EMBRYO DEVELOPMENT AND EMBRYO TRANSFER IN MEISHAN AND LARGE WHITE PIGS

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SUMMARY

This paper reports studies of the rate and variability of embryonic development in the first 10 days of pregnancy in Meishan and Large White pigs, and the effects of reciprocal embryo transfer between the breeds on survival and fetal growth at day 30 of pregnancy. After adjustment for a breed difference in the time of ovulation, no evidence of differences in the rate of embryonic development between breeds was found. No overall difference between breeds in the amount of variability between embryos within females was observed, although there were hints of greater variation in between embryos from a few Large White females. After embryo transfer, no differences in survival between recipient breeds were observed, but lower survival in embryos from Meishan donors suggested that they were intolerant to the transfer procedures used. Fetal growth at day 30 was greater both in Large White embryos and in Large White gilts, but no significant interaction between breed of embryo and breed of gilt was observed.

INTRODUCTION

The Chinese Meishan pig has a larger litter size than any Western breed, with approximately 4 more piglets born alive than the Large White (Bidanel *et al.*, 1989; Haley and Lee, 1990). The Meishan could be of direct value in Western breed improvement programmes, but regardless of this, it could be of immense value as a tool for the identification of the mechanisms and ultimately the genes responsible for prolificacy. Crossbreeding studies have shown that the genes responsible act maternally (Bidanel *et al.*, 1989; Haley and Lee, 1990). Furthermore, although there is a difference in ovulation rate in favour of the Meishan, it has a higher level of prenatal survival at a given ovulation rate than the Large White and this is the main reason for its prolificacy (Haley and Lee, 1990).

The work of Bazer *et al.* (1988) suggests that Meishan embryos are less variable in size and more rapidly developing than Large White embryos between 8 and 12 days of pregnancy. Reduced embryonic variation could enhance prenatal survival by reducing asynchrony between the embryo and mother (Wilmut *et al.*, 1985; Pope, 1988). The study of Bazer *et al.* (1988) did not indicate the time at which Meishan embryos became less variable than Large White embryos and the work described here attempts to address that problem. Additionally, observations on embryos or crossbreeding studies cannot show whether maternal genes act to enhance prenatal survival via an improved quality or less variable ova, or via an improved *in utero* environment. Here we describe a reciprocal embryo transfer experiment in which the effects of embryo and dam on embryonic survival were measured.

MATERIALS AND METHODS

Experiment 1 - Fifteen Meishan and 10 Large White 2nd parity sows were examined for the onset of a natural heat at 08.00, 16.00 and 24.00 hrs. daily. Meishan sows were examined by laparoscopy for ovulation at 30, 40 or 50 hours after observed onset of heat, 5 animals at each time period. Large White sows were examined for ovulation 30 or 40 hours after the onset of heat, 5 animals at each time period. The data (ovulated or not ovulated) were analysed using generalised linear models assuming a binomial error as implemented in the GLIM computer package (Baker and Nelder, 1978). From this model the time at which 50% of females in each breed would have ovulated was predicted.

Experiment 2 - Meishan and Large White 2nd parity sows were examined for the onset of a natural heat at 08.00, 16.00 or 24.00 hrs daily.and mated to Large White boars at the onset of heat and 16 or 24 hours later. Sows were slaughtered between 18 and 60 (group A) or between 140 and 220 hours (group B) after the estimated time of ovulation. Embryos were recovered 1 to 6 hrs after slaughter.

The developmental stage of each group A embryo was recorded on an 11 point scale (i.e. 1 = early post-fertilisation 1 cell; 2 = early pro-nuclear 1 cell; 3 = late pro-nuclear 1 cell; 4 = syngamy; 5 = 2 cell; 6 = 3-4 cell; 7 = 5-8 cell; 8 = 9 cell to pre-morula 9 = morula; 10 = compact morula; 11 = early blastocyst). The data from each sow were categorised into developmental stages and were analysed using a generalised linear model with a poisson error as implemented in GLIM. To look for breed differences in developmental rate, the interactions between breed and developmental stage and between breed, developmental stage and estimated interval between ovulation and slaughter were included in the model. To look for breed differences in within female variation of developmental stage, the residual within sow deviation, after fitting a model including sow and its interaction with mean developmental stage of embryos in a sow was examined for each breed separately.

