental structure during gestation associated with large and small pig fetuses^{1,2}

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ABSTRACT: The efficiency of nutrient transport from the pregnant female pig to the developing fetus depends on the size and function of the placenta. It has been reported that maternal and fetal blood vessels are arranged in a cross-countercurrent arrangement within placental microscopic folds. Thus, the blood supplies are in close apposition to each other within these microscopic folds, and maternal and fetal blood flows in approximately opposite directions perpendicular to the plane of the placenta. This arrangement indicates that the width of the microscopic folds influences placental efficiency. The objective of this study was to determine whether differences in pig placental microscopic fold development are associated with differences in fetal size or are influenced by selection for ovulation rate or uterine capacity. Gilts from a randomly selected control line, a line selected for ovulation rate, and a line selected for uterine capacity were slaughtered, and uterine wall samples were collected within the placentas associated with the largest and smallest fetuses in each litter on d 45, 65, 85, and 105 of gestation. The uterine wall

samples were processed for histology and analyzed using computer-assisted morphometry. Average width of the placental folds and average width of the placental stroma above the folds were measured. To measure fold complexity, the length of the epithelial bilayer for a given length of placenta was also measured. The width of the folded bilayer increased significantly from d 65 to 105 and was greater in placentas associated with small fetuses compared with large fetuses on d 105 of gestation. In contrast, the width of the placental stroma above the folded bilayer decreased with gestation and decreased more rapidly in placenta associated with the smallest compared with the largest fetus. These results indicate that the width of the microscopic folds of the placental trophoblast/endometrial epithelial bilayer is increased in placenta associated with small fetuses, which we hypothesize will increase the surface area for interaction between maternal and fetal blood supplies, thus improving placental efficiency in response to reduced placental size.

Key words: fetus, pregnancy, trophoblast

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INTRODUCTION

The efficiency of nutrient exchange between the maternal and fetal blood supplies affects the development of the pig fetus and thus influences litter size, birth weights, and postnatal survival. Biensen et al. (1998) introduced the concept that the fetal weight:placental

weight ratio (**FPR**) could be used as a measure of placental efficiency in the pig, and Wilson et al. (1999) reported that divergent selection for the FPR resulted in differences in litter size. However, a more extensive study in which pigs were divergently selected for the FPR indicated no difference in litter size among selected groups (Mesa et al., 2005). Thus, better ways to assess the efficiency of the placenta are needed to improve this important reproductive trait.

Placental efficiency is likely to be influenced by its size (which determines access to the uterus), its structure influencing nutrient exchange, and the existence of nutrient-specific mechanisms that facilitate transport (e.g., maternal and fetal hemoglobin oxygen affinities and their effect on oxygen transfer). According to Leiser and Dantzer (1988), the maternal and fetal blood supplies are arranged in a cross-countercurrent arrangement within the microscopic folds of the pig placental trophoblast/endometrial epithelial bilayer. The effi-

¹Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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ciency of exchange of nutrients, given this arrangement, will be influenced by the distance across the epithelial cell bilayer (because this influences the rate of diffusion from mother to fetus) and the width of the folded region of the bilayer (because this influences the period that units of maternal and fetal blood interact). The objective of the following experiment was to determine whether the width, complexity, or both, of the placental microscopic folded structure differs during gestation between placenta associated with large and small fetuses in a crowded uterine environment or is affected by selection for ovulation rate or uterine capacity.

MATERIALS AND METHODS

Animal handling protocols were approved by the US Meat Animal Research Center Institutional Animal Care and Use Committee.

Gilts for this experiment were a subset of the gilts used by Freking et al. (2007). Briefly, gilts from a randomly selected control line, a line selected for ovulation rate (OR), and a line selected for uterine capacity (UC) were unilaterally hysterectomized-ovariectomized (UHO) at approximately 160 d of age. This treatment has been used in numerous studies to induce intrauterine crowding in gilts (Knight et al., 1977; Christenson et al., 1987; Vallet and Christenson, 2004). Gilts were allowed to recover from surgery and then were observed for estrous behavior beginning about 250 d of age. Gilts were naturally mated at standing estrus to boars from the same lines and were slaughtered on d 45 (n = 2, 4, 3 for control, OR, and UC, respectively), d 65 (n = 6, 10, 7), 85 (n = 7, 6, 6), and d 105 (n = 7, 7, 7) of gestation.

