EFFECT OF THREE METHODS OF SELECTION FOR LITTER SIZE IN MICE ON PREIMPLANTATION EMBRYONIC DEVELOPMENT

By

SALIH ABDU AL-SHOREPY

Bachelor Of Science
King Faisal University
Al-Ahsa, Saudia Arabia
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Thesis Approved:

ARCHIE C.

Thesis Adviser

David S. Buchanan

Rodney D. Geisert

Thomas C. Collins

Dean of the Graduate College

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NOMENCLATURE

OR	Ovulation rate
PB	Percentage of blastocyst
PD	Percentage of degenerating embryos
PEB	Percentage of early blastocyst
PEC	Percentage of eight-cell
PEXB	Percentage of expanded blastocyst
PFC	Percentage of four-cell
PM	Percentage of morula
PTC	Percentage of two-cell
PWFO	Percentage of unfertilized ova
STAGE	Stage of embryo development
STD	Standard deviation

CHAPTER I

INTRODUCTION

Increasing prolificacy, or litter size at birth, is a major avenue for improving animal productivity. Increasing the number of young produced in a pregnancy was cited by Tess et al. (1983) as the most important factor in decreasing the cost of producing marketable body weight in swine. Direct selection for increased litter size in swine was unsuccessful in the one long-term study completed (Olivier, 1982). Ollivier found no significant response to direct selection for litter size in swine after 11 generations . Mice have been used as a laboratory model for litter-bearing species. Experiments with mice have spanned more generations and selection for litter size has been more successful. Litter size was increased 1.6 pups after 31 generations of selection by Falconer (1960, 1971) and resulted in a realized heritability of .15. Bakker (1978) reported an increase of 6 pups with a realized heritability of .11 after 29 generations of selection. In sheep, intense screening of the population for prolific ewes has had significant response in some studies (Turner, 1978).

The number of young born is determined by several factors. The number of ova shed, the proportion of ova fertilized and the proportion of fertilized ova resulting in viable offspring are components of litter size. Fertilization rate in swine and mice is high and does not significantly influence variation in litter size. Selection for the ratio of pups to ovulation rate, weighted by the number of pups , was successful in increasing both prenatal survival and litter size in mice (Bradford, 1979). Direct selection for ovulation rate in swine increased the number of ova shed, but did not result in a corresponding increase in litter size (Johnson et al., 1984). Johnson et al. (1984) suggested selection on an optimum index of ovulation rate and embryo survival which would place different emphasis on the components than the natural index of litter size. The index was derived so that ovulation rate was expected to increase while prenatal survival remained constant. Selection for number born from unilaterally hysterectomized-ovariectomized gilts has been hypothesized as a method for improving genetic merit for uterine capacity (Christenson et al., 1987). Clutter et al. (1990) and Gion et al., (1990) reported significant response when they evaluated those alternative methods of selection with mice.

Understanding genetic variation associated with physiological aspects of number born will help develop new selection methods. Variation in stage of embryonic development and delayed embryonic development have been suggested as sources of embryonic loss (Wilmut et al., 1986; Pope et al., 1988). Several studies have found genetic differences among inbred mouse strains in the time of the first cleavage division (Niewa et al., 1980; Shire and Whitten, 1980), and a line of mice selected for increased litter size had a greater percentage of advanced embryos on the second day of gestation (Durrent et al., 1980). Alleles of a single gene that influences the rate of embryonic development in mice were associated with larger litter size (Warner et al., 1991).

The purpose of this study was to determine if lines of mice with varying genetic merit for litter size differ in stage of, or uniformity within a litter for, embryonic development prior to implantation. With the results from this study, it may be possible to determine the effects of genetic selection for litter size or its components (e.g. ovulation rate, uterine capacity) on characteristics of embryonic development to the time of implantation.

CHAPTER II

LITERATURE REVIEW

Selection for Litter Size

Direct Selection: Swine

Few studies of direct selection for increased litter size in the pig have been published. In a study by Rutledge (1980), three lines were selected for increased litter size within full-sib families. One line was standardized to six pigs per litter within 24 h of birth (S) and one was not standardized (N). Males were randomly selected. An unstandardized, randomly selected control (C) was used. After three generations of selection, mean litter size was 11.3, 10.2 and 10.8 for S, N and C, respectively.

Ollivier (1982) reported results from a study in which direct selection for litter size was practiced for 11 generations. A response of 0.15/ pigs/ litter/ generation was achieved in the first five generations of selection within sire families for large litter size. The line consisted of 80 females and 8 males per generation from a purebred Large White population. However, this response did not continue in subsequent generations. The genetic change after 11 generations was zero. Ollivier cited lower heritability and delayed puberty contributing to a low selection intensity as a possible reason for the lack of continued response to selection.

Direct Selection: Mice

Direct selection for litter size in the mouse has been successful in several studies. In a study by Bradford (1968), five closed lines were generated from a four-way cross of inbred lines. Mass and intra-litter selection for litter size (lines S_1 and S-IL, respectively) were compared to an unselected control (line C). Also, mass selection for litter size in superovulated females (line S-T) was compared to a randomly selected, superovulated control (line C-T). In addition, an untreated line selected for larger litter size (S_2) was generated from an eight-way cross of inbred lines.

The lines initiated from the four-way cross decreased in litter size after one generation of selection. This decline might be attributed to the maximum degree of heterozygosity of the dams in the base population. There was significant response to selection for increased litter size in S_1 and S_2 lines through generation two (generation one equaled base generation). Realized heritabilities were $.13\pm.07$ and $.22\pm.06$ for S_1 and S_2, respectively. After did not continue in subsequent generations. The genetic change after 11 generations was zero. Ollivier cited lower heritability and delayed puberty contributing to a low selection intensity as a possible reason for the lack of continued response to selection.

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Results from 15 generations of selection for large (high line) and small (low line) litter size in mice was reported by Joakimsen and Baker (1977). A randomly selected control was also maintained. In each line, 90 females and 30 males were mated each generation. From the analysis of the base population through generation 15, the paternal half-sib estimate of heritability was .15±.20 for total number born. At generation 15, divergent selection for number born in the high and low lines resulted in a difference of 7.6 pups between the two lines. It was observed that during generation 8 through 15, there was a continued increase in the high line while the change in the low line was found to be not significant.

Eisen (1978) conducted a study of direct selection for litter size as a part of larger study. The best 20 females were chosen in each selection line based on total number of pups born. The control line was maintained by randomly selecting one male and one female from each full-sib family. After sexing, litters were standardized to eight young at 1 day of age. Litter size was recorded as the number of fully formed pups. The heritability for number born estimated from the regression of offspring on dam, pooled over lines, was .17±.04. Estimated heritability for number born from the intra-class correlation among full-sib families, pooled over lines, was .11±.02. The realized direct response in litter size through generation 12 was significant. An increase of approximately .3 pups per generation or a total response of 1.5 phenotypic standard deviations was observed. Realized heritability for litter size after 11 generation of selection was .19±.04.

Bakker et al. (1978) reported results from 29 generations of direct selection for increased litter size in mice. In each generation, the 24 largest litters were selected. Litters were not standardized. The change in litter size, measured as a total dead and alive, from 29 generation of selection was 6 pups. The realized heritability was .11±.01. Greater litter size in the selection line was mainly caused by greater ovulation rate, while lesser pre- and post-implantation losses also contributed to the difference.

Selection on the Components of Litter Size

The two major components of litter size are ovulation rate and prenatal survival. Alternatively, litter size may be considered to be determined by the number of potentially viable embryos and uterine capacity (Bennett and Leymaster, 1989). Uterine capacity was defined by Bennett and Leymaster as the number of potentially viable embryos that the female can carry from ovulation to birth. When the number of potentially viable embryos exceeds uterine capacity, reduction is seen as competition between embryos / fetuses for limited uterine resources. Variation in these components contribute to variation in number born.

Selection for Ovulation Rate: Swine

Experiments selecting for ovulation rate have been conducted to measure the correlated response in litter size. Zimmerman and Cunningham (1975) reported the results of five generations of selection for ovulation rate in a population of swine composed of fourteen breeds. Two lines (control and select) were established. In both lines, ovulation rate was measured by counting corpora lutea nine to eleven days following the second estrus. Based on ovulation rate, replacement gilts in the select line were chosen by mass selection. Replacement gilts in the control line were randomly chosen from each ovulation rate class in proportion to the number of gilts in that class. Replacement boars were chosen randomly in both lines and matings were made such that inbreeding was minimized. At generation zero, ovulation rate of the control line exceeded that of the select line by .2 ova. The ovulation rate of the select line exceeded that of the control by 2.5 ova in generation five. The regression of the line mean on generation number was .40 and .16 for the select and control lines, respectively. Realized heritability, calculated by using the regression of the line difference in each generation on the cumulative weighted selection differential, was .52±.10.

Cunningham et al. (1979) reported the results of continued selection for ovulation rate through generation nine as well as correlated change in litter size. Selection was based on corporalutery (CL) counts at second estrus. At generation nine, mean ovulation rate for the select line exceeded the control line by 3.71 ova, and the regressions of line mean on generation number was .30 for the select line and .14 for the control line. Realized heritability was .42±.06. However, there were no significant differences between the lines for total number born, number born alive, number weaned, litter birth weight, or litter weaning weight. Realized genetic correlations of ovulation rate with the measures of litter size were small and nonsignificant. The author ruled out failure to maintain the ovulation rate advantage at subsequent estrous cycles as a cause for the lack of correlated response in litter size.

Commenting on this study, Johnson et al. (1984) cited higher embryo and/or prenatal loss in the select line as failure for an increase in litter size. Embryo survival rate, calculated from line means for ovulation rate and assuming 100% fertilization, declined 1.6±0.05% per generation. Ninth generation gilts and sows and tenth generation gilts were slaughtered at either 30 or 70 days of gestation. The select line had more live fetuses than did the control females. The difference was about one-half of the select line's superiority in ovulation rate. The select line averaged from 5.4 and 10.6% lower embryo survival at 30 and 70 days, respectively.

Selection for Ovulation Rate: Mice

Extensive studies of selection for ovulation rate have been conducted in mice. Selection for high and low natural ovulation rate was studied by Land and Falconer (1969). Females were mated at six to eight weeks of age and ovulation rates measured after the first litter of a female was weaned. The lines included a line selected for high natural ovulation rate (HN), a line selected for low natural ovulation rate (LN), and a randomly selected control (C). Selection was within full-sib families. At generation 12, ovulation rate had changed from about 16 eggs to 21 in the HN line and 14 in the LN line. The realized heritability for the divergence was .31. The litter size in the select lines did not differentiate. However, lower litter sizes were observed in the selected lines than the control in the last five generations of selection. The authors hypothesized that the LN line declined in litter size in accordance with the decreased of ovulation rate, while the HN line must have experienced an increase in prenatal loss to the extent that it more than offset the increase in ovulation rate.

