The Effect of Uterine Environment on Meishan and Yorkshire Fetal Development and Placental Size and Vascularity

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Summary and Implications

When Meishan (M) and Yorkshire (Y) embryos were cotransferred to Y recipients that were then allowed to farrow, it was observed that the birth weights of M and Y littermates were similar averaging 1.15 ± .06 kg. In contrast, placentae matched to M piglets were markedly smaller (♂70% lighter) and more vascular (♂two-fold) than Y placentae. To investigate the effect of uterine environment on conceptus development to term, M and Y embryos were cotransferred to M recipients (n=3) that were slaughtered one day before expected parturition (day 113). Fetal weight, placental weight, and placental surface area were recorded. Additionally, a section of the intact maternal placental interface was excised, fixed, embedded, sectioned, and stained to allow quantitation of the placental vascular density (PVD). As observed when M and Y fetuses were cogestated by Y recipients, littermate M and Y fetuses gestated in M uteri were similar in weight (1.04 ± .03 vs. 1.03 ± .05 kg) at term. Further, M conceptuses exhibited markedly reduced (P<.03) placental weights (170 ± 19 vs. 249 ± 10 g) and surface areas (1017 ± 70 vs. 1506 ± 96 cm^2) compared with their Y littermates. As was the case for conceptuses gestated in Y uteri, the similarity in fetal weight between the two breeds with very different placental sizes appears to result from an increased (P<.09) M PVD compared with littermate Y fetuses (2.5 ± .3 vs. 1.4 ± .4%). These data indicate that in both the M and Y uterine environment, the reduced size of the M compared with Y placenta is compensated for by an increase in PVD. Currently it is unknown whether M placentae contain a greater amount of vasculature (total volume) or a similar amount of vasculature simply squeezed into a smaller space. To investigate breed differences in total placental vasculature we have perfused placentae of M and Y conceptuses cogestated in M uteri on d 113 of gestation with vascular casting material and will compare the volumes of the corrosion casts.

Introduction

For the last decade, we and others have made use of the prolific Chinese Meishan pig as a model to understand the controls of litter size. Meishan gilts give birth to three to five more pigs per litter than our domestic breeds such as the Yorkshire, while exhibiting a similar ovulation rate and uterine size. Several investigators have repeatedly demonstrated that throughout gestation, the Meishan conceptus is considerably smaller than similar age conceptuses from U.S. and European breeds. Previously, we had shown that if Meishan and Yorkshire embryos were cotransferred to Yorkshire recipient females, that were then allowed to farrow, Meishan and Yorkshire littermate piglets were born at similar weights. In contrast to the piglets, Meishan placentae were markedly smaller (70% lighter) and more vascular (♂two-fold) than Yorkshire placentae. The similarity in birth weight of Meishan and Yorkshire piglets with markedly different placental weights was compensated for by a marked increase in placental vascular density in Meishan compared with Yorkshire placentae. The objective of this experiment was to further characterize the role of differential placental vascularization in compensating for differences in littermate placental size.

Materials and Methods

Study 1. Meishan and Yorkshire gilts of similar reproductive age were used in this study. When three gilts exhibited estrus simultaneously (two Meishan + one Yorkshire gilt), one Yorkshire and one Meishan gilt were mated to a boar of their same breed at zero and 24 hours after first detection of estrus (donor gilts) and the remaining gilt (Meishan) served as a nonmated recipient. Embryos were collected from donor gilts on day 2 to 3 of gestation. Ten Meishan embryos and 10 Yorkshire embryos were transferred to the recipient.

At day 113 of gestation recipient females were slaughtered and gravid uteri recovered. The fetuses were weighed and their crown-rump lengths determined. A section of intact utero-placental tissue near the umbilical cord was excised and fixed in 10% neutral buffered formalin for later evaluation of vascular density. The placentae were weighed and then spread out on butcher's paper and traced. The placental tracings were later used to quantify placental surface area by using a planimeter. Fixed utero-placental tissues were sectioned perpendicular to the utero-placental interface and stained. Stained sections were then projected onto a video screen by using a microscope fitted with a camera. The image on the video screen was then traced onto an acetate sheet to include the limits of the placental and endometrial tissue visible and the interior of visible blood vessels. An image analysis system was then used to quantitate the areas traced and to calculate the percentage of placental tissue occupied by blood vessels.