At slaughter, the uterus was opened along the anti-mesometrial side of the uterus, each individual fetus was weighed, and the placentas associated with the largest and smallest fetus within each litter were identified. Rectangular sections of the uterine wall were collected such that 1 edge of the section was the anti-mesometrial cut edge, with the rectangle extending toward the mesometrial side of the uterus. The section was collected approximately midway between where the umbilicus enters the placenta and the paraplacental zone. The sections were placed into small, plastic cassettes (Sakura Finetek USA Inc., Torrance, CA) and fixed in buffered formalin overnight at room temperature on a rocking platform. Uterine wall samples were then incubated in 70% ethanol overnight at 4°C. The samples were then incubated in a graded series of ethanol (2 h 95% ethanol, 2 h absolute ethanol, overnight absolute ethanol), xylene (2 × 2 h xylene, overnight xylene), and paraffin (2 × 2 h paraffin, overnight paraffin). Sections were trimmed and embedded in fresh paraffin for sectioning. Care was taken to orient the tissues such that the uterine wall could be sectioned in the same direction as the long axis of the uterine horn, which was perpendicular to the microscopic placental folds present in the pig. The paraffin-embedded tissues were then sectioned (6 μm), placed on coated glass

slides, processed through a graded series of xylene and ethanol, stained with hematoxylin and eosin, processed through a graded series of ethanol and xylene, and coverslipped using Permount (Fisher Scientific, Pittsburgh, PA).

Morphometric measurements were made on individual sections (1 section for each large and small placenta from each gilt) using a Zeiss Axioplan 2 microscope fitted with a charge-coupled device camera (Qioptic Imaging Solutions, Fairport, NY) and BQ Nova Prime (version 6.90.10) software (Bioquant Image Analysis, Nashville, TN). To obtain an average width of the placental folds within each measured field, the tops and bottoms of the placental folds within view were tagged, and these points were joined together to form a polygon. Individual lines were drawn from the top of each fold to the intersection of the polygon at the bottom of the folds. Then a line linking the center of each of these lines was drawn across the length of the polygon, and the length of this line was recorded. The average width of the polygon around the enclosed surface was calculated as the area of the polygon divided by the length of the polygon through its center. The width of the placental stroma above the placental folds was also measured by drawing a line from the top of each fold to the stromal border and averaging the widths obtained for that field (Figure 1). These measurements were obtained for 2 fields for each section. The total width of the placenta was calculated as the width of the folded bilayer plus the width of the stroma above the folds.

Statistical Analysis

Correlation analysis between the 2 measurements made for each slide for average width of the microscopic folds, average width of the stroma above the folds, and length of the folds per length of the placenta was used to assess the repeatability of these measurements. Simple correlations among the morphometric measurements, fetal weights, placental weights, and the FPR were also calculated. Measurements for the fold length per unit length of placenta, placental stromal width, placental fold width, and the total width of the placenta for each slide were averaged, and the averages were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC), using a model that included the fixed effects of line (control, OR, or UC), day of gestation (45, 65, 85, and 105), the line × day interaction, relative size of the fetus (largest or smallest), and the size × line, size × day, and size × line × day interactions. Gilt within line × day was included as a random effect. In addition, fold length per unit of placental length was analyzed using the same model described above but including the fold width as a covariate. This was done to assess potential treatment effects on fold complexity after accounting for effects on fold width.

When interactions were significant, individual interaction contrasts were performed to further delineate

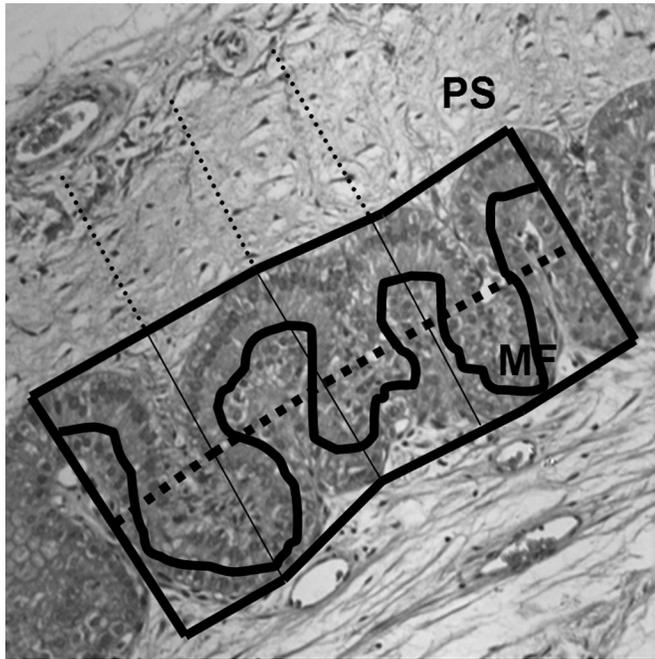


Figure 1. A photomicrograph of a placental section illustrating the strategy used for morphometric analysis. A rectangular box (solid thick black line) was constructed by connecting the tops and bottoms of the microscopic folds (MF). Then, lines (thin solid black) were drawn connecting the top of each fold with the bottom of the rectangle. A line (thick dashed black) was drawn connecting the center of each side of the rectangle and each thin black line within the rectangle. The software measured the area of the rectangle and the length of the thick broken line through the center of the rectangle. The average width of the rectangle was calculated as the area divided by the length of the line through the center, thus giving a measure of the average width of the folds. In addition, a line (solid thick black) was traced along the center of the microscopic folds themselves, to obtain a length for the epithelial bilayer. This length was divided by the line through the center of the rectangle to obtain the length of the microscopic fold per length of placenta. Finally, lines (thin dashed black) were drawn from the tops of the folds to the border of the placental stroma (PS) above the microscopic folds, thus providing a measure of the width of the stroma above the folds.