Selection for ovulation rate in mice was studied concurrently with selection for litter size (Bradford, 1969). All female progeny up to four per litter from first litters were mated to non-littermate males from their own line. At d 16 of gestation, the following data were collected: corpora lutea count in the live animal (CL), implants (I) and normal fetuses (N). Males and females from the dam's second litters were then selected on the basis of an index of full- and half-sister information for CL number. A control line was also maintained. The realized heritability for ovulation rate was slightly less than .10 after ten generations of selection. Litter size was not significantly different from the control, despite an increase in number of implantations paralleling the increase in number of CL. Ovulation rate in the select line was four to five ova greater than the control after 15 generations (Bradford, 1979). During the last five generations of selection for ovulation rate and the following 15 generations of random selection within litters, litter size increased slowly in a significant manner. It was suggested that natural selection among embryos after implantation had improved prenatal survival.

Pomp et al. (1988) analyzed the results from the study conducted by Eisen mentioned earlier to determine the genetic correlation between increased litter size and ovulation rate through 23 generations. It was observed that selection for increased litter size resulted in an increase of 8.5 ova in the selection line relative to the control line. In other words, selection for large litter size led to a correlated increase in natural ovulation rate.

Selection for Increase Prenatal Survival
: Mice

Selection for embryo survival weighted by number of normal fetuses (N) at day 16 of gestation (N^2/CL) in naturally ovulating females (line E) and superovulated females (line E-T) was carried out concurrently with the study described above by Bradford (1969). N^2/CL selection criterion was used as an attempt to increase embryo survival without affecting ovulation rate. Embryo survival in line E, measured by N/CL, increased from .81 in generation 1 to .90 in generation 11. Net increase of ovulation rate in line E was similar to that in the line selected for natural ovulation rate. As a result, litter size in this line increased more than 25% that of the control. Line E-T responded positively to direct selection in litter size. In this line, treated and untreated second-litter females increased $.13 \pm .07$ and $.10 \pm .12$ pups per generation, respectively.

Selection on an Index

Johnson et al. (1984) described an ovulation rate-embryo survival model in swine. The authors suggested selection on an optimum index of ovulation rate and the proportion ova resulting in offspring as a more efficient method of improving genetic merit for litter size in swine. Their calculations indicated that the natural index of litter size puts less than optimum weightings on these two components. The optimum index they derived was expected to increase ovulation rate, while maintaining prenatal survival. Thus, litter size would increase with the increase in ovulation rate without being negated by a large decrease in prenatal survival.

Neal (1987) reported results from five generations of selection in a line that was a composite population of the Large White and Landrace breed of swine using an index of I = 10.6 x (ovulation rate) + 72.6 x (embryo survival). Laparotomy was performed at day 50 of gestation to count both corpora lutea and fetuses. The 43 highest ranking of 150 females were allowed to farrow, while the remainder were culled. Sire were selected from the 15 highest indexing females. The control line averaged 42 litters by 15 sires per generation. Litters were standardized to 10 pigs when possible. After 5 generation, the index line averaged 2.97 more ova than the control. Regression of the difference between means of the lines on generation number were .57±.11 for ovulation rate, -.13±.0096 for embryo survival, .20±.20 for number of fetuses at 50 day of gestation and .19±.14 for total number born. The realized heritability of the index was .30±.09. The authors concluded that the index selection was effective in increasing ovulation rate, while number of fetuses at day 50 of gestation and number of pigs at birth increased but not in a significant manner. The decline in prenatal survival rate did not completely offset the increase in ovulation rate.

Selection on Uterine Capacity

Christenson et al. (1987) hypothesized that the size of the natural litter does not reflect the female's ability for carrying a large number of viable offspring when ovulation rate is not limiting. Therefore, they proposed the use of unilateral hysterectomy-ovariectomy (UHO) in swine to allow estimation of uterine capacity per uterine horns unconfounded by ovulation rate. An experiment was conducted to evaluate the hypothesized model. Gilts were assigned randomly to control or UHO on day 8 to 12 following first observed estrus. Gilts within each group were assigned to be either slaughtered at day 86 of gestation or allowed farrow. Gilts assigned to farrow were observed by laparotomy to count corpora lutea at about day 40 of gestation.

Ovulation rate in UHO gilts was similar to that of the control gilts. Litter size of UHO gilts was 5.7 pigs compared to 9 pigs in the control. The regression of litter size on ovulation rate, averaged over time of ovulation, was .51 and .24 pigs/CL for control gilts and UHO gilts, respectively. In UHO gilts that were farrowed, ovulation rate accounted for only .4% of the variation in litter size. Therefore, the UHO procedure was successful in decreasing the effect of ovulation rate on litter size.

To address the concern that UHO gilts simulates the litter size per uterine horn of intact gilts, data for ovulation rate and litter size on UHO gilts that were farrowed were multiplied by two to scale the bivariate distribution to a total uterine basis. Comparison of linear regression equations predicting litter sizes from ovulation rates for the raw and scaled data implied that UHO gilts simulated one-half the litter size of intact gilts with doubled ovulation rates.

Bennett and Leymaster (1989) developed a potentially viable embryo-uterine capacity model and discussed its ramifications. The model assumed that the number of eggs was reduced to the number of potentially viable embryos by factors inherent to the ovum and embryo. Furthermore, the number of potentially viable embryos that the female can carry successfully is uterine capacity. Consequently, litter size is the minimum of these two components in the model. This model is based on ovulation rate, the probability of an ovum becoming a potential embryo and uterine capacity. It was suggested that dealing either with ovulation rate or uterine capacity independently will not achieve large changes in litter size. As a result, they proposed that a hormonal manipulation, single genes or a nutritional regime would be unlikely to increase litter size and that combinations of factors are needed to increase litter size.

Evaluation of Alternative Selection Methods: Mice

An experiment evaluating alternative method of selection to increase litter size was reported by Clutter et al. (1990)and Gion et al. (1990). A base population of outbred mice was used to generate lines for implementation of the following selection criteria:

LS: Direct selection based on number born.

IX: Selection based on an index of ovulation rate and ova success (ratio of number of pups to number of ova).

UT: Selection based on number born in females that underwent unilateral ovariectomy at four weeks of age. This criterion was designed to serve as a model for selection on uterine capacity.

LC: An unselected control.

Besides generating lines for the selection criteria described above, additional pairing of base stock were made to produce full- and half-sib families. Their daughters were used to provide data for estimating parameters in the base population to construct an index to be used in the IX line. Females were sacrificed at day 17 of gestation and the following data were collected: ovulation rate in both sides, number of fetuses in the left and right uterine horns and corresponding ova success. Results revealed that ovulation rate, litter size and ova success were all higher in the right side than in the left side. Heritability estimates were .18, .33 and .15 for litter size, ovulation rate and ova success, respectively. The linear index, derived for a quadratic definition of breeding value, was used in the IX line. The linear index was I= (1.21 x total ovulation rate (TOR) + 9.05 x total ova success(TOS)) was chosen for use.

To determine the best procedure to be used in the UT line, data were also collected. Ovulation rate in unilaterally ovariectomized females was 95% of that in females with both ovaries. It was concluded that unilateral ovariectomy provides a physiological state to measure and practice selection for uterine potential in the mouse.

Selection criteria described above were applied in three replicates. Within each selection line (IX, LS, and UT), litters of the 16 highest ranking dams were chosen as breeders for each generation. In the LC line, one female and one male were chosen from each of the 16 litters. After 13 generations , the advantage in total fetuses of IX, LS and UT over LC was 1.97, 1.66 and .79, respectively. The regression of breeding value for litter size in each line, which was estimated as response in the generation 13 evaluation divided by cumulative realized selection differential, was .11±.02, .08±.01 and .05±.03 for IX, LS and UT, respectively. Regression of response in number born on generation number was .17±.01, .15±.04 and .10±.02 for IX, LS and UT, respectively.

Physiological Determinants of Litter Size

Uterine Capacity and Embryo Survival : Swine

Several studies have addressed sources of variation in uterine capacity and embryo survival. Dziuk (1968) altered the uterine space available per embryo in swine by varying the number of embryos or the length of the uterus in the gilts he studied. This author found that embryo survival was generally unaffected by increasing or decreasing the uterine space per embryo relative to control. Only when the average number of embryos was at least 14, did it appear that intra-uterine crowding became a possible limiting factor in embryonic survival. In other words, intra-uterine crowding is unlikely to play an important factor in early embryonic losses under normal conditions.

In a study by Wu et al. (1989), a number of pregnant gilts were laparotomized at day 3 of gestation, uterine horn dimensions were measured and corpora lutea (CL) were counted in order to determine uterine space needed for each embryo. It was found that each fetus surviving to day 50 of gestation was associated with 36 cm of initial uterine length , but fetal survival was not associated with number of CL. It was concluded that when the number of ovulations exceeded the maximum capacity of the uterine horns, the number of fetuses was correlated with initial uterine length but not with the number of CL.

The relationships between the length of the uterine horn, the number of fetuses and prenatal mortality were studied by Wu et al. (1987). Gilts were sacrificed at 3,5,7,9,11,13 and 15 weeks of gestation. The length of each horn, the number of fetuses in each horn and number of CL in each ovary were measured. Embryonic loss was determined by the proportion of the number of missing fetuses relative to the number of CL. The relationship between length of a single uterine horn and fetuses at 13 to 15 weeks of gestation was found to be constant. That is, with each additional fetus the uterus increased about 10 cm in length. As the number of CL increased, prenatal mortality also increased. It was also observed that an increase in CL was accompanied by increase in live fetuses up to 14. It can be concluded from this study and others (e.g. Blichfeldt and Almid, 1982) that uterine length seems to be an important limiting factor to litter size in pigs when number of ovulations is high.

Bazer et al. (1988a, 1988b) studied the conceptus development from widely differing breeds of pigs, Large White and Chinese Meishan, on days 8, 10, 11, 12, 14 and 30 of gestation. Meishan pigs expressed an earlier age of puberty, a greater number of piglets born and less prenatal mortality than Large White pigs. It was observed that the Meishan had smaller (mass and dimensions) uteri than Large White. But the greater weight of Large White uterus might account for much of this difference. Despite the fact that Chinese Meishan pigs had smaller uteri, they had 2.3 more fetuses at day 30 of gestation coming from 4.4 fewer ovulations. The Meishan embryos developed faster and with less variation than the embryos of Large White. This may explain some of the differences in embryonic survival between breeds.

The distribution and development of pig embryos in relation to the number of embryos and their positions within the uterine horn was investigated by Anderson and

Parker (1976). It was found that reduced embryo and placental development during prenatal period was unrelated to location of the embryo within the uterine horn.

Fetal and Maternal Interaction: Swine

Pope (1988a) has reported that the degree of asynchrony between the uterus and the embryo at the time of implantation is the cause of considerable prenatal mortality; less developed embryos are likely candidates for embryonic loss. Willmut et al. (1986) suggested that the variation in the stage of preimplantation development among embryos contributes to embryo loss. As a result, the more advanced embryos have increased potential to survive at the expense of those that are less advanced.

