The effect of breed and intrauterine crowding on fetal erythropoiesis on day 35 of gestation in swine[1]

J. L. Vallet[2], H. G. Klemcke, R. K. Christenson, and P. L. Pearson

USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE 68933

ABSTRACT: In a previous report, it was suggested that intrauterine crowding impaired fetal erythropoiesis and that fetal erythropoiesis was accelerated in Meishan pigs during early pregnancy. Because these conclusions were based on limited numbers of observations, the present experiment was undertaken to provide a more extensive investigation of these phenomena. Intact white crossbred gilts, unilaterally hysterectomized-ovariectomized (UHO) white crossbred gilts, and intact Meishan gilts (n = 13 to 16 per group) were mated after at least one estrous cycle of normal duration (17 to 23 d). Gilts were laparotomized at d 35 of pregnancy, the uterine horns were exteriorized and opened near each fetus, and a blood sample was collected from each fetus. The uterine horn was then surgically removed, and each fetus and placenta was weighed. All fetal blood samples were measured for hematocrit, red blood cell number, and hemoglobin. Erythropoietin and the percentages of nucleated cells and reticulocytes were also measured in blood samples from the largest and smallest living fetus in each litter. Fetal hematocrits were not affected by treatment. Blood cell counts were greater ($P < 0.01$) in fetuses of Meishan gilts than in White crossbred intact or UHO gilts. Hemoglobin was less ($P < 0.01$) in fetuses of Meishan gilts than in fetuses of White crossbred intact or UHO gilts. The percentages of nucleated (immature) cells and reticulocytes were both less ($P < 0.01$) in fetuses of Meishan intact gilts. Erythropoietin was also lower ($P < 0.01$) in fetuses of Meishan gilts. As observed previously, fetal weight was correlated (r = 0.38; $P < 0.01$) with blood hemoglobin concentration. These results confirm that fetal erythropoiesis in Meishan gilts is accelerated compared with White crossbred gilts. These results are consistent with the hypothesis that faster blood cell development could be beneficial to fetal survival in swine.

Key Words: Blood, Fetus, Pigs, Pregnancy

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Introduction

Uterine capacity, or the number of fetuses that can be maintained by the uterus until farrowing, is an important component of litter size in swine (Christenson et al., 1987; Bennett and Leymaster, 1989). However, factors influencing this trait are not well understood. A previous study comparing the onset of fetal erythropoiesis during early gestation (d 24 to 40) in white crossbred intact, white crossbred unilaterally hysterectomized-ovariectomized (**UHO**; an animal surgical model used to estimate uterine capacity and known to increase intrauterine crowding; Christenson et al., 1987), and Meishan (a breed suggested to have greater uterine capacity; Haley and Lee, 1993) intact gilts suggested that fetal erythropoiesis was impaired under crowded intrauterine conditions and was accelerated in the Meishan breed (Pearson et al., 1998). From these results, it was hypothesized that defects or improvements in fetal erythropoiesis may influence uterine capacity during early pregnancy (Pearson et al., 1998). However, in Pearson et al. (1998), low blood volumes on d 24 and 30 of pregnancy necessitated pooling of blood samples on these days, limiting the data collected. Furthermore, in order to examine more traits, fetal traits associated with erythropoiesis (hematocrit, hemoglobin, etc.) were examined in only three fetuses per gilt on d 40 in the previous experiment. The objective of the current study was to provide more extensive data on intrauterine crowding effects on fetal erythropoiesis and more fully characterize fetal erythropoiesis in Meishan gilts during early pregnancy.

Materials and Methods

This study was reviewed and approved by the Meat Animal Research Center Animal Care and Use Commit-

[1]Names 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 USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2]Correspondence: P.O. Box 166, State Spur 18D (phone: 402-762-4187; fax: 402-762-4382; E-mail: vallet@email.marc.usda.gov).