Study 2. An additional four embryo transfers were accomplished as described for study 1, to address the question of whether there were marked differences in total vascular volume between Meishan and Yorkshire fetuses cogestated in a common Meishan uterine environment.

Pregnant Meishan females were slaughtered on day 113 of gestation and gravid uteri were collected. After transporting the gravid uterus to the laboratory, a small incision was made overlying a viable conceptus. The umbilical cord was the exteriorized through the incision. Both umbilical arteries were dissected free of the surrounding connective tissue, and an 18-gauge needle (with the end made blunt) was inserted into each artery. The umbilical vein was then cut and the placental vasculature perfused with physiologic saline. After all the blood had been flushed from the placenta, latex vascular casting material (BioDur, Germany) was perfused into the placental vasculature with the aid of a Harvard peristaltic pump. The same process was then repeated for each conceptus in the uterus. The tract was then allowed to cure for 24 hours before being placed in a bath containing KOH in water (4% wt/vol) to digest the tissue. After marked digestion of both uterine and fetal tissue had occurred, placental vascular casts were removed from the initial digestion bath and placed in fresh KOH in water to finish digesting the remaining placental membranes before being rinsed with copious amounts of water and allowed to dry.

Study 1. The average number of conceptuses for the three litters collected was 8.7 ± 1.7, with both Meishan and Yorkshire fetuses present in every litter. The average uterine horn length was 252 ± 14 cm. As can easily be seen in Figure 1, there was no overall difference ($P>.90$) in fetal weight between the Meishan and Yorkshire fetuses. Additionally, there was a marked overall difference ($P<.03$) in placental weight (Figure 1) and placental surface area (Figure 2) between Meishan and Yorkshire conceptuses. Interestingly, to compensate for the decreased placental size, Meishan conceptuses had a increased ($P<.09$) placental vascular density compared with Yorkshire littermates (Figure 2).

Study 2. From the vascular casts we obtained, we could see the marked differences in placental size between Meishan and Yorkshire conceptuses. Unfortunately, as we were not able to ensure that each placenta was perfused at the same pressure or to the same end point, we found that the resulting casts exhibited marked differences in their degree of perfusion. As a result of this technical hurdle, we are unable at this time to make any quantitative statement about differences in placental vascular volume between Meishan and Yorkshire conceptuses. We are continuing to make placental corrosion casts and hope in the near future to be able to use them in a quantitative manner.

Discussion

In agreement with a previous study using Yorkshire recipient females, we found in the current study that Meishan and Yorkshire late term fetuses cogestated in a Meishan uterus exhibit similar weights on day 113 of gestation, even though Meishan placentae are markedly smaller (surface area and weight) than Yorkshire placentae. The lack of difference in fetal weight between the Meishan and Yorkshire fetuses in the face of markedly different placental sizes appears to be a result of a compensatory increase in the density of placental blood vessels in the Meishan compared with Yorkshire placenta. These observations beg the question as to whether both fetal breeds (Meishan and Yorkshire) have similar amounts of total vasculature, simply packed into different size placentae. Although many nice casts of the placental vasculature were obtained from Meishan and Yorkshire conceptuses, we were unable to conclude anything about the relative difference, or lack thereof, in total placental vascular volume between Meishan and Yorkshire placentae. This resulted from our inability to adequately control perfusion pressure and thus vessel distensibility and overflow of casting material into the venous system. The previously described difference in placental size, however, was obvious from the corrosion casts.