Variation in stage of embryo development within a litter may be due in part to variation in time of ovulation. In an attempt to investigate the effect of embryonic uniformity in crossbred gilts, Pope et al. (1988b) compared the morphological variation among littermate embryos on day 11 in sham-operated control gilts and in gilts whose non-ovulated follicles were destroyed by electrocauterization. It was observed in this experiment that cauterizing 6 to 8 mm follicles in gilts with both follicles and corpora hemorrhagica (CH) resulted in more embryonic uniformity than in gilts with only CH. These authors suggested that gilts seem to ovulate the majority of their follicles during a short period of time, with the rest following over a relatively longer period of time. Smaller embryos within a litter may result from the later-ovulating follicles, accordingly, the small littermate embryos are likely candidates for loss, especially under competition.

In regard to the hormones that influence uterine function, Wild et al. (1988) reported that more advanced pig blastocysts produced a greater amount of estradiol compared to less advanced blastocysts; this may create quick uterine response to allow more developed embryos to survive at the expense of less developed littermate embryos. In other words, increasing the degree of uniformity perhaps reduces the likelihood of embryonic loss, and accordingly may increase litter size.

Fetal and Maternal Interaction : Mice

Gates, (1965) found that there was a considerable variation in development among mouse eggs by the time they reached the uterus. The most retarded eggs recovered were about one day behind the most rapidly developed eggs. This delay could be enough to cause asynchrony with that of the uterine endometrium, consequently this may lead to embryonic mortality. Variation in the stage of embryo development due to the genotype of the embryo has been investigated in the mouse and found to be associated with alleles of the preimplantation embryonic development (Ped) gene (Warner et al., 1987). Galdbard and Warner (1982) found that the Ped gene in the mouse influenced the time of first cleavage division. However, no influence was detected on time of fertilization nor time of ovulation. Ped gene alleles that resulted in increased rate of development have more recently been associated with larger litter size and heavier litter weight (Warner et al., 1991).

Inbred strains of mice differed in the time of first cleavage of embryos (McLaren and Bowman, 1973). Five strains of mice belonging to the randomly bred Q strain and to the inbred strains C57BL/McL, C3H/BiMcL, JU/Fa and RIII/Fa were used. On the fourth day of gestation, embryos were recovered and examined with a dissecting microscope. There was a significant difference between strains both in the percentage of embryos which reached the blastocyst stage and the number of cells per embryo. C57BL embryo had most cells while C3H/BiMcL had the fewest. These authors found that the difference in cell number per embryo in these two lines did not depend on a difference in time of mating nor on the genotype of the male, but was the result of an advantage of some four hours in the time at which cleavage begins. Niwa et al. (1980) found that there was a significant difference among the four strains of mice tested in the proportion of eggs cleaved 20 h after the time of insemination, illustrating the fact that strain differences also exist in the time of the first cleavage.

Fetal and Maternal Interaction: Sheep

Wilmut et al. (1985) reported that since many of the factors that lead to embryo loss also influence progesterone secretion in sheep, the level of progesterone and the timing of changes in the levels may well be an important means by which embryo loss arises. Furthermore, observations made by Quirke et al. (1979) on ewes have shown that progesterone level during the estrous cycle were higher in ewes with two ovulations than those with only one. Subsequently, there might be a greater chance of asynchrony between the dam and her embryos due to the change in the level of progesterone.

Literature Review Summary

Direct selection for increased litter size in swine was successful in the first few generations of selection in the two studies reviewed here. However, the only study to have proceeded through 11 generations was not successful in increasing litter size. Direct selection in mice has been successful. Evaluations of selection response has indicated that increases in number born is a function of increased ovulation rate with some decrease in embryo loss.

Therefore, selection response in number born can be explained primarily by decreased embryonic survival. Litter size at birth is a function of its components, such as ovulation rate and ova success, and the variation in these components has been evaluated. Successful selection for ovulation rate has been reported in mice and pigs; however, the increase in ovulation rate did not necessarily result in an increase in litter size, mostly due to an accompanying increase in prenatal loss. Selection for decreased prenatal mortality was successful in mice and resulted in an increase in litter size.

Selection on an index of ovulation rate and embryo survival in swine increased ovulation rate, but embryo survival was lower than expected. Selection for number born from unilaterally hysterectomized-ovariectomized gilts and mice has been proposed as a method for putting selection emphasis on the ability to produce a large number of viable offspring when ovulation rate is not a limiting factor. This method is applied more easily in the mouse due to the fact that the mouse has a duplex uterus which doesn't allow transuterine migration. Index selection and selection for uterine capacity resulted in significant response in mice.

Source of variation in uterine capacity and embryo survival have been addressed by a number of studies. Variation in stage of embryo development was suggested as a source of losses. The more advanced embryos survive at the expense of those that are less advanced. Variation in stage of embryo development within a litter may be due in part to variation in time of ovulation. In the pig, variability in preimplantation development of littermate embryos may contribute to embryonic loss. Studies have found that inbred strains of mice differed in time of first cleavage division and alleles of a single gene that influences the rate if embryonic development in the mouse was associated with larger litter size.

CHAPTER III

EFFECT OF THREE METHODS OF SELECTION FOR LITTER SIZE IN MICE ON PREIMPLANTATION EMBRYONIC DEVELOPMENT

Introduction

Litter size is determined by the number of ova shed, fertilization rate, and pre- and post-implantation embryonic mortality. Direct selection for increased litter size in mice was successful and resulted in an increase in ovulation rate (Falconer, 1960; Joakimsen and Bakker, 1977). A decrease in prenatal survival was responsible for response to selection for small litter size in mice (Batman, 1966; Joakimsen and Bakker, 1977). It was concluded in studies involving the crossing of selected lines of mice that the two components of embryonic survival, pre- and post-implantation viability, are controlled by different genes (Bradford and Nott, 1969). Response to direct selection for litter size in swine in the one study to proceed 11 generations was relatively unsuccessful (Ollivier, 1982).

Selection on an index of ovulation rate and embryo survival was suggested as a more efficient method of improving genetic merit for litter size in swine (Johnson et al., 1984). Number of fetuses sustained following unilateral hysterectomy-ovariectomy in gilts has been suggested as a physiological model to measure uterine capacity independent of ovulation rate (Christenson et al.,1987). A significant response was found when those alternative methods of selection were evaluated in mice (Clutter.et al., 1990; Gion et al., 1990)

Understanding of genetic variation associated with physiological components of litter size is needed to design optimal selection strategies. Delayed embryonic development due to the genotype of the embryo has been observed in the mouse (Warner et al., 1987) and is associated with embryonic loss (Gates, 1965).

The purpose of the present study was to determine if lines of mice with varying genetic merit for litter size differ in stage of, or uniformity within a litter for, embryonic development prior to implantation.

Materials and Methods

Source of Animals

Animals for the present study were sampled from a population of mice at the University of Nebraska (Clutter,

1986) in which alternative methods of selection for increased litter size and its components were evaluated. The selection criteria compared were:

IX: Selection based on an index of ovulation rate and ova success (number born/ovulation rate) as suggested by Johnson et al. (1984)

UT: Selection based on number born in females that had undergone unilateral ovariectomy at four weeks of age. This was designed as a model for selection on uterine capacity in the pig as described by Christenson et al. (1987).

LS: Direct selection for total number born. This selection represented the classical method of selection for litter size and served as a comparison for determining the relative effectiveness of alternative methods.

LC: Unselected control.

Each of the criterion described above were replicated three times, with all four selection criteria contemporary within a replicate. Lines used here refers to a replicate-criterion population.

Females evaluated in the present study reflected 21 generations of selection on the above criteria. Following generation 21, the selection was relaxed and lines were maintained with 32 mating pairs in each generation in the Animal Science Small Animal Laboratory at the University of Nebraska. For the present study, one to two females per litter were randomly sampled from each line in all three replicates of generation 27. Following weaning at three weeks of age, mice were transported to the Animal Science Department at Oklahoma State University for the remainder of the study.

Animal Management and Data Collection

Animals had ad libitum access to a rodent block diet containing 24% crude protein, 4% fat and 4.5% crude fiber. Automatically timed lights came on at 700h and off at 1900h. Animal room temperature was maintained at approximately 24°C. Females were mated at approximately nine weeks of age with males from the same line. An attempt was made to minimize inbreeding when assigning females to a male. Each mating cage contained one male and three to four females.

Females were checked daily for mating plugs and mating dates were recorded. Observation of a mating plug was regarded as day 0 of gestation. Females were sacrificed at day 3.5 of gestation via cervical dislocation. Left and right uterine horns were immediately transferred separately to a clean petri dish containing a small amount of Saline-NaN_3 (.15 M NaCl). Left and right ovaries were also transferred to separate dishes containing small amounts of Saline to prevent drying.

Once the excess fat was removed from the uterine horn and a small part of the upper part of the uterine horn was cut to allow a 5-ml syringe to be inserted, embryos were flushed into a small petri dish with 2 ml Saline. Embryos were then examined under a 150x magnification microscope and classified according to cleavage stage as follows: two-cell embryo, four-cell embryo, eight-cell embryo, morula, early blastocyst, blastocyst or expanded blastocyst (Table 1). Unfertilized ova and degenerating embryos were also identified.

Ovulation rate was determined by counting corpora lutea. After removing the bursa and excess fat, corpora lutea were counted with the aid of dissection and 10x magnification.

Statistical Analysis

Earlier evaluations of this population have revealed a greater ovulation rate, ova success and number of fetuses at term on the right side of the reproductive tract than on the left side (Clutter et al., 1990). Estimates of phenotypic and genetic parameters associated with litter size and its components in the base population and responses to selection in IX, LS and UT have also varied with side of the uterus (Clutter et al., 1990; Gion et al., 1990, respectively). Consequently, analyses in the present study were conducted by uterine side using the model shown

TABLE 1

EMBRYO/OVA CALSSIFICATION

		Cleavage stage						
		1	2	3	4	5	6	7
Unfertilized ovum	Degenerating embryo	Two-cell embryo	Four-cell embryo	Eight-cell embryo	Morula	Early blastocyst	Blastocyst	Expanded blastocyst

in Table 2. Degrees of freedom for the effect of criterion were partitioned to test the orthogonal comparisons of selection to a control (mean of LS, IX, and UT vs LC) selection for litter size to that for a single component of litter size (Mean of LS, and IX vs UT) and direct selection to index selection (LS vs UT). Replicate effects were considered random and comparisons were tested using the criterion X replicate interaction as the error term.

To test the effect of selection criterion on the average distribution of embryos within a uterine horn among the pre-implantation stages of development, proportions of embryos in each stage were converted to an angular scale using a weighted arc sine transformation (Mosteller and Youtz, 1961). The transformation was designed to provide a valid analysis of variance for proportions with respect to the underlying statistical assumptions and was weighted to account for differing numbers of total embryos. The resulting transformed values were analyzed by using both univariate and multivariate analysis of variance (MANOVA) procedures with the model and contrasts described in Table 2. The MANOVA procedures provided comparisons of the average distributions for the criteria.