The maximum diameter of group B embryos was recorded and analysed, after transformation to logarithms to remove an association between mean and variance, using GLIM assuming a normal error. Effects fitted were breed, estimated interval between ovulation and slaughter and their interaction. The total within sow variance of log transformed maximum embryo diameter was calculated for each breed separately and the breed within female variances were compared as a ratio, with significance assessed against a two tailed distribution.

Experiment 3 - Forty Meishan gilts and 40 Large White x Landrace gilts aged 7-8 months were treated with 20 mg of allyltrenbolone (Regumate; Hoechst, UK) for 18 days to synchronize oestrus. Following cessation of Regumate, gilts were examined at 08.00 and 16.00 hrs daily for onset of heat. At oestrus, gilts were assigned to be embryo donors or recipients to achieve approximately equal numbers of transfer pairs in the four possible combinations of donor and recipient breed. Donor gilts were mated to boars of the same breed at 0 and 12 hrs after onset of oestrus. Embryos were recovered from Meishan gilts at day 5 (where day 0 is at the onset of oestrus) and from Large White gilts on day 4 using established procedures (Polge, 1982). This difference in timing allows for the observed difference in timing of ovulation (see results of experiment 1). Embryos were classified into developmental stages as were group A embryos in experiment 2 and transferred to non-mated recipients within 2 hours of recovery. Recipient gilts which had not returned to oestrus by day 30±1 were slaughtered and their reproductive tract recovered. Numbers of corpora lutea, total fetuses and viable fetuses were recorded, as was the volume of allantoic sac, crown-rump length and weight of each fetus.

Data on embryo developmental stage were analysed in the same way as for group A in experiment 1. For each donor/recipient pair, the estimated time between donor ovulation and transfer, the estimated asynchrony between the time of ovulation of donor and recipient and the mean developmental stage of the transferred embryos was calculated. The proportion of the transferred embryos present as viable fetuses at slaughter was analysed after arc-sine transformation to angles. Analysis was performed using GLIM assuming a normal error and including terms for breed of donor and recipient and their interaction, number of embryos transferred, mean stage of transferred embryos, estimated time after ovulation of donor at transfer and estimated asynchrony between donor and recipient. Analyses of fetal growth were performed using GLIM assuming a normal error and including terms for breed of donor and recipient and their interaction, mean stage of transferred embryos, day of pregnancy at slaughter and total number of fetuses in the same horn.

RESULTS

Experiment 1 - In the Meishan breed, no sows out of 5 had ovulated when examined at either 30 or 40 hours after onset of heat, all 5 had ovulated when observed at 50 hours. Two Large White sows out of 5 had ovulated when observed at 30 hours after the onset of heat and all 5 had ovulated at 40 hours after the onset of heat. Assuming the onset of heat occurred at the midpoint between the time at which it was observed and the last observation at which the sow was not on heat, the predicted time of ovulation of 50% of sows is 34.3 and 49.0 hours after the onset of heat in the Large White and Meishan breeds, respectively. These intervals were used when estimating the timing of ovulation relative to the onset of heat in experiments 2 and 3.

Experiment 2 - In group A, embryos from 9 Meishan sows and 13 Large White sows were assessed. The mean estimated interval between ovulation and slaughter was 37.0 ± 2.1 hours with no significant difference between breeds. There was no evidence of a difference between breeds in the developmental stage, nor of a difference between breeds in the relationship between estimated time after ovulation and developmental stage. The mean developmental stage of embryos from single females are shown plotted against estimated time after ovulation in figure 1. To reveal variation in embryo developmental stage within females, the deviation of the observed from the fitted values after fitting sow and the interaction between sow and the mean developmental stage of embryos from a sow, is shown in figure 2. There was little evidence of greater residual variation in Large White sows than in Meishan sows, but the two most deviant embryos were from Large White sows.

In group B, embryos from 14 Meishan sows and 7 Large White sows were assessed. The mean estimated interval between ovulation and slaughter was 161.7 ± 5.3 hours, with no significant difference between breeds. There was no evidence of a breed difference in log maximum embryo diameter or of the regression of log maximum embryo diameter on estimated time after ovulation. The overall regression of log maximum embryo diameter on estimated interval after ovulation was positive and significantly different from zero (0.027 ± 0.002). The mean log maximum diameter of embryos from each female is shown plotted against estimated time from ovulation in figure 3.