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tee. White crossbred (¼ Yorkshire, ¼ Landrace, ¼ Chester White, ¼ Large White) gilts were UHO at 160 d of age. White crossbred intact (n = 16), UHO (n = 14), and Meishan intact (n = 13) gilts were observed for estrous behavior beginning at approximately 200 d of age and were mated to mature boars of the same breed after at least one estrous cycle of normal duration (17 to 23 d). On d 35 of pregnancy, gilts were anesthetized, and the reproductive tract was exposed through a mid-ventral incision. The uterus was opened adjacent to each fetus and a blood sample was collected from that fetus into a 1-mL heparinized syringe. The fetus was then removed and weighed. After all fetuses were collected, the uterine horn(s) were surgically removed and each placenta was recovered and weighed in such a way that each placenta remained matched to each fetus. The number of live fetuses and corpora lutea (**CL**) were recorded. Finally, the liver of each fetus was dissected and weighed.

Hematocrits, hemoglobin, and total red blood cell concentrations were measured for each fetal blood sample as described previously (Pearson et al., 1998). Blood smears were prepared for blood samples collected from the largest and smallest fetus from each gilt and were evaluated for the percentage of nucleated cells (after Wright-Giemsa staining; Sigma Chemical Co., St. Louis, MO) and the percentage of reticulocytes (after Accustain staining; Pearson et al., 1998). The percentage of nucleated cells was evaluated rather than evaluating differential cell types as described previously (Pearson et al., 1998) because this percentage could be more objectively measured and still produce a measure of the maturity of the red blood cell population. Blood samples were then centrifuged and the plasma was collected and assayed for erythropoietin as previously described (Pearson et al., 1998).

Statistical Analysis

Fetal weights, placental weights, fetal blood cell counts, fetal hematocrits, fetal blood hemoglobin, fetal blood percentage of nucleated cells, and fetal blood percentage of reticulocytes were averaged for each gilt. Gilt averages for each of these traits, the number of CL, and the number of live fetuses were analyzed by ANOVA using a model that included the effect of breed or UHO (White crossbred intact, UHO, or Meishan intact). To further evaluate differences between means, the following contrasts were performed: 1) White crossbred intact gilts were compared to White crossbred UHO gilts (effect of intrauterine crowding), and 2) White crossbred intact gilts were compared to Meishan intact gilts (effect of breed). Because previous work indicated a positive correlation between fetal weight and hematocrit and hemoglobin, the relationships between fetal weight and blood traits were evaluated, both within breed/UHO groups and across breed/UHO groups. Regression analysis was performed for blood traits from each fetus using a model that included the effects of breed or UHO group, fetal weight, and the breed or UHO group × fetal weight interaction, and used the interaction of fetal weight with gilt within breed or UHO group as the error term.

Results

As expected, the number of CL did not differ between White crossbred intact and UHO gilts. Unexpectedly, there was also no difference between White crossbred and Meishan intact gilts (Table 1). In contrast, litter size was reduced ($P < 0.01$) in White crossbred UHO gilts compared with either of the intact groups, which did not differ. Thus, these data indicate that intrauterine crowding had already caused significant fetal mortality by d 35 of pregnancy. Placental weights were less ($P < 0.01$) in both White crossbred UHO and Meishan than in White crossbred intact gilts. Fetal weights were also less in Meishan ($P < 0.01$) than in White crossbred intact gilts. However, UHO had no effect on fetal weight. Fetal liver weights did not differ for the three groups.

Fetal hematocrits, hemoglobin, red blood cell counts, percentage of reticulocytes, and plasma erythropoietin did not differ between the UHO and White crossbred intact gilts (Table 1). However, the percentage of nucleated blood cells was less ($P < 0.01$) in the White crossbred UHO group compared with the White crossbred intact gilts.

In contrast to the White crossbred intact vs. UHO gilt comparison, several of the blood traits differed in fetuses of Meishan-intact gilts when compared with White crossbred intact gilts. Fetal hematocrits did not differ between breeds. However, fetal hemoglobin concentrations were lower ($P < 0.01$) in Meishan intact gilts compared with White crossbred intact gilts. Paradoxically, fetal red blood cell counts were greater in the Meishan-intact gilts compared with White crossbred intact gilts. Also, both the percentage of nucleated cells and the percentage of reticulocytes were lower in fetuses of the Meishan-intact gilts compared with White crossbred intact gilts. Taken together, the increased cell counts, decreased nucleated cells, and decreased reticulocytes in Meishan fetal blood at this stage of pregnancy strongly suggest that fetal red blood cells mature earlier in Meishan gilts compared with White crossbred gilts, confirming our previous observations (Pearson et al., 1998).