The effect of selection on average developmental stage in each horn was also studied. Two-cell embryo through expanded blastocyst stages were assigned sequential scores Table 1, and each embryo within a class was givin the

TABLE 2

STATISTICAL MODEL AND CONTRASTS

Effect	Contrast of criterion effects		
Selection criterion(S)[1]			
Replicate(R)	$\overline{\text{LS, IX. UT}}$	vs	LC
SxR	$\overline{\text{LS, IX}}$	vs	UT
Residual	IX	vs	LS

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[1] Tested using S x R as the error term.

corresponding score (e.g. blastocysts were each given a score of 6). Mean stages were analyzed with the model and contrasts in Table 2. Within uterine horn, standard deviations for developmental stage were calculated to quantify variability among littermate embryos under each of the selection criteria.

Proportions of recovered ova and embryos from each uterine horn that were unfertilized ova and degenerating embryos were converted to an angular scale using the weighted arc sine transformation described above. The resulting transformed values for each trait were analyzed with least-squares procedures using the model and contrasts in Table 2.

Results

Data were collected from a total of 424 female mice (Table 3). Least squares means for ovulation rate (OR), embryonic developmental stage (STAGE), and standard deviation (STD) for developmental stage by selection criterion and uterine side are presented in Table 3.

Means for ovulation rate are present graphically in Figure 1. Mean percentages of two-cell (PTC), four-cell (PFC),eight-cell (PEC), morula (PM), early blastocyst (PEB), blastocyst (B), and expanded blastocyst (PEXB) are presented in Figures 2 and 3 for each selection criterion by side of the uterus and in figures 4 through 9 for each

TABLE 3

LEAST SQUARES MEANS FOR OVULATION RATE, AVERAGE DEVELOPMENTAL STAGE AND STANDARD DEVIATION IN STAGE OF DEVELOPMENT BY SELECTION CRITERION AND UTERINE SIDE

		OR		STAGE		STD	
Line	n[a]	Left side	Right side	Left side	Right side	Left side	Right side
LS	113	8.27(.22)[b]	8.94(.17)	5.51(.19)	5.48(.23)	.77(.06)	.77(.03)
IX	105	7.48(.23)	8.79(.18)	5.58(.20)	5.46(.23)	.74(.06)	.83(.03)
UT	116	7.13(.22)	7.88(.17)	5.41(.19)	5.56(.22)	.83(.06)	.74(.03)
LC	87	6.22(.25)	7.21(.20)	4.94(.22)	5.09(.26)	.81(.07)	.88(.04)

[a] Number of females measured

[b] Standard error of the mean in parentheses

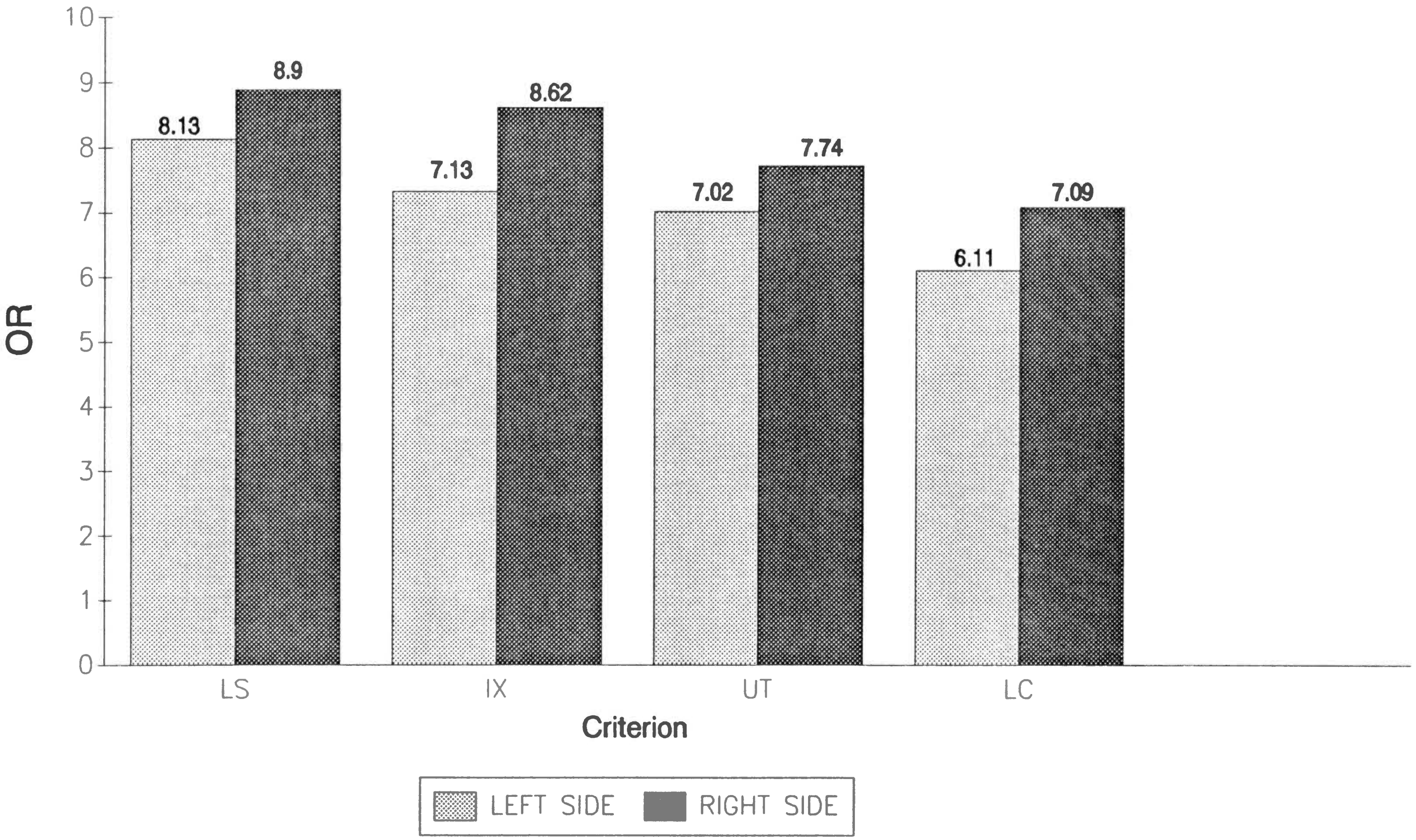

Figure 1. Mean Ovulation Rate (OR) for each Selection Criterion by Side of the Uterus.

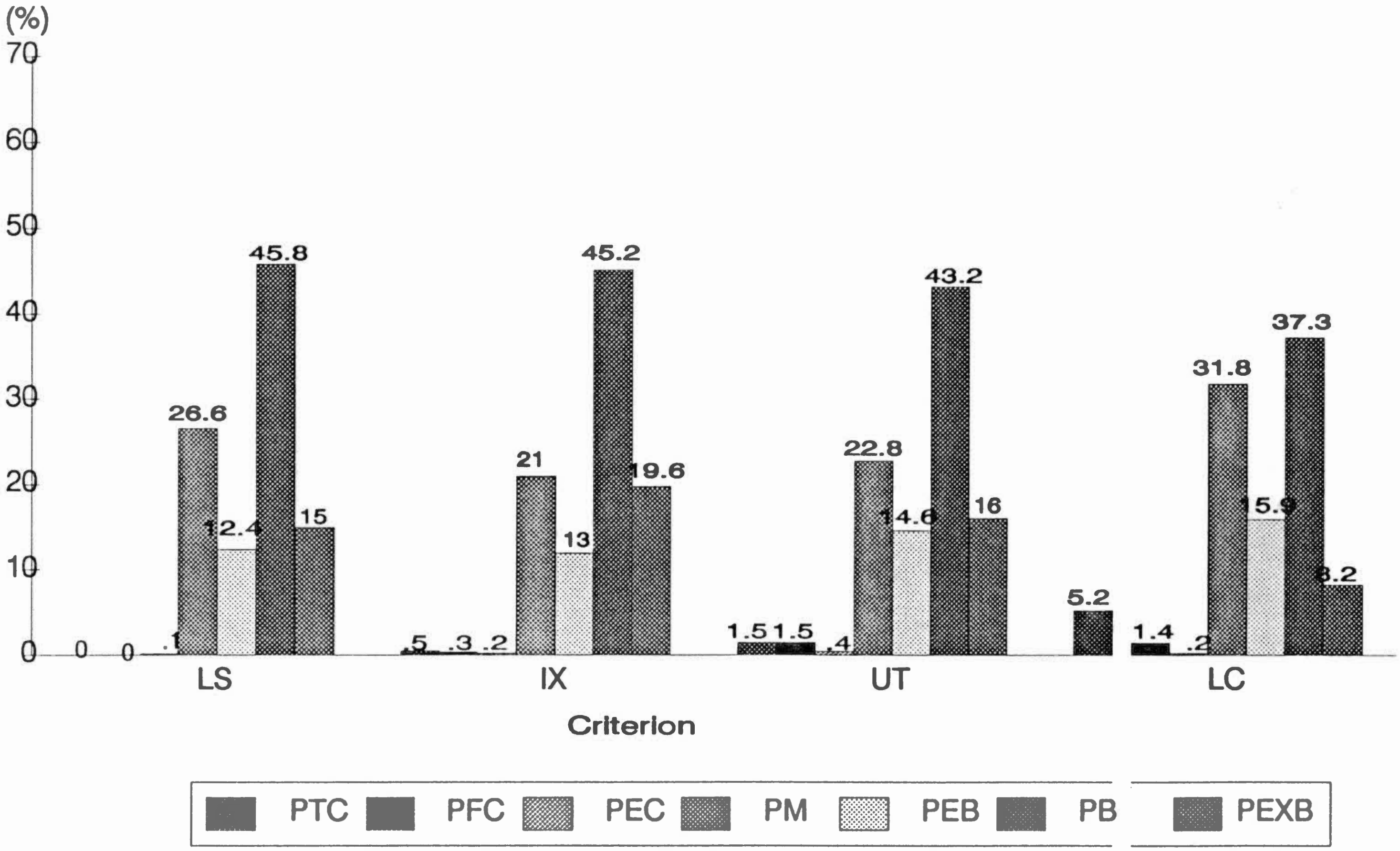

Figure 2. Average Distributions of Developmental Stage for each Selection Criterion by Left Side of the Uterus