the source of the interaction. Where main effects were significant, contrasts were used to further delineate main effects. The FPR was analyzed using a model similar to that used for the morphometric traits. Placental and fetal weights were analyzed after \ln transformation (to equalize variances among treatment groups) using a model similar to that used for the morphometric traits. In addition, \ln -transformed fetal and placental weights were used to compare allometric relationships between fetal and placental weights, using methods similar to those described by Vallet and Freking (2006) and Freking et al. (2007). Briefly, regression analysis

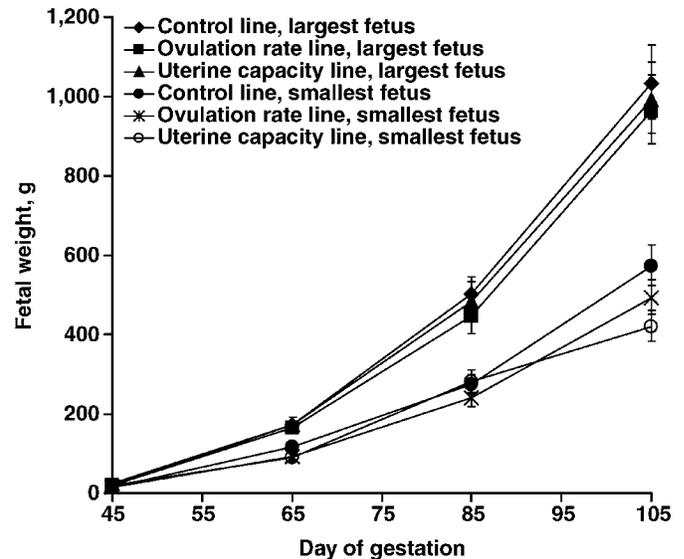


Figure 2. Means for fetal weights for the largest and smallest fetuses from a randomly selected control line, a line selected for ovulation rate, and a line selected for uterine capacity examined on d 45, 65, 85, and 105 of gestation. No line effects were detected. However, as expected, a significant day \times fetal size interaction was present ($P < 0.01$). Visual appraisal of the results indicated that the interaction was due to greater growth rates for the largest fetuses in the litter compared with the smallest fetuses in the litter.

was used to obtain the slope of the relationship between \ln fetal weight and \ln placental weight. According to Huxley (1932), the slope of this relationship represents the growth rate of the fetus divided by the growth rate of the placenta. A slope of 1 indicates proportionate growth, a slope less than 1 indicates that fetal weights increase disproportionately to placental weights (i.e., a fetal sparing effect). Effects were considered statistically significant when $P \leq 0.05$.

RESULTS

Fetal and placental weights are summarized in Figures 2 and 3. A significant day \times fetal size interaction was obtained for fetal weights. This interaction is likely due to the slower growth rate of the smallest fetuses in the litter compared with the largest. Results of the analysis of placental weights indicated significant effects for selected line ($P = 0.01$), day ($P < 0.01$), and fetal size ($P < 0.01$). Placental weights were less for both the ovulation rate and uterine capacity-selected lines compared with the control line. Placental weights increased significantly between each day of gestation, although placental weight increased less from d 85 to 105 than during earlier pregnancy. Finally, placental weights were reduced for placenta associated with the smallest fetus compared with the largest fetus in the litter. A more extensive analysis of fetal and placental

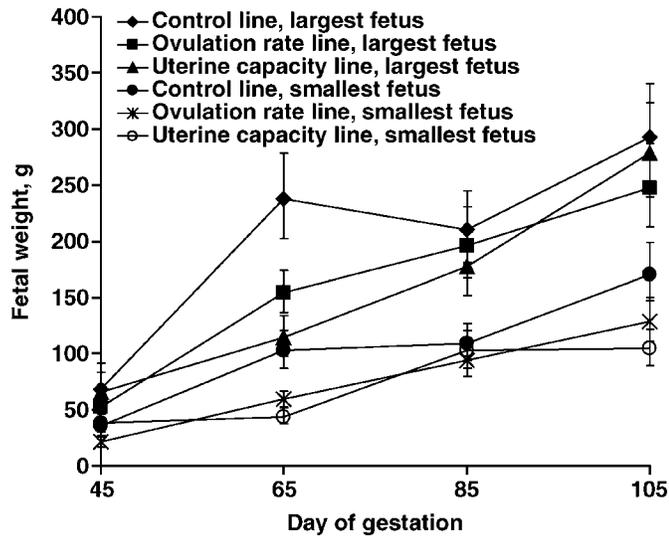


Figure 3. Means for the placental weights for the largest and smallest fetuses from a randomly selected control line, a line selected for ovulation rate, and a line selected for uterine capacity examined on d 45, 65, 85, and 105 of gestation. Statistical analysis indicated significant effects ($P \leq 0.01$) of line, day, and fetal size. Placental weights were less in the ovulation rate line and uterine capacity line compared with the control line. Placental weights increased progressively on each day of gestation and were reduced for placentas associated with the smallest fetuses in the litter compared with the largest fetuses in the litter.

weights from the selected lines throughout gestation can be found in Freking et al. (2007).