Regression analysis of the relationship between the fetal blood traits measured and fetal weight indicated an overall relationship between hemoglobin concentration in fetal blood and fetal weight ($r = 0.38$; $P < 0.01$). No significant overall relationships were observed between fetal weight and hematocrit, red blood cell count, percentage of nucleated cells, percentage of reticulocytes or erythropoietin.

Discussion

The results of this experiment confirm and extend our previous observations concerning the effects of in-

Table 1. Least squares means for traits measured on d 35 of pregnancy in White crossbred intact (WC-INT), White crossbred unilaterally hysterectomized-ovariectomized (UHO), and Meishan intact gilts

	Treatments		
Variable	White crossbred intact	White crossbred UHO	Meishan intact
Number of gilts	16	14	13
Number of corpora lutea	14.4 ± 0.6	13.9 ± 0.7	15.1 ± 0.6
Litter size	11.4 ± 0.6	8.6 ± 0.7[a]	12.7 ± 0.7
Placental weight, g	32.7 ± 1.8	21.7 ± 1.9[a]	21.5 ± 2.0[a]
Fetal weight, g	3.7 ± 0.1	3.6 ± 0.2	3.1 ± 0.2[a]
Fetal liver weight, g	0.36 ± 0.01	0.34 ± 0.02	0.34 ± 0.02
Hematocrit, %	20.3 ± 0.8	19.3 ± 0.9	21.2 ± 0.9
Hemoglobin, g/dL	5.2 ± 0.2	4.7 ± 0.2	4.2 ± 0.2[a]
Red blood cells, $\times 10^6$/L	1.08 ± 0.08	1.18 ± 0.09	1.71 ± 0.09[a]
Nucleated blood cells, %	29.5 ± 2.2	18.5 ± 2.4[a]	17.2 ± 2.4[a]
Reticulocytes, %	45.1 ± 1.7	48.7 ± 1.9	32.9 ± 1.8[a]
Erythropoietin, mU/mL	30.1 ± 2.0	25.2 ± 2.4	17.3 ± 2.2[a]

[a]Mean differs from WC-INT gilts, $P < 0.01$.

trauterine crowding and breed on litter size and fetal erythropoiesis during early pregnancy. These data confirm that litter size is reduced in UHO gilts on d 35 of pregnancy as previously shown by Vallet and Christenson (1993). Results also may indicate that intrauterine crowding accelerated the transition from nucleated (immature) to nonnucleated (mature) erythrocytes that occurs during this period of pregnancy because UHO treatment reduced the percentage of nucleated cells in fetal blood. Results confirmed that hemoglobin content of blood is associated (r = 0.38) with fetal weight. Finally, these results strongly suggest that the maturation of the fetal blood supply, which includes both an increase in red blood cell number and maturity, occurs earlier in the Meishan breed, suggesting that this may contribute to the greater uterine capacity of this breed.

Ovulation rate was not significantly different in Meishans compared with White crossbred gilts, although ovulation rate did tend to be greater in Meishans. This result is similar to some of the results described by Haley and Lee (1993), in which both higher and lower ovulation rates were obtained when Meishans were compared with various white breeds. However, the current results contrast with the results of Christenson (1993), who reported greater ovulation rates in Meishans vs. White crossbred gilts at ages substantially similar (approximately 200 d of age) to those of gilts used in this experiment. Haley and Lee (1993) also summarized the results of several experiments in which embryonic survival (survival to d 30) was examined. Similar to ovulation rates, both greater and lower embryonic survival was obtained in Meishan than in various White breeds. Christenson (1993), using gilts that were substantially similar to those used in the current experiment, found no difference between Meishan and White crossbred gilts in litter size measured at d 30. In the current study, no difference in litter size between breeds was observed on d 35. These results indicate that the greater fertility of the Meishan is probably primarily due to greater uterine capacity in this breed, as previously suggested (Christenson, 1993; Haley and Lee, 1993). The current prevailing hypothesis that the Meishan accomplishes greater fertility by reductions in placental and fetal size is consistent with this concept (Christenson, 1993; Wilson and Ford, 2001; Vallet et al., 2002). Unfortunately, because ovulation rate was relatively low in this study and litter size measurements were taken before the full effects of uterine capacity were observable (see below), we cannot address this question with the current data. Further experiments, possibly using the UHO surgical model, are needed to appropriately address this question.