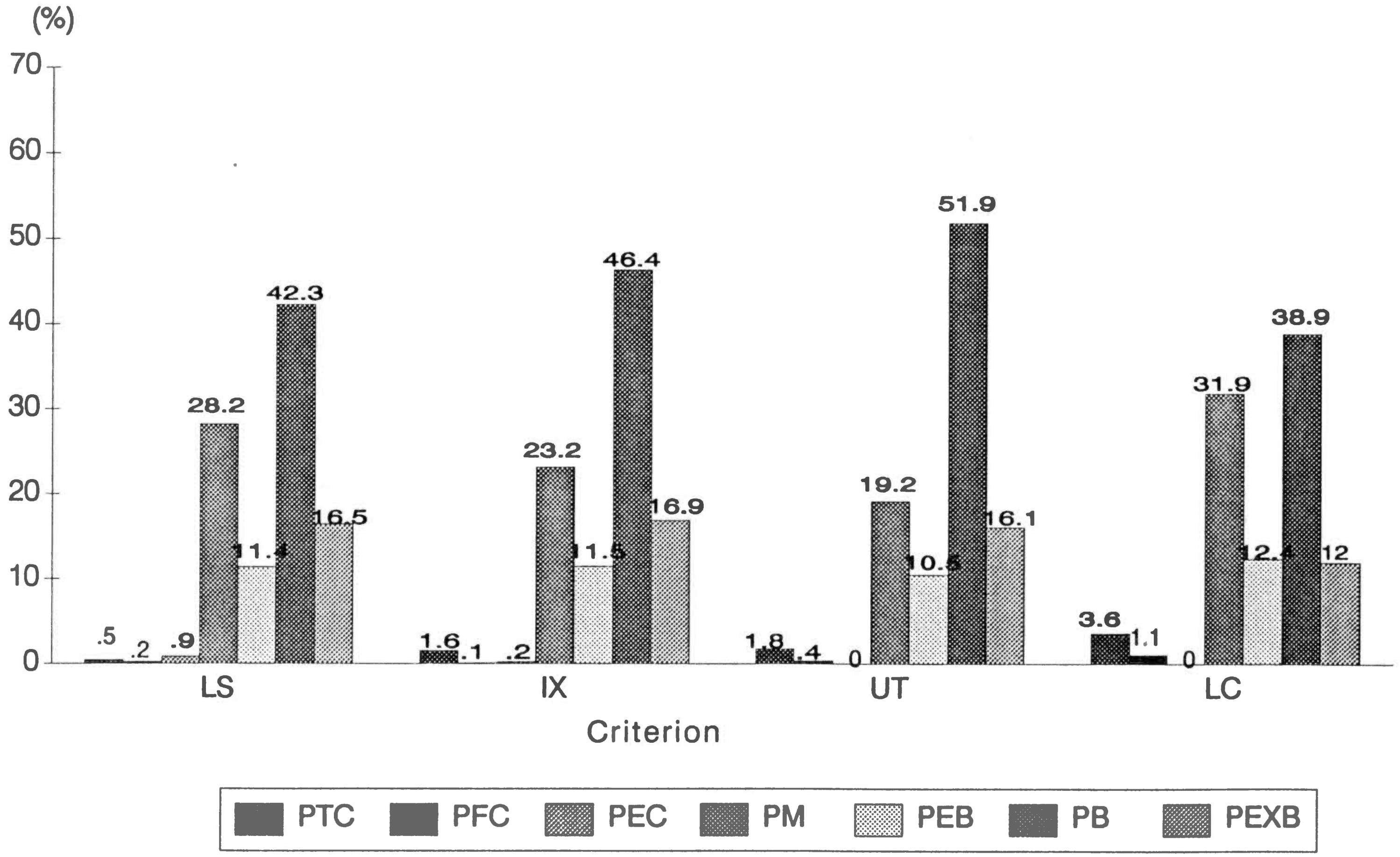

Figure 3. Average Distributions of Developmental Stage for each Selection Criterion by Right Side of the Uterus

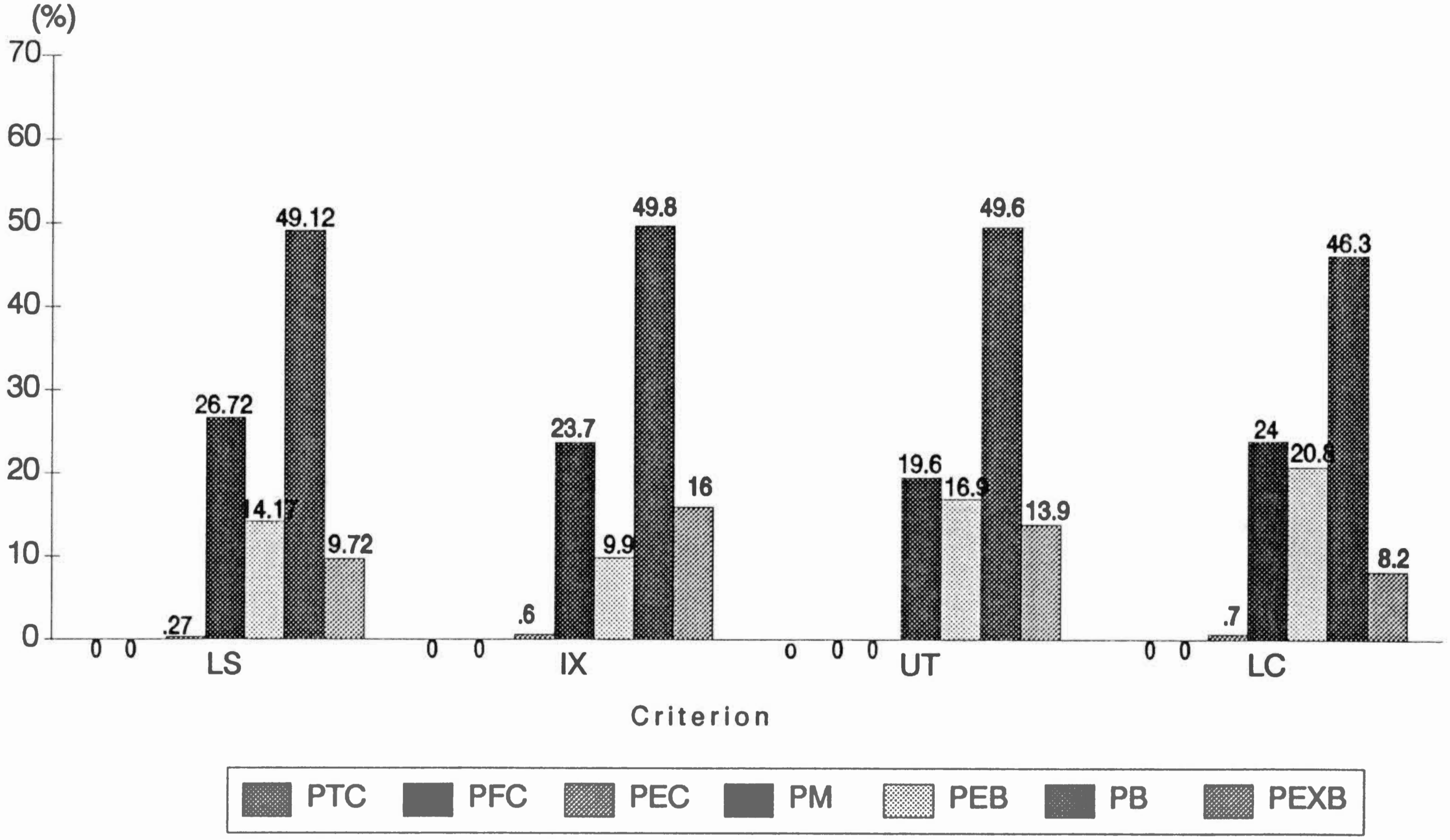

Figure 4. Average Left-Side Distributions of Developmental Stage for each Selction Criterion in Replicate I

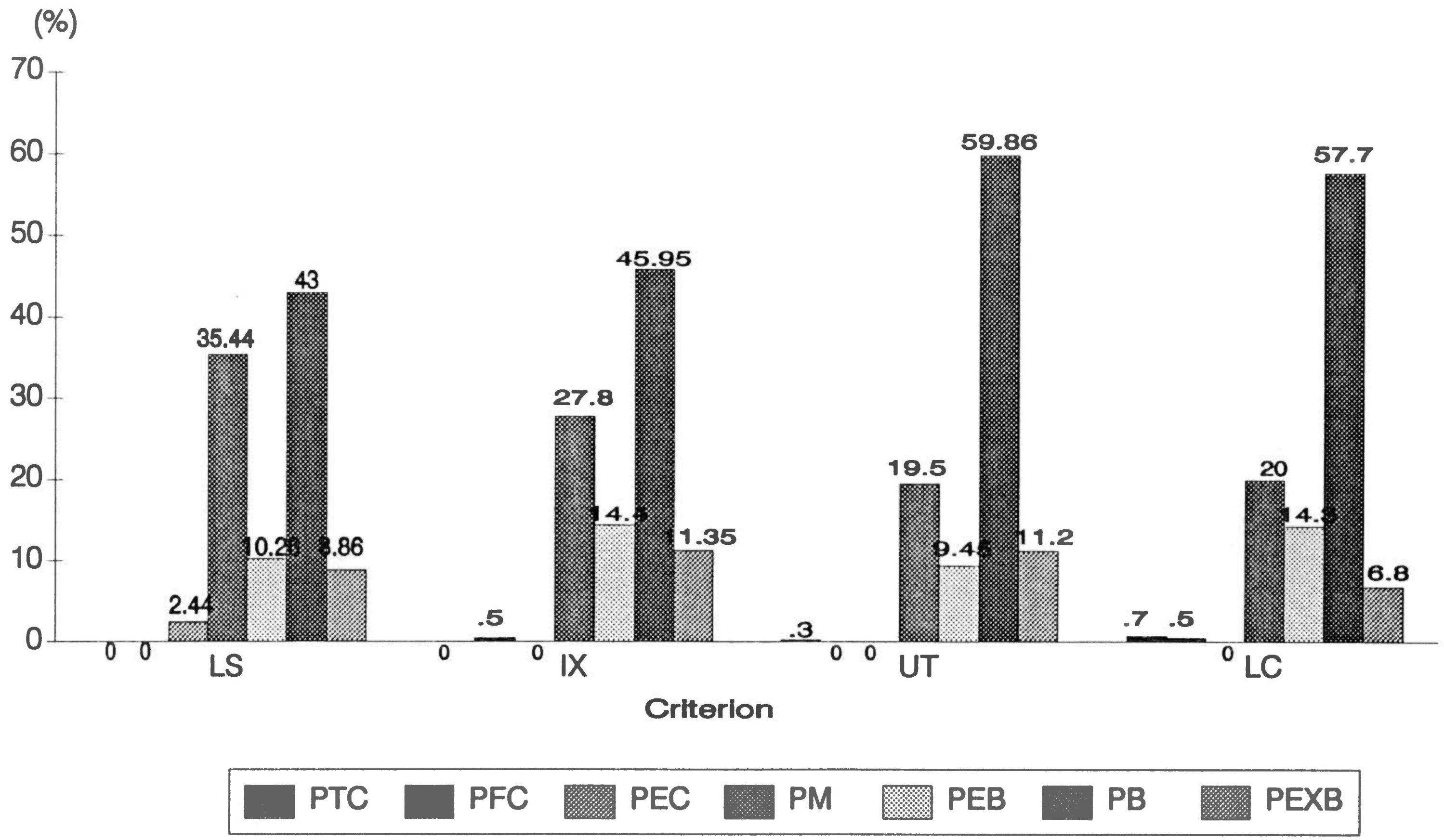

Figure 5. Average Right-Side Distributions of Developmental Stage for each Selection Criterion in Replicate I