Least squares means for the FPR are presented in Table 1. Significant effects of fetal size ($P < 0.01$) and

day of gestation ($P < 0.01$) were obtained. Line effects, interactions with line, and the day of gestation \times fetal size interaction were not statistically significant. Results indicated that the FPR increased with advancing gestation and was greater for placentas of small fetuses compared with large fetuses.

We also assessed whether the allometric relationships between fetal and placental weights were similar across the different treatment groups in this experiment. Allometric slopes for the ln of fetal weight vs. the ln of placental weight were affected by a day of gestation \times fetal size interaction ($P < 0.05$), and no line differences were obtained (Table 1). This analysis indicates that the relationship between fetal weight and placental weight varies according to both day of gestation and fetal weight, with the growth of fetuses relatively more affected by variation in the size of the placenta during later pregnancy compared with early pregnancy and for the smallest fetuses compared with the largest fetuses within a litter. These results confirm that the use of the FPR is inappropriate as a measure of placental efficiency for the range of placental and fetal weights obtained in this experiment.

Figure 4 illustrates representative placental sections stained with hematoxylin and eosin collected throughout gestation. Visual assessment of the stained sections suggested that the folded structure of the epithelial bilayer of the placenta became wider and more complex with advancing gestation, and morphometry was used to further assess these changes. Table 1 presents the means obtained for the morphometric measurements collected in this experiment. Repeatabilities of placental fold length per length of the placenta, placental stromal width above the folds, and width of the folds were 0.65, 0.61, and 0.87, respectively. Repeatability of

Table 1. The fetal weight:placental weight ratio and morphometric traits for placentas associated with the largest and smallest fetuses in litters of gilts from a randomly selected control line, a line selected for ovulation rate, and a line selected for uterine capacity

Item ²	d 45 ¹		d 65		d 85		d 105	
	Large	Small	Large	Small	Large	Small	Large	Small
Fetal weight:placental weight ratio ³	0.35 \pm 0.20	0.56 \pm 0.20	1.12 \pm 0.12	1.60 \pm 0.12	2.51 \pm 0.13	2.67 \pm 0.13	3.72 \pm 0.12	3.80 \pm 0.12
Allometric slope ⁴	0.36 \pm 0.14	0.41 \pm 0.10	0.29 \pm 0.11	0.46 \pm 0.06	0.45 \pm 0.07	0.59 \pm 0.05	0.32 \pm 0.10	0.73 \pm 0.07
Fold length (μm) per micrometer of placenta ⁵	4.22 \pm 0.32	4.30 \pm 0.32	3.92 \pm 0.20	4.15 \pm 0.20	4.74 \pm 0.21	5.08 \pm 0.21	5.75 \pm 0.20	6.18 \pm 0.20
Stromal width above fold, ⁶ μm	244 \pm 24	218 \pm 24	244 \pm 15	108 \pm 15	111 \pm 16	94 \pm 16	80 \pm 15	61 \pm 15
Fold width, ⁷ μm	191 \pm 20	203 \pm 20	214 \pm 12	212 \pm 12	239 \pm 13	256 \pm 13	315 \pm 12	361 \pm 12
Total placental width, ⁶ μm	434 \pm 30	421 \pm 30	458 \pm 19	319 \pm 19	349 \pm 20	352 \pm 20	395 \pm 19	422 \pm 19
Number of observations	9	9	22	22	19	19	21	21

¹d = day of gestation.

²There was no effect of selection line for any traits, so the data were combined for presentation.

³Significant ($P < 0.01$) effects of day and fetal size were detected.

⁴A significant day \times fetal size interaction ($P < 0.05$) was detected.

⁵Significant effects of day ($P < 0.01$) and fetal size ($P < 0.05$) were detected.

⁶A significant day \times fetal size interaction was detected ($P < 0.01$).

⁷Significant day ($P < 0.01$) and fetal size ($P < 0.05$) effects were detected.