The total within female variance of log maximum embryo diameter was 0.041 (d.f. 223) and 0.072 (d.f. 73) in Meishan and Large White sows respectively, the variance ratio is 1.78 and highly significant (p < 0.001). Inspection of the data reveals that this result is largely due to a single Large White sow with a within sow variance of 0.360, over 3 fold of that of the next most variable sow (a Meishan sow). Omitting this Large White sow, the within Large White sow variance becomes 0.046 (d.f. 67), the variance ratio becomes 1.14 and non significant.

Experiment 3 - Embryos from 15 Meishan gilts and 17 Large White gilts were assessed. The mean estimated interval between ovulation and measurement was 71.5 ± 1.5 hours with no significant difference between breeds. There was no evidence of a difference between breeds in the developmental stage, nor of a difference between breeds in the relationship between estimated time after ovulation and developmental stage. The mean developmental stage of embryos from single females are shown plotted against estimated time after ovulation in figure 1. To reveal variation in embryo developmental stage within females, the deviation of the observed from the fitted values after fitting sow and the interaction between sow and the mean developmental stage of embryos from a sow, is shown in figure 4. There was little evidence of greater residual variation in Large White sows than in Meishan sows, although the few most deviant embryos were from Large White sows.

Fig. 1. Developmental stage of embryos from Experiment 2 Group A and Experiment 3.

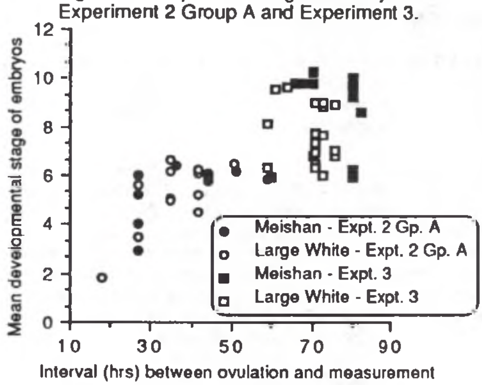


Fig. 2. Variation in embryo developmental stage within females in Experiment 2 Group A.

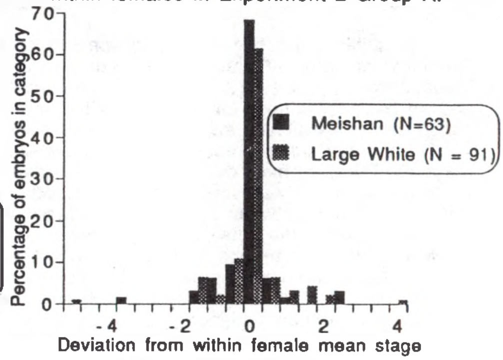


Fig. 3. Mean log maximum diameter of embryos from Experiment 2 Group B.

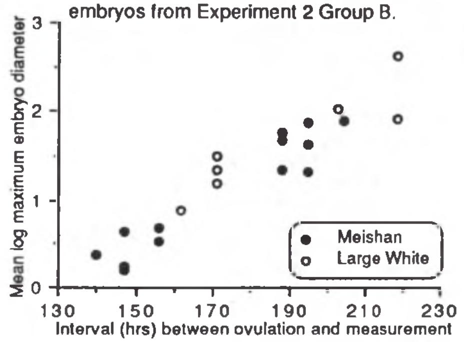
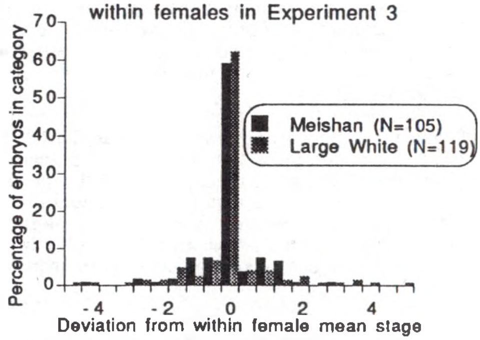


Fig. 4. Variation in embryo developmental stage within females in Experiment 3



Twenty (63%) recipient gilts remained pregnant until day 30. There was a significant effect of breed of donor on the proportion of embryos surviving in favour of the Large White. There was no effect of breed of recipient or interaction between breed of donor and recipient. The number and mean stage of embryos transferred, estimated time after ovulation of donor and estimated asynchrony between donor and recipient did not explain the observed effect of donor breed. The observed proportions surviving are shown in table 1.