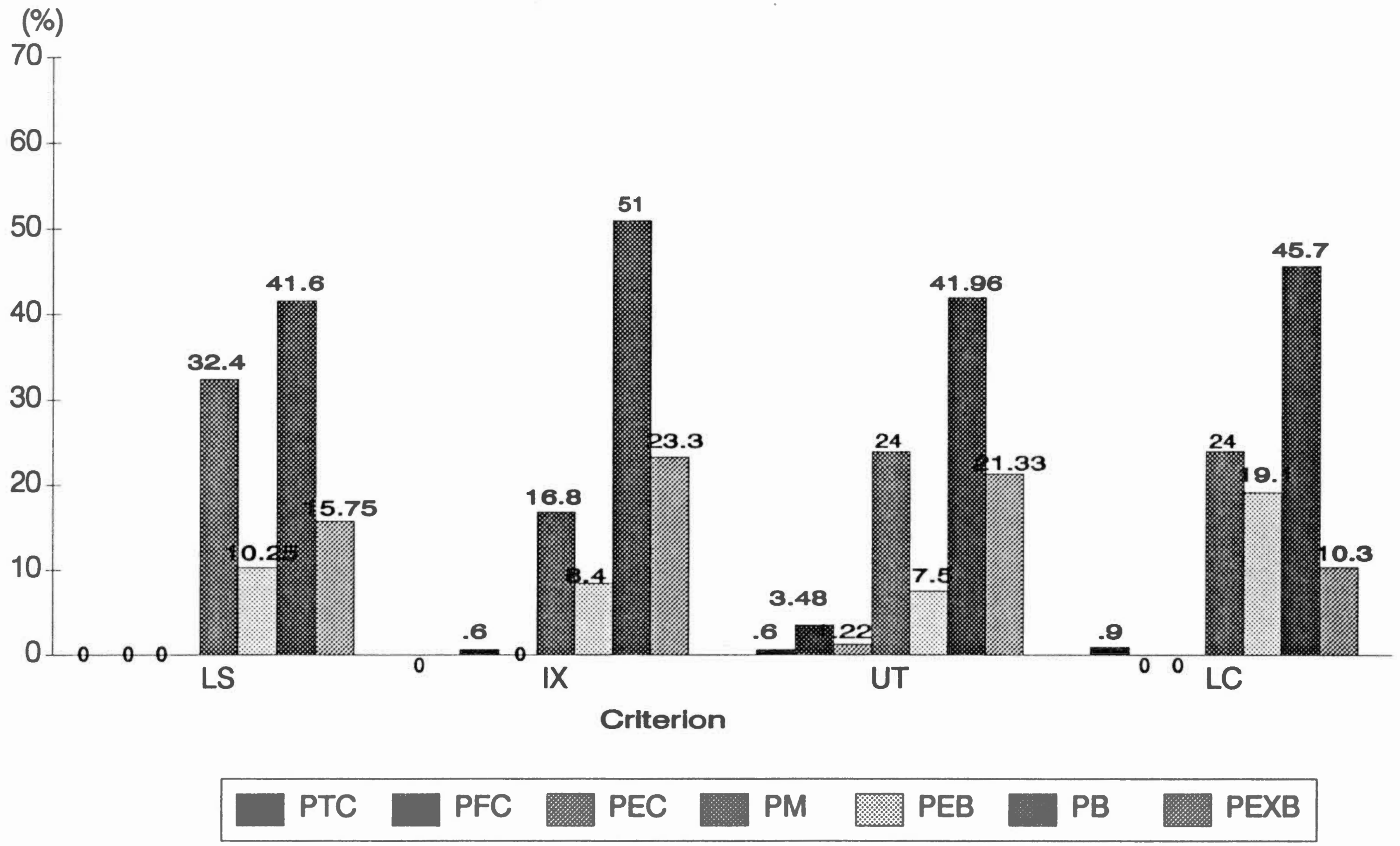

Figure 6. Average Left-Side Distributions of Developmental Stage for each Selection Criterion in Replicate II

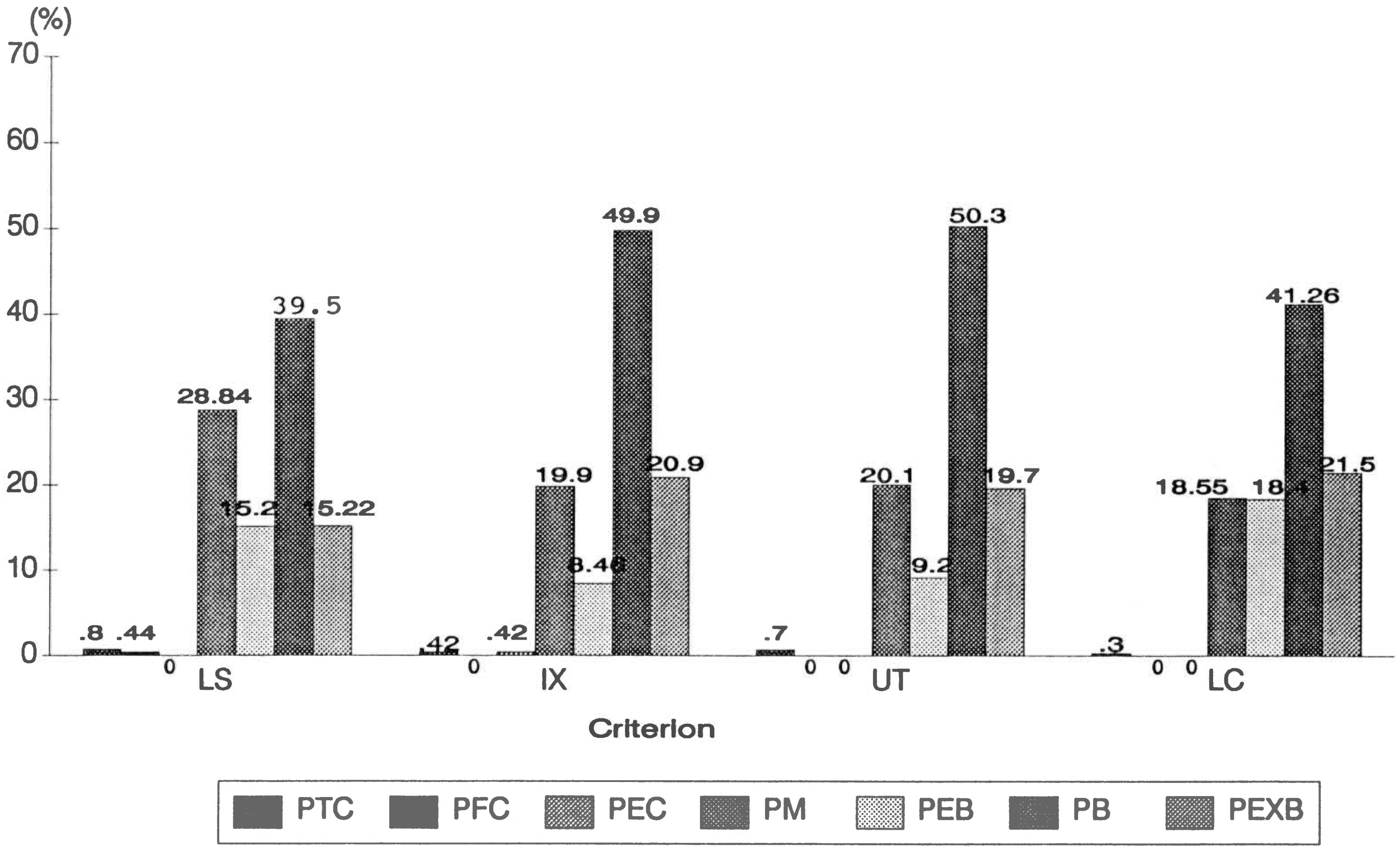

Figure 7. Average Right-Side Distributions of Developmental Stage for each Selection Criterion in Replicate II

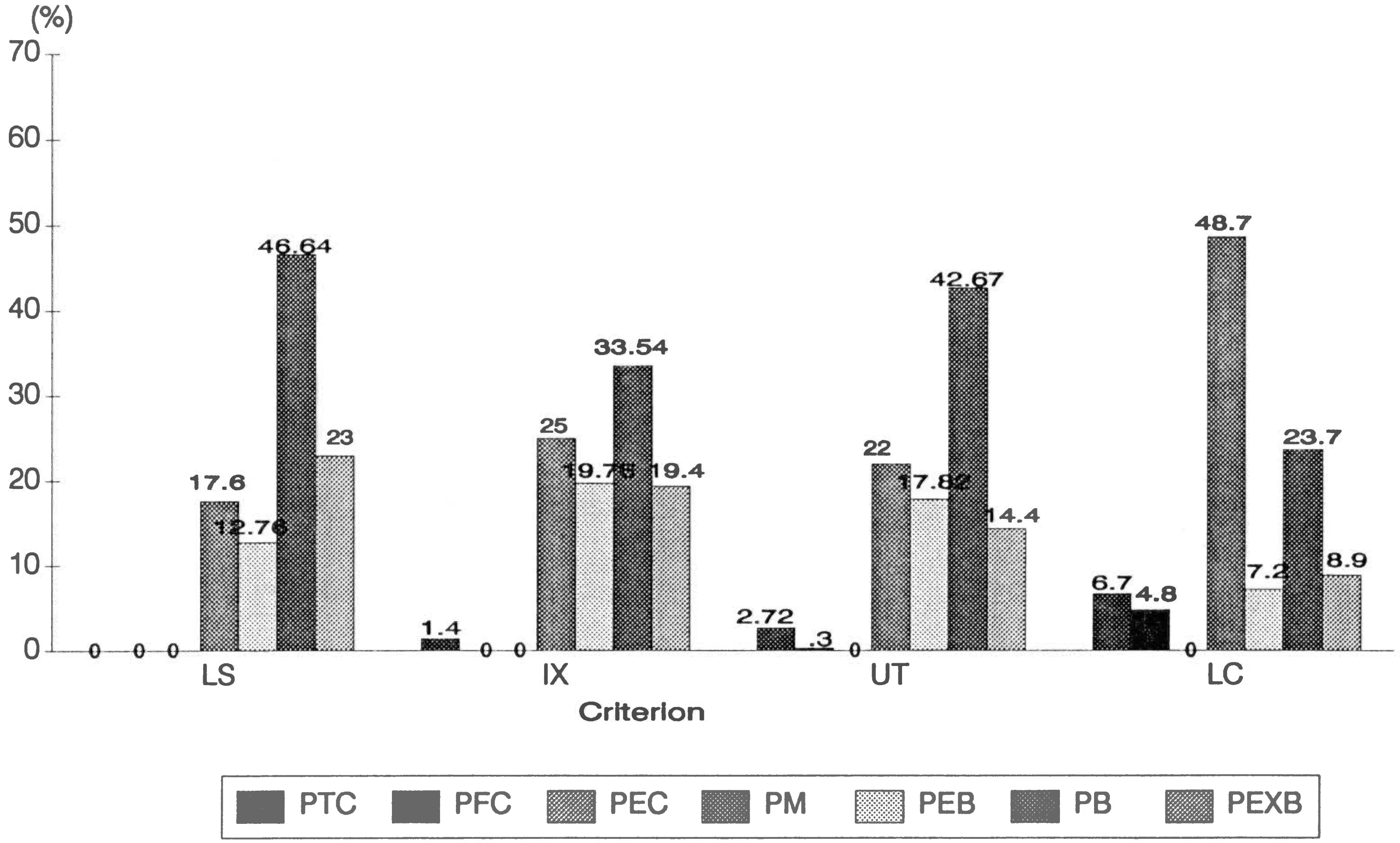

Figure 8. Average Left-Side Distributions of Developmental Stage for each Selection Criterion in Replicate III