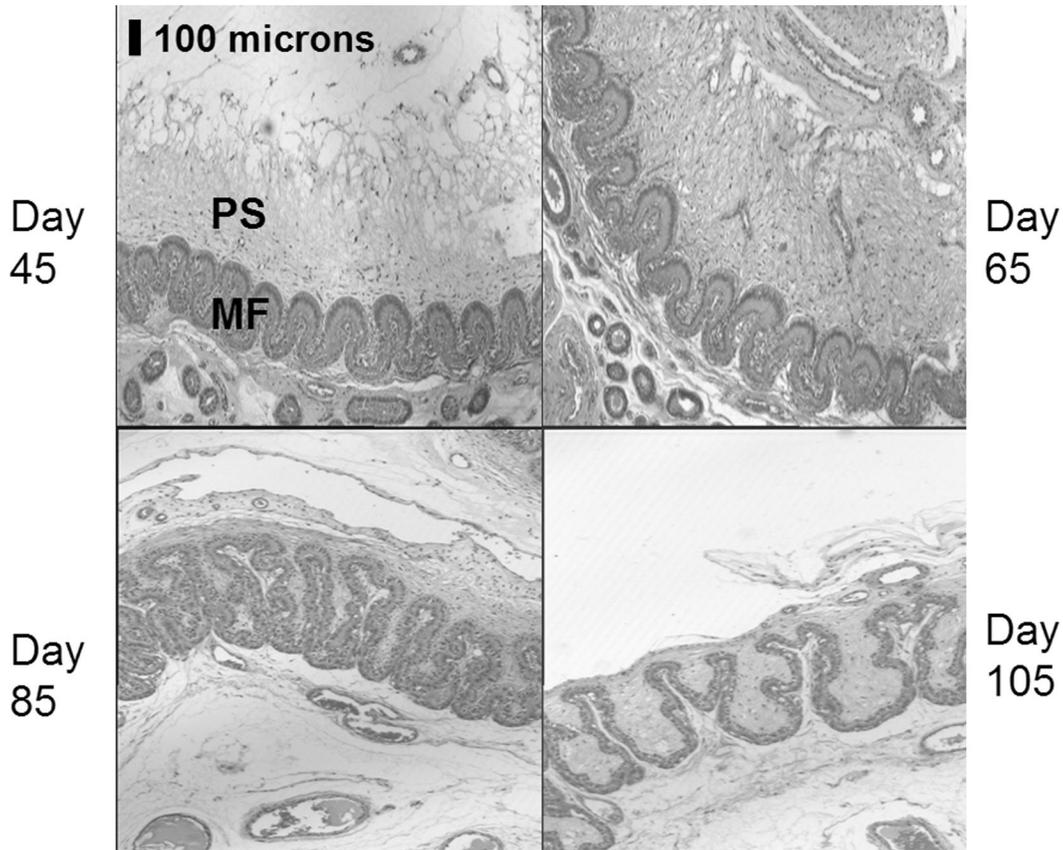


Figure 4. Photomicrographs of representative placental sections obtained from d 45, 65, 85, and 105 of gestation and stained with hematoxylin and eosin. Note the microscopic placental folds (MF) and placental stroma (PS), which get progressively taller and thinner, respectively, with advancing gestation.

the average of multiple measurements is $1 - (1 - r)/n$, with r being the repeatability of a single measure and n the number of replicates. Thus, the repeatability of the average of duplicate measures is 0.83, 0.81, and 0.94, respectively, and the repeatability of the average of triplicate measures would be 0.88, 0.87, and 0.96, indicating that an additional measurement of each slide beyond duplicate measurements would only reduce the variance of the measures by 2 to 6%.

Analysis of the total width of the placenta throughout gestation indicated a significant day \times size of fetus interaction ($P < 0.01$). No differences among the selected lines were obtained, and results are presented after combining the different selected lines (Table 1). Further analysis of the least squares means indicated that the width of the placenta was significantly reduced in placenta associated with the smallest fetus in the litter on d 65 of gestation ($P < 0.01$). Subsequently, the width of the placenta did not differ between the largest and smallest fetuses during the remainder of gestation, thus explaining the interaction.

Analysis of the width of the placental stroma above the folded bilayer indicated a significant day \times size of the fetus ($P < 0.01$) interaction (Table 1) for this trait. As for total width of the placenta, no selection line effects were detected, so data from the 3 selection lines

were combined for presentation. The width of the placental stroma above the folded bilayer was less in placenta associated with the smallest fetus in the litter, and this difference was particularly evident on d 65 of gestation, essentially explaining the interaction and also the difference in total width between placentas associated with the largest and smallest fetuses within a litter on d 65. On d 105 of gestation, for the placenta associated with the smallest living fetus in the experiment, the placental stroma above the folded bilayer was absent (Figure 5).

Analysis of the width of the placental folds indicated significant effects of day of gestation ($P < 0.01$) and fetal size ($P < 0.05$). In contrast to the stroma above the placental folds, the width of the placental folds increased with advancing gestation and was greater for placenta associated with the smallest fetus compared with placenta of the largest fetus in the litter. This difference was especially evident on d 105 of gestation. No line effects were observed, thus data were combined across selection lines for presentation (Table 1).