As uterine capacity would appear to be the ultimate limitation to litter size, the prolificacy of the Meishan appears to be a result of a limited placental growth. Previously we demonstrated that both the breed of the conceptus and the breed of the pregnant female can both exert effects on conceptus size. In addition, we have shown that the size of the fetus and piglet is determined not only by the size of its placenta, but instead a combination of placental size and vascularity (i.e., surface area and relative efficiency). More recently we demonstrated that each conceptus develops on a placenta that is of a size and vascularity determined by the unique genotype of that conceptus, as placental size and resultant efficiency (piglet weight:placental weight ratio) can vary by as much as four-fold within a litter. Based on the results of this study, we have further demonstrated that the key difference between the prolific Chinese Meishan and the Yorkshire (or other domestic pig breed), is that the Meishan conceptus develops a smaller, more vascular placenta allowing an increased number of viable conceptuses to survive to term in the limited uterine space available.

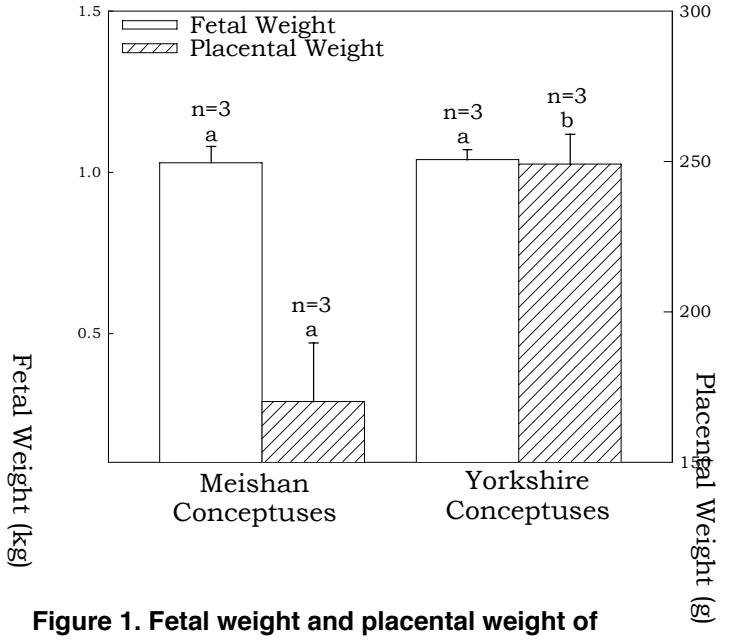

Figure 1. Fetal weight and placental weight of Meishan and Yorkshire conceptuses cogestated by Meishan recipient females to day 113. Means ± SEM with different letters within a measure differ (P<.03).

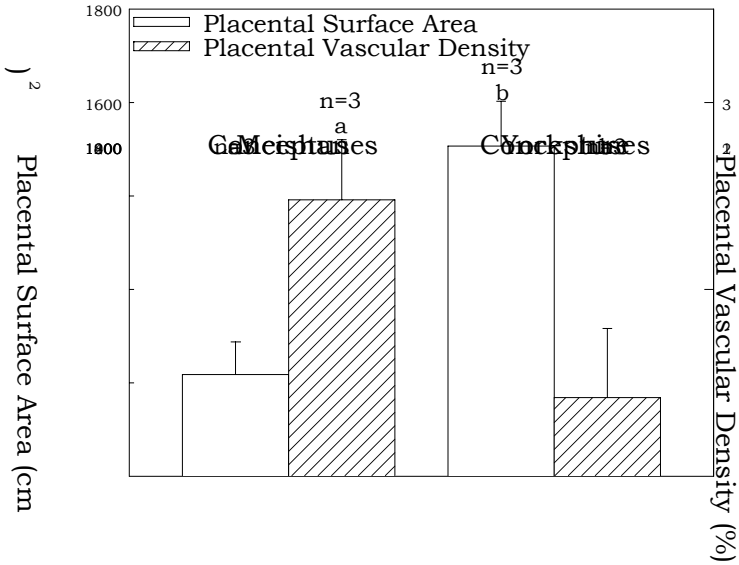

Figure 2. Placental surface area and placental vascular density of Meishan and Yorkshire conceptuses cogestated by Meishan recipient females to day 113. Means ± SEM with different letters differ (P<.02).