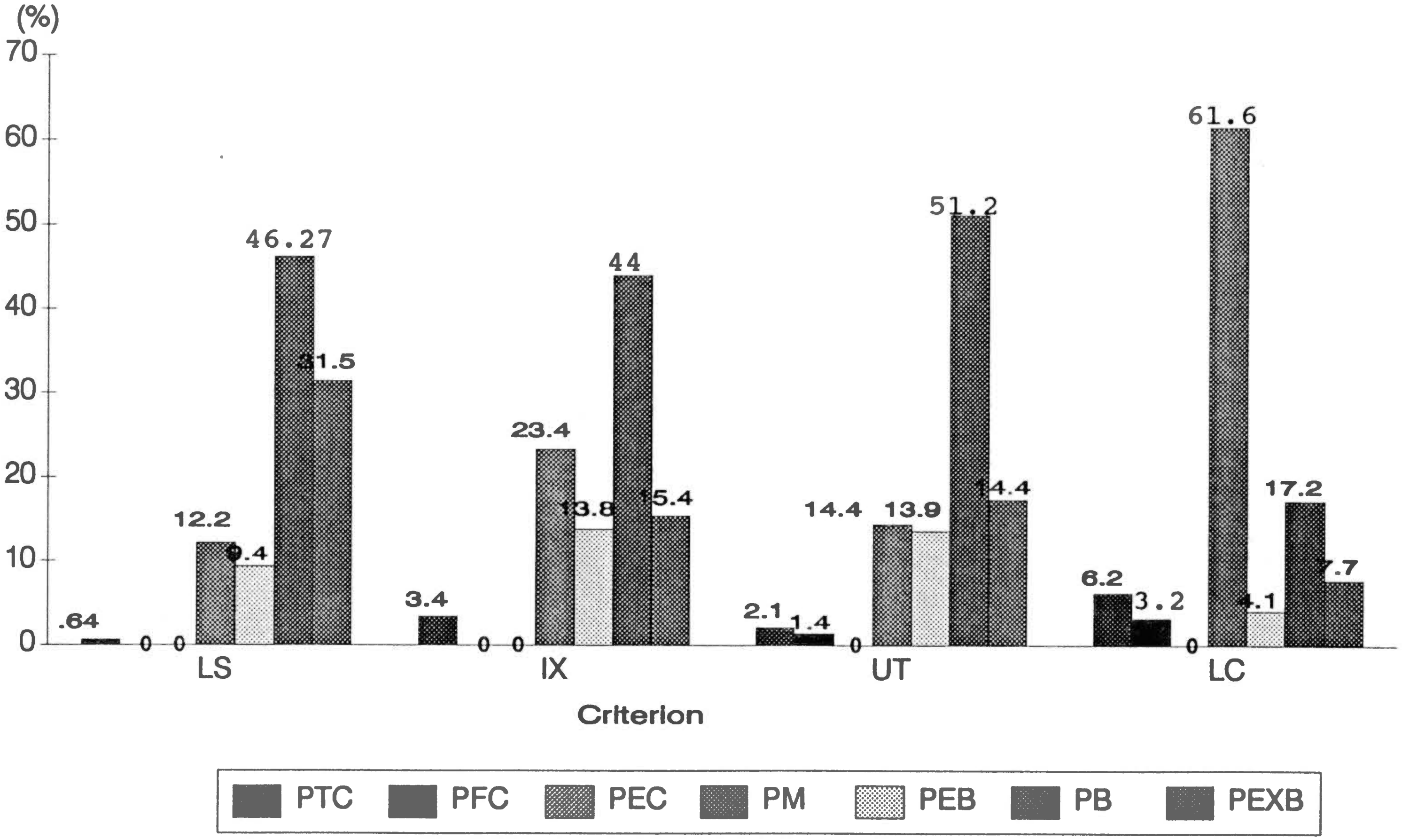

Figure 9. Average Right-Side Distributions of Developmental Stage for each Selection Criterion in Replicate III

selection criterion by replicate and side of the uterus. Mean for percent unfertilized ova (PUFO) and percent degenerating embryos (PD) are shown in Figures 10 and 11, respectively.

Significance levels of orthogonal contrasts of selection criterion means are presented in Table 4 for each of the univariate analyses. On both sides of the uterus, the three selection criteria (IX, LS, and UT) increased (P<.01) OR relative to the control. Ovulation rate increased to a greater extent in IX and LS than UT (P=.05) on the left ovary and the right ovary (P<.01). Ovulation rate of LS was greater (P=.06) than that of IX on the left ovary; differences between IX and LS for ovulation rate on the right ovary were not significant. Mean percent of recovered embryos and ova (number of observation/OR) was not affected by criterion and averaged 77%.

The univariate analysis of left-side percentages in each class indicated that LS, IX and UT increased (P=.01) PEXB and tended to decrease (P=.09) PTC. Percentages for eight-cell embryos (PEC) for the LS, IX and UT tended to increase (P=.13) relative to the control; increased (P=.10) PEC were also evident in IX and LS versus UT and to a greater amount in IX than LS (P=.03). The multivariate analysis of left-side percentages revealed that selection tended to affect average distribution of embryonic development relative to the control (P=.10, Table 5).

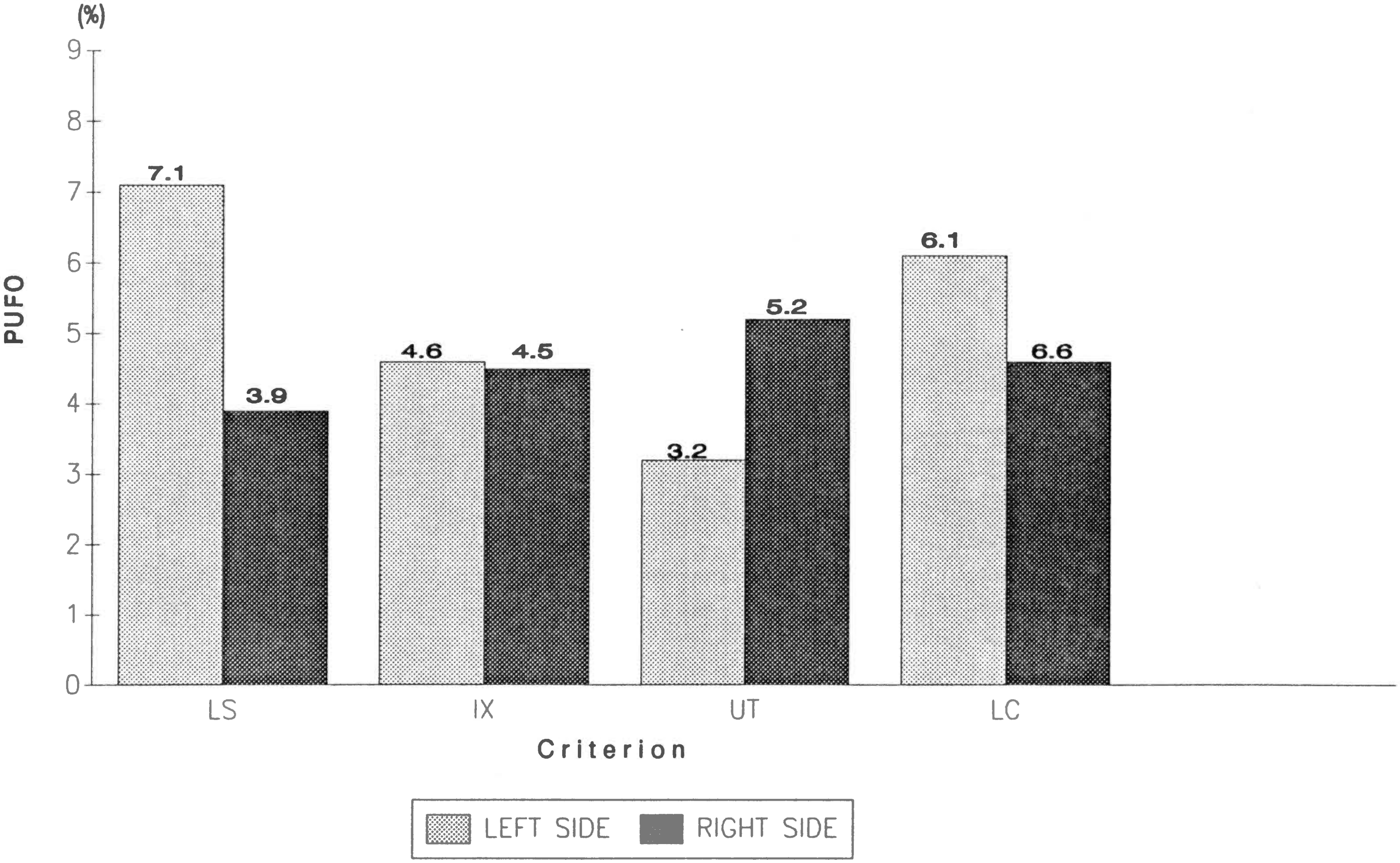

Figure 10. Mean Percent Unfertilized ova (PUFO)for each Selection Criterion by Side of the Uterus