Analysis of the length of the folded bilayer per unit length of the placenta indicated significant main effects of day of gestation ($P < 0.01$) and fetal size ($P < 0.05$), with no effects of selection line. Similar to the width of the folds, the length of the folds per unit of placental

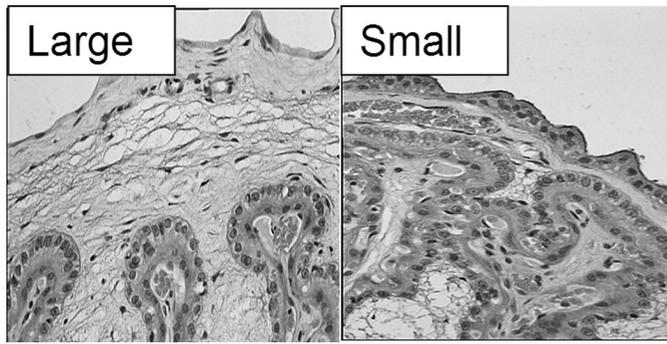


Figure 5. Photomicrographs showing the placentas from the largest and smallest fetuses from a litter collected at 105 d of gestation. The small fetus had the least weight in the experiment for d 105 of gestation. Note that the placental stroma above the microscopic folds remains in the placenta from the largest fetus but has been completely penetrated by the developing microscopic folds in the placenta from the smallest fetus.

length increased as gestation progressed and was greater in placenta associated with the smallest fetuses compared with the largest fetuses. Upon reanalysis of the data with the width of the placental folds added as a covariate, the effect of fetal size was no longer statistically significant, and the effect of day of gestation was reduced but remained significant ($P < 0.01$). With the inclusion of the width of the placental folds as a covariate, the length of the folds per unit of placental length increased ($P \leq 0.05$) from d 65 to 105 of gestation. This increase is likely due to the increase in complexity of the folding of the epithelial bilayer observed on d 85 and 105 of gestation.

Correlation coefficients between the morphometric traits measured in this experiment are reported in Table 2. The average fold length per unit of placental length was strongly positively correlated ($P < 0.01$) with the width of the placental folds. The width of the placental folds was negatively correlated ($P < 0.01$) with the width of the placental stroma above the folds. These correlations suggest that (1) the fold length per unit of placental length increases primarily by increased width of the folds and (2) increased width of the folds occurs at the expense of the stroma above the folds.

DISCUSSION

This is the first report to investigate the influence of selection for ovulation rate or uterine capacity, day of gestation, and the size of the fetus in a crowded uterine environment on the microscopic structure of the pig placenta using morphometric analysis. The overall width, the width of the folded trophoblast/endometrial epithelial bilayer, and the width of the placental stroma above the folded bilayer were measured. Results indicated that selection for ovulation rate or uterine capacity had little effect on these parameters. Overall, the

width of the placenta decreased until d 85 of gestation and then was greater on d 105 of gestation. The width of the folded bilayer increased during late gestation and increased more rapidly in placenta of the smallest fetus compared with the largest fetus. The width of the placental stroma decreased with advancing gestation and was less in placenta of the smallest fetus compared with the largest fetus. One possibility explaining the differences in placental development between the smallest fetus and the largest fetus is that these differences could be a compensatory response to reductions in access to uterine space by increasing the surface area available for interaction between the maternal and fetal blood supplies.

The efficiency of transport of nutrients to the developing fetus by the placenta must influence its health and growth. Thus, a useful measure of the ability of the placenta to transport nutrients is essential, if selection for placental function is to be performed. The FPR has been proposed as a selection tool enabling selection for improved placental function, increased fetal survival, and litter size (Wilson et al., 1999). However, subsequent results indicated that selection using the FPR does not result in improvements in litter size (Mesa et al., 2005). There are several difficulties with selection based on the FPR. According to Weil (1962), the use of a ratio like the FPR, is only appropriate if (1) the 2 variables in the ratio increase or decrease together in a linear fashion, (2) the relationship between the 2 variables passes through the origin, and (3) the variance of both variables increases with the magnitude of each variable. The relationship between fetal weight and placental weight is not linear. Vallet (2000) reported that the relationship between fetal weights and placental weights was curvilinear over the range of fetal and placental weights measured in UHO gilts at 105 d of gestation. This curvilinear relationship indicated that fetal weights were not dependent on placental weight when the placenta weighed greater than 200 g and became increasingly dependent on placental weight as placental weight decreased.

The allometric relationships calculated in the current experiment are consistent with this concept. The allometric slopes for the ln of fetal weight vs. the ln of placental weight for the largest fetuses in a litter are consistently much less than 1, indicating that the weight of these fetuses depends little on the weight of the placenta. In contrast, the allometric slope of the smallest fetus is consistently greater than the allometric slope for the largest fetus and approaches 1 as gestation advances. These observations indicate that although small fetuses are more dependent on the size of the placenta than large fetuses, the resulting slope is still less than 1 on d 105. Thus, even in small fetuses, mechanisms must exist that maintain the growth of the fetus. Differences in expression of these compensatory mechanisms with placental weight is consistent with the observation that the FPR is not a fixed characteristic in pigs, the FPR becomes greater as intrauterine