Our previous results (Pearson et al., 1998) and the results of others (see Vallet, 2000 for review) indicated that loss of fetuses increased significantly between d 30 and 40 of pregnancy under crowded intrauterine conditions. The present results confirm this observation; litter size was reduced in UHO White crossbred gilts on d 35. Anecdotally, during the course of performing this experiment, many conceptuses were observed that appeared to have died recently. Thus, as observed previously (Vallet and Christenson, 1993; Knight et al., 1977; Vallet, 2000), the period between d 30 and 40 of pregnancy represents a period when the pig conceptus becomes unusually sensitive to the effects of intrauterine crowding.

The fetal weight:placental weight ratio has been suggested as a measure of placental efficiency in pigs (Biensen et al., 1998). However, the current results indicated that placental weights were reduced by intrauterine crowding, whereas fetal weights were unchanged. This agrees with previous observations indicating that fetal growth rate is less sensitive to intrauterine crowding than placental growth rate (Vallet and Christenson, 1993). Thus, at this stage of pregnancy, the efficiency of placental transport can compensate for a reduction in placental size caused by intrauterine crowding. Indeed, placental weights were nearly identical between the White crossbred UHO group and the Meishan intact group, whereas fetal weights were greater in the White crossbred UHO group compared with intact Meishan gilts. This suggests that placental efficiency in the UHO group actually exceeded that of the Meishan group at this stage of pregnancy. Previous studies have indicated that placental efficiency of Meishan conceptuses is greater than that of Occidental breeds (Biensen et al., 1998). Taken together, these observations support the concept that the true potential for placental efficiency of a given conceptus is not manifested unless the conceptus is in a crowded intrauterine environment, with its associated reduction in placental weight, as has been suggested previously (Vallet, 2000).

The percentage of nucleated cells in the fetal blood supply was significantly less in White crossbred UHO gilts compared with intact gilts. The best explanation for this result is that the intrauterine crowding resulting from UHO accelerated the transition from immature to mature red blood cells. The more mature nonnucleated red blood cells might be expected to carry oxygen more efficiently than immature nucleated cells. Thus, the acceleration in maturation may be a component of the increase in placental efficiency noted above. However, an alternative explanation is that the crowded intrauterine environment selected against fetuses in which maturation proceeded more slowly. We cannot currently distinguish between these two possibilities, and further experiments are necessary.

Several of the blood traits measured in this experiment indicated that erythropoiesis in Meishan fetuses is accelerated compared with White crossbred fetuses. The concentration of red blood cells was greater and the percentage of nucleated cells and percentage of reticulocytes were less in Meishan fetuses compared with White crossbred fetuses. Paradoxically, fetal erythropoietin was less in Meishans. However, Pearson et al. (1998) indicated that this hormone decreased from d 24 to 40 of pregnancy, coincident with the onset of red blood cell maturation. Thus, lower erythropoietin in Meishans may suggest that the normal decrease in erythropoietin that occurs during this period may be accelerated in Meishans, which is consistent with previous observations (Pearson et al., 1998). In seeming contrast, hematocrit was unchanged and hemoglobin content of blood was actually lower in Meishan fetuses than in White crossbred fetuses. However, immature red blood cells are larger and therefore take up more volume compared with mature red blood cells (Bloom and Fawcett, 1968), making hematocrit a somewhat unreliable measure at this stage of fetal development. Furthermore, an examination of previous results (Pearson et al., 1998) indicates that hemoglobin content per red blood cell in that experiment decreased from a range of 5.1 to 5.6 pg of hemoglobin per red blood cell on d 24 to a range of 2.36 to 2.77 pg of hemoglobin per red blood cell on d 40. Similar results are described by Wintrobe and Schumaker (1936), who suggested that these differences were explained primarily by differences in cell size and not in hemoglobin concentration within the cell. These reports indicate that hemoglobin per fetal red blood cell decreases dramatically as the erythron matures during this period. Thus, the fall in hemoglobin per red blood cell observed in Meishans in the current experiment actually supports the conclusion that the fetal blood supply is maturing more rapidly in Meishan fetuses than in White crossbred fetuses. It is possible that the more rapid maturation of the erythron may contribute to the improved uterine capacity of the Meishan breed.