Fetuses from Large White donors were longer with larger allantoic sacs than embryos from Meishan donors. Fetuses in Large White recipients were longer, heavier and had larger allantoic sacs than fetuses in Meishan recipients. There was no evidence for interactions between breed of donor and recipient. The effects of mean stage of embryos transferred, day of pregnancy and total number of fetuses in the same horn did not explain the observed breed effects. Observed fetal lengths, weights and allantoic sac volumes are shown in table 1.

Table 1 Fetal survival and fetal growth on day 30 of pregnancy after embryo transfer.

Breed of donor:	Meishan		Large White	
Breed of recipient:	Meishan	Large White	Meishan	Large White
Number of gilts	7	5	4	4
Observed proportion survival	43.3 ± 6.4	45.6 ± 8.4	70.8 ± 8.4	68.3 ± 7.5
Observed allantoic fluid volume (ml)	109.4 ± 6.7	113.5 ± 7.8	130.5 ± 7.5	174.4 ± 7.3
Predicted allantoic fluid volume (ml)*	116.7	126.0	128.8	171.3
Observed crown rump length (mm)	21.6 ± 0.3	23.6 ± 0.3	23.5 ± 0.3	24.8 ± 0.3
Predicted crown rump length (mm)*	22.2	23.5	23.2	25.6
Observed fetal weight (g)	0.76 ± 0.03	1.06± 0.04	0.96 ± 0.04	1.17 ± 0.04
Predicted fetal weight (g)*	0.84	1.04	0.91	1.12

*Predicted means after fitting of full model and adjustment for effects of covariates.

DISCUSSION

The difference between the Meishan and Large White breeds in the timing of ovulation relative to the onset of heat estimated in this experiment (14.7 hrs) is similar to that of 12 hrs reported by Martinat-Botte *et al.* (1989). We have attempted to adjust for the difference in the timing of ovulation and we find no evidence for a difference in the rate of development of embryos between breeds in the period between ovulation and 220 hour post-ovulation.

In all three studies reported there is some hint of greater variation within Large White females in the form of the few most deviant embryos in group A experiment 2 and in experiment 3 and one Large White female with a very variable embryos in experiment 3. However, there is no large general tendency for less variability between embryos within Meishan females than within Large White females, and it is uncertain whether the small effects observed could, if real, explain the overall difference in prenatal survival between the breeds. The effects are in any event much less than those observed by Bazer *et al.* (1988), who reported an approximately 4 fold difference between the breeds in the variance of embryos between days 8 and 11 of pregnancy. However, the report of Bazer *et al.* (1988) does not make it clear whether between sow variance was included in the estimate of between embryo variance which would tend to inflate the estimates and could introduce a bias.

The results of experiment 3 indicate lower survival of embryos from Meishan donors. The levels of survival of embryos from Large White donors (approximately 70%) are good, indicating no overall technical problem with the experiment and also no evidence of enhanced survival of embryos in a Meishan *in utero* environment. The results suggest that the Meishan embryos are intolerant of the routine embryo transfer procedures used, although why this is so is uncertain and it could be due to any one of a number of factors, from the medium used to incubate the embryos, to the use of Regumate to synchronise the gilts.

The observations on the size of the fetuses at day 30 suggest that both fetal and maternal factors influence the growth and development of the feto-placental unit. Inspection of the predicted means of the four groups in table 1 reveals that for allantoic fluid volume and fetal length the maternal and fetal effects contribute approximately equally to the total breed difference, whereas for fetal weight the maternal contribution is approximately 3 fold that of the (non significant) fetal effect. This compares with weight at birth, where maternal genetic effects explain virtually all of the breed difference (D'Agaro *et al.*, 1990). It seems possible that Meishan sows control the growth of their fetuses *in utero* in order to minimise fetal competion and consequent mortality in the latter part of pregnancy.

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