Table 2. Correlations among fetal and placental weights and the morphometric measurements obtained on placental sections from d 45, 65, 85, and 105 of gestation (all data) for d 45 and 65 combined and for d 85 and 105 combined

Item	ln Fetal weight	ln Placental weight	Epithelial bilayer length per length of placenta	Microscopic fold width	Width of the placental stroma above the folded bilayer	Total placental width
All data						
Fetal weight:placental weight ratio	0.75**	0.29**	0.62**	0.68**	-0.70**	-0.17*
ln Fetal weight		0.82**	0.45**	0.52**	-0.42**	-0.01
ln Placental weight			0.19*	0.25*	0.02	0.23**
Epithelial bilayer length per length of placenta				0.81**	-0.50**	0.14
Microscopic fold width					-0.44**	0.36**
Width of the placental stroma above the folded bilayer						0.68**
d 45 and 65 combined						
Fetal weight:placental weight ratio	0.50**	-0.15	0.20	0.29*	-0.57**	-0.47
ln Fetal weight		0.76**	-0.16	0.24	0.03	0.10
ln Placental weight			-0.28*	0.05	0.46**	0.46**
Epithelial bilayer length per length of placenta				0.60**	-0.07	-0.09
Microscopic fold width					-0.07	0.23
Width of the placental stroma above the folded bilayer						0.95**
d 85 and 105 combined						
Fetal weight:placental weight ratio	0.23*	-0.29**	0.40**	0.51**	-0.60	0.10
ln Fetal weight		0.86**	0.17	0.19	0.05	0.23*
ln Placental weight			-0.05	-0.08	0.37**	0.17
Epithelial bilayer length per length placenta				0.78**	-0.34**	0.55**
Microscopic fold width					-0.36**	0.76**
Width of the placental stroma above the folded bilayer						0.34**

* $P < 0.05$; ** $P < 0.01$.

crowding increases (e.g., calculated from Knight et al., 1977), and is consistent with our observed differences in FPR between large and small fetuses throughout gestation. Finally, using a ratio like the FPR as a selection tool does not provide an accurate prediction of genetic gain using heritability estimates (Gunsett, 1987), perhaps because of shifting selection emphasis between the 2 traits in the ratio (MacNeil, 2005). Because of these difficulties, measurable traits other than the FPR are needed to select for placental efficiency.

The pig placenta is epitheliochorial, such that neither the trophoblast nor the endometrial epithelium is eroded. Thus, nutrients must pass through both epithelia during transport from mother to fetus. Capillary density, surface area, and distance between capillaries influence the efficiency of transport. In addition, the arrangement of fetal and maternal blood flows in the porcine placenta described by Leiser and Dantzer (1988) suggested that the width of the folded bilayer should contribute to the efficiency of nutrient transfer in the porcine placenta. This arrangement suggests that relatively nutrient-rich maternal and fetal blood occurs at the top of the folds, and relatively nutrient-depleted maternal and fetal blood occurs at the bottom of the folds. Increased width of the folds would increase the interaction distance between the 2 blood supplies

and improve the transfer of nutrients from mother to fetus. The increase in width of the folds from d 65 to 105 of gestation is consistent with this hypothesis and with previous histological evidence (MacDonald, 1976; Leiser and Dantzer, 1988) and likely contributes to improved placental efficiency as gestation advances. Interestingly, the width of the folds increases more rapidly in placentas associated with small fetuses. It has been reported that the intrauterine crowding induced by the UHO treatment reduces fetal and placental weights and placental area, and it has been suggested that the reduced fetal weight is due to placental insufficiency (Knight et al., 1977). Our results are consistent with the possibility that the lack of availability of uterine nutrients caused by the reduction in placental surface area may alter placental development such that the microscopic folds become wider, potentially improving nutrient transfer from sow to fetus. It seems possible that this could contribute to the fetal sparing effect (allometric slope less than 1) that remains even in small fetuses during late gestation.

Although our results suggest that the placenta associated with small fetuses should transfer nutrients more efficiently than placenta of larger fetuses in the same litter, there is little evidence in the literature to support this concept. Placental efficiency varies depending on

the nutrient examined and would seem to depend on the size of the placenta, various aspects of placental development such as the depth of folds, increased complexity of the epithelial bilayer and the distance between the maternal and fetal capillaries (Friess et al., 1980), and individual nutrient-specific mechanisms that may enhance the transport of that particular nutrient. Examples of mechanisms that enhance specific nutrients include differences between maternal and fetal hemoglobin oxygen affinity and their effect on oxygen transfer (Comline and Silver, 1974; Wilkening and Meschia, 1992); the possible existence of placental glucose transporters and their effect on glucose transfer (Baumann et al., 2002); the secretion of endometrial uteroferrin and retinol-binding protein and their effects on iron and retinol transport, respectively (Roberts and Bazer, 1988); and AA transporters and their effects on the transport of specific AA (Wu et al., 1995; Finch et al., 2004). The transport of other nutrients, such as FFA, would seem to be mostly excluded (Thulin et al., 1989). To our knowledge, the *in vivo* transport efficiency for placentas associated with large and small fetuses has never been measured for any specific nutrient in the pig, nor has the contribution of placental size or structure been examined for transport of any specific nutrient. Reynolds et al. (1985) reported that fetal umbilical blood concentrations and placental uptake of oxygen, glucose, α -amino nitrogen, and urea nitrogen did not differ from d 70 to 110 of gestation. The results of Reynolds et al. (1985) suggest that the modifications in the placental folds described here and by MacDonald (1976), along with the reductions in the distance between maternal and fetal capillary beds described by Friess et al. (1980), do not contribute significantly to the efficiency of placental transport for these nutrients during late gestation. The relationships between placental size and structure and the efficiency of transport of individual nutrients require further investigation.