Both the current results and the results of Pearson et al. (1998) are consistent with an earlier transition from nonnucleated to nucleated cells in Meishan vs. White crossbred gilts. Lee et al. (2001) reported that in mice this transition is controlled by the onset of erythropoietin production by the fetal liver and aorta-gonado-mesonephras areas. However, Klemcke et al. (2001) reported that erythropoietin messenger RNA (**mRNA**) expression was highest in fetal livers on d 24, at a time when red blood cells are mostly nucleated, and decreases on d 30 and 40 as the red blood cells mature. Lee et al. (2001) also reported that mRNA for erythropoietin receptor is present during both the primitive (generating nucleated red blood cells) and definitive (generating nonnucleated red blood cells) stages of erythropoiesis in mice. In contrast, Pearson et al. (2000) reported that erythropoietin receptor mRNA is nearly absent from fetal liver on d 24 and increased dramatically on d 30 and 40. One interpretation of these results is that the transition from nucleated to nonnucleated red blood cells may be controlled by the onset of expression of erythropoietin receptor in the pig. If true, the more rapid maturation of the blood supply in the Meishan could be due to differences in the eythropoietin receptor gene. However, erythropoietin receptor gene expression did not differ in Meishans compared with White composite gilts (Pearson et al., 2000). Information is currently lacking on differences in erythropoietin receptor protein levels between the two breeds. Furthermore, differences between the two breeds in the erythropoietin receptor coding sequence or in erythropoietin receptor second messenger systems are also possible explanations for the earlier maturation in the Meishan. Thus, differences between the Meishan and White crossbred breeds in these or other genes involved in erythropoiesis could be responsible for the earlier maturation in the Meishan.

Previous results (Pearson et al., 1998; Vallet et al., 2002) indicated positive associations between fetal weight and hematocrit and hemoglobin. One of the purposes of this experiment was to confirm these associations with more observations. An overall positive association between hemoglobin and fetal weights was obtained, confirming previous results (Pearson et al., 1998). This occurred in spite of the observation that maturation of the blood supply is associated with decreasing hemoglobin per cell. There was no overall association between hematocrit and fetal weight, in contrast to results reported previously (Pearson et al., 1998; Vallet et al., 2002). However, as previously indicated, the differences in the sizes of the red blood cells present at d 35 of pregnancy may make it unlikely that a significant relationship would be expected. It may be that a significant influence of intrauterine crowding on hemoglobin occurs by d 35 but occurs later for hematocrit as the fetal blood supply finishes converting to all nonnucleated cells.

Although it is clear from these and previous results that intrauterine crowding is associated with changes in fetal erythropoiesis, it remains unclear whether changes in fetal erythropoiesis have consequences for uterine capacity. The effects of intrauterine crowding on fetal erythropoiesis may not be sufficient to result in poorer survival of smaller fetuses, in which case other mechanisms should be explored. Methods to specifically alter fetal erythropoiesis while other fetal and placental functions remain unchanged are currently lacking. The approach we are currently taking is to search for variation in genes affecting fetal erythropoiesis, such as erythropoietin receptor. Only through methods specific to fetal erythropoiesis can cause and affect relationships with uterine capacity and litter size be demonstrated.

Implications

These results confirm that intrauterine crowding influences the transition from embryonic to fetal erythropoiesis and that the blood supply of Meishan fetuses matures more rapidly during this critical stage of pregnancy. This may play a role in the greater uterine capacity of this breed. Results further indicate that on d 35 of pregnancy, placental efficiency can increase in response to intrauterine crowding, lending support to the concept that true placental efficiency can only be measured when the size of the placenta is below a critical limit, as has been suggested previously. These results may indicate that improvements in the efficiency of fetal erythropoiesis during this period of pregnancy will result in improvements in uterine capacity and lead to increases in litter size of swine.

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