Inclusion of the width of the microscopic folds as a covariate reduced the significance of the effect of gestation on the length of the epithelial bilayer per length of the placenta, but the effect of gestation length on this trait remained significant. This is consistent with the increased complexity of the folding pattern of the epithelial bilayer with gestation noted here and in previous experiments (MacDonald, 1976). During later gestation, secondary ridges appear between primary microscopic folds, which develop during earlier gestation, and the secondary ridges further increase the surface area available for exchange between the mother and fetus. Almost nothing is known about how the folds and ridges develop within the epithelial bilayer. Our hypothesis that the folds develop by invasion of the placental stroma suggests 2 mechanisms that may be tested in further experimentation. First, invasion of the epithelial bilayer into the placental stroma would require the secretion of enzymes that are specific to the components of the placental stroma. We have begun examining placental content of hyaluronan and placen-

tal expression of hyaluronidases to begin to understand the potential role of these molecules in placental development, but roles for many other extracellular matrix components and their degrading enzymes in this process are also likely. Second, in both prostate (Lopes et al., 1996) and human endometrial glandular epithelium (Hempstock et al., 2004), cell height correlates with secretory activity. The location of the tall columnar trophoblast epithelial cells at the tops of the folds suggests that these cells may play a central role in invasion of the epithelial bilayer into the placental stroma via the secretion of enzymes needed to invade the stroma. Interestingly, the trophoblast cells at the top of the secondary ridges also have the tall columnar phenotype, consistent with a role for these cells in invasion into the stroma. These cells have been implicated in macromolecule transport by the placenta (Friess et al., 1980), but there is little evidence indicating that this is their function. Further characterization of these cells is essential to an understanding of their role in the pig placenta.

Correlation analysis of the data obtained provided several interesting results. Most notable among these is a strong overall correlation between the FPR and microscopic fold width. Comparing early with late pregnancy indicated that this correlation is greatest during later pregnancy, which is consistent with the increase in fold development during later pregnancy playing a role in improved placental efficiency. However, as discussed previously, the FPR itself may not be a good estimate of placental efficiency, so these correlations require some caution as to their interpretation. Another result is the contribution of the stroma to overall placental weight and placental thickness during gestation. The correlation between stromal thickness and overall placental thickness during early gestation indicates that the stroma explains most of the variation in placental thickness and a significant portion of the variation in placental weight. During later pregnancy, these correlations are much less. Furthermore, a strong negative contribution of microscopic fold width to both placental weight and stromal width was evident. These correlations, along with the overall negative correlation between the width of the placental folds and the width of the overlying stroma are all consistent with the hypothesis that the microscopic folds develop at the expense of the overlying placental stroma. Furthermore, the width of the overlying placental stroma was much less in the placenta of small fetuses, whereas the width of the folds was greater. Clearly, this competition between the 2 structures must be limited by the available placental stroma and suggests the hypothesis that in small fetuses, growth of the folds can exceed the availability of the stroma, completely penetrating this layer, as is illustrated in sections from the placenta of the smallest living fetus on d 105 for this experiment. It seems possible that this could provide an explanation for fetal losses that occur during late pregnancy in a crowded intrauterine environment (Christenson et al., 1987;

Freking et al., 2007). Once the microscopic folds penetrate the available placental stroma, no further widening of the folds is possible, which may lead to the inability of the placenta to compensate further for the lack of uterine space, resulting in the death of the fetus. Further experimentation is required to determine the validity of this hypothesis.

In summary, we have demonstrated significant changes in the development of the placenta associated with the largest and smallest fetuses in litters subjected to intrauterine crowding caused by the UHO procedure. We were unable to demonstrate any effect of selection for ovulation rate or uterine capacity on placental development. Our results indicated that the increased crowding experienced by the smallest fetus within the UHO uterine environment was associated with increased placental microscopic fold width, increased length of the placental epithelial bilayer per unit length of the placenta, and reduced width of the stroma above the folded epithelial bilayer. We hypothesize that these changes may compensate for the crowded intrauterine environment experienced by these fetuses but also may result in fetal losses if this compensatory mechanism is exceeded. Further experiments are required to determine the role of the development of the microscopic folds in placental efficiency, health of the fetus, and litter size in pigs.

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