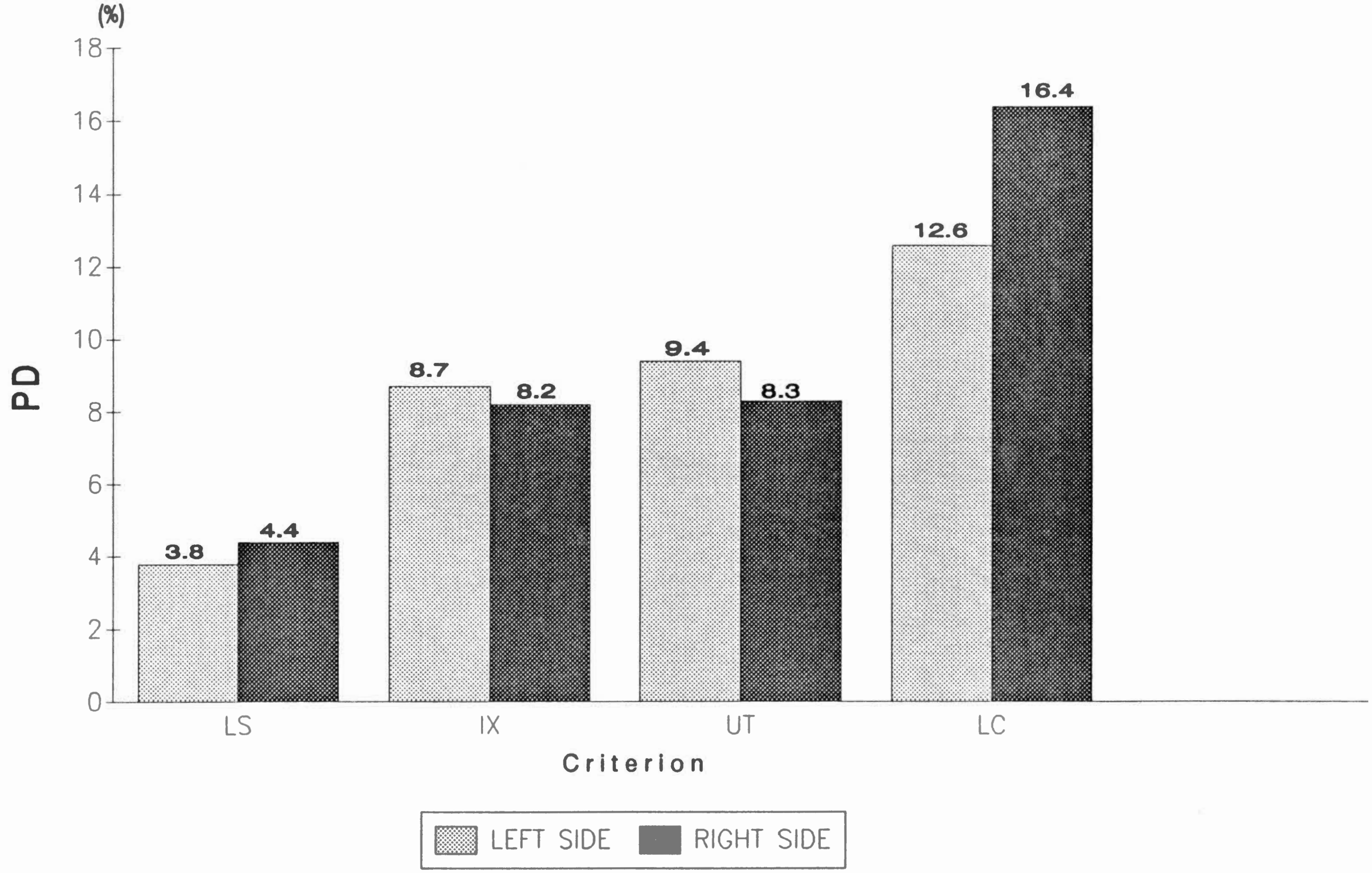

Figure 11. Mean Percent Degenerating Embryos (PD) for each Selection Criterion by Side of the Uterus

TABLE 4

SIGNIFICANCE LEVELS OF ORTHOGONAL CONTRASTS OF SELECTION CRITERION MEANS FROM UNIVARIATE ANALYSES

	Left side			Right side		
Chracteristic	IX, LS, UT vs LC	IX, LS vs UT	IX vs LS	IX, LS, UT vs LC	IX, LS vs UT	IX vs LS
OR	.004	.04	.05	.001	.004	NS
PUFO	NS[a]	NS	NS	NS	NS	NS
PD	.20	NS	NS	.05	NS	NS
PTC	.09	NS	NS	.04	NS	NS
PFC	.20	.20	NS	.04	NS	NS
PEC	.13	.10	.03	.04	NS	NS
PM	NS	NS	NS	NS	NS	NS
PEB	NS	NS	NS	NS	NS	NS
PB	.19	NS	NS	NS	NS	NS
PEXB	.01	NS	.19	NS	NS	NS
STAGE	.07	NS	NS	NS	NS	NS
STD	NS	NS	NS	.06	.16	NS

[a] Nonsignificant (P>.20).

TABLE 5

SIGNIFICANCE LEVELS OF ORTHOGONAL CONTRASTS OF SELECTION CRITERION MEANS FROM MULTIVARIATE ANALYSES BY REPLICATE AND SIDE OF THE UTERUS

	Left side			Right side		
Chracteristic	IX, LS, UT vs LC	IX, LS vs UT	IX vs LS	IX, LS, UT vs LC	IX, LS vs UT	IX vs LS
R1	NS[a]	NS	NS	NS	.12	NS
R2	.046	.045	.19	NS	NS	NS
R3	.0001	NS	.11	.0001	NS	.08
OVERALL	.10	NS	NS	NS	NS	NS

[a] Non significant ($P > .20$).

Univariate analyses for the right side of the uterus indicated that the three selection criteria had a similar decrease (P<.05) in percentages of pre-morula (PTC, PFC, and PEC), but contrasts of the average distributions with MANOVA were not significant (P> .20).

On the left side of the uterus, LS, IX and UT tended to increase (P=.07) average stage of development relative to the control (Tables 3 and 4). Females from LS, IX and UT also had greater numeric values for average stage of development than LC on the right side of the uterus, but the difference was not statistically significant. On the right side of the uterus, average STD for developmental stage was less (P=.06) for the three selection criteria relative to the control; differences among criteria for average STD on the left side of the uterus were not significant.

From the orthogonal comparison with MANOVA for each replicate and side of the uterus (Table 5), selection affected average distributions of embryonic development relative to the control (P<.01) on both sides of the uterus in the third replicate and on the left side of the uterus in the second replicate (P<.05). None of the orthogonal contrasts of the average distributions with MANOVA associated with selection for the first replicate were significant. In tables 6 and 7 are mean percentages for ova and embryos pooled across replicate for left and right

TABLE 6

MEAN PERCENTAGES FOR THE OVA AND EMBRYOS POOLED ACROSS REPLICATE BY LEFT SIDE OF THE UTERUS

	Ovum and Embryo					
Criterion	PUFO	PD	PTC	PFC	PEC	PM
LS	7.60(3.46)[a]	3.75(1.87)	0	0	.08(.08)	22.55(5.7)
IX	4.42(2.32)	9.2(3.34).	.37(.35)	.18(.17)	.18(.15)	18.85(4.5)
UT	3.2(1.51)	9.5(3.68)	1.05(.68)	1.2(.96)	.34(.34)	19.5(3.5)
LC	6.12(3.16)	12.75(4.14)	1.85(.83)	.65(.56)	.2(.19)	25.2(5.2)

	Embryo		
Criterion	PEB	PB	PEXB
LS	11.05(2.41)	40.7(4.61)	14.25(3.17)
IX	10.8(2.77)	39.1(5.1)	16.9(4.51)
UT	12.5(3.1)	39.0(5.3)	14(3.4)
LC	13.30(3.44)	32.33(5.47)	7.6(3.19)

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[a] Standard error in parentheses

TABLE 7

MEAN PERCENTAGES FOR THE OVA AND EMBRYOS POOLED ACROSS REPLICATE BY RIGHT SIDE OF THE UTERUS

	Ovum and Embryo					
Criterion	PUFO	PD	PTC	PFC	PEC	PM
LS	4.0(2.35)[a]	4.5(2.53)	.42(.42)	.13(.13)	.75(.75)	23.45(4.23)
IX	4.5(2.1)	8.75(3.75)	.85(.63)	.16(.15)	.12(.12)	20.4(4.1)
UT	5.0(2.0)	8.1(2.99)	1.0(.77)	.46(.46)	0	15.4(3.5)
LC	4.55(2.29)	16.3(5.23)	1.62(.95)	.83(.57)	0	24.4(4.72)

	Embryo		
Criterion	PEB	PB	PEXB
LS	10.7(2.28)	39.25(4.9)	16.8(3.42)
IX	10.5(2.31)	40.15(4.68)	13.65(3.31)
UT	9.5(2.58)	46.5(5.31)	13.96(3.5)
LC	10.3(3.3)	32.2(4.84)	9.8(3.0)

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[a] Standard error in parentheses

side of the uterus.

For PUFO, none of the orthogonal comparisons were significant for either side. On the right side of the uterus, Percentages of degenerating embryos for LS, IX and UT were lower (P=.05) than those in the control. Females from LS, IX and had lower numeric values for degenerating embryos than LC on the left side, but the difference was not significant.

Discussion

Differences between selection criteria for ovulation rate were observed. These differences are consistent with an earlier evaluation of these lines conducted after 5 and 13 generations of selection (Gion et al., 1990). Generally, ovulation rate responded to selection, but in greater amount to direct (LS) or index (IX) selection for litter size than to selection for uterine capacity (UT). Across lines the right side was higher in ova shed than the left side. These differences were also in agreement with earlier results from this population (Clutter et al., 1990; Gion et al., 1990) and with characterizations of other mouse populations (Wielbold et al., 1987).

The present study represents the first evaluation of the effect of genetic selection for litter size or its components on these characteristics of pre-implantation embryonic development. Although differences between criteria were only highly significant for proportions of embryos in some of the individual classifications, a tendency for a higher frequency of expanded blastocysts and lower frequency of pre-morula observations in LS, IX and UT vs LC was indicated. Analysis of average stage of development revealed that selection resulted in more advanced embryos at day 3.5 in the left uterine horn. These results agree with observations that direct selection for litter size in the mouse increased the proportion of embryos developed beyond the eight-cell stage by day 2 of gestation (Durrant et al., 1980)

A greater percentage of advanced embryos due to selection may be the result of a change in the timing of ovulation, of fertilization or of first cleavage division, the rate of subsequent cell division and embryonic development or some combination of these factors. Each factor is likely affected by several genes and by environmental variation. The selection criteria which were used in the present study, each attempt to increase allelic frequencies associated with ultimate litter size through complex physiological processes from ovulation to the end of gestation (or those independent of ovulation rate in the case of UT). While single genes may have a significant effect on variation in litter size, by affecting a single process or through pleiotrophy, these selection criteria undoubtedly addressed many loci. The results of this study suggest that selection has changed the frequencies of alleles that affect some determinant(s) of embryonic stage at implantation.

Alleles of the pre-implantation embryonic development (ped) gene, linked to the major histocompatibility complex (MHC) in the mouse, have been shown to affect rate of cleavage division (Galdbard and Warner, 1982). Ped gene alleles that resulted in an increased rate of development have more recently been associated with larger litter size and heavier litter weight (Warner et al., 1991). Although changes in the timing of ovulation rate or fertilization can not be ruled out, it is interesting to speculate that alleles of the ped gene may have been one target of these selection criteria.

Normally developing mouse embryos are expected to be at or beyond the morula stage by 3.5 days post coitum (Smith and McLaren, 1977). It was reported that of mouse embryos at the morula, early blastocyst, blastocyst or expanded blastocyst stage by 3.5 days post coitum, those in the latter two stages had greater implantation success and post-implantation survival (Gates, 1965). A greater proportion of embryos in LS, IX, and UT were observed to be at stages of development associated previously with increased survival.

Delayed embryonic development in the mice and pigs (Gates, 1965; Wilmut et al., 1986, respectively) and variability in the stage of pre-implantation development among littermate pig embryos has shown to ultimately contribute to embryonic loss (Pope et al., 1986); the more advanced embryos survive at the expense of those that are delayed or less advanced. Littermate embryos from the highly prolific Meishan breed of pigs developed faster and with less variability than their Large White contemporaries, perhaps explaining some of the differences in embryonic survival between the breeds (Bazer et al., 1988a, 1988b). Results from the present study reveal that these selection criteria decreased variability among embryos within the right uterine horn at day 3.5 of gestation. Selection may have altered the distribution of ovulations over time as described in the pig (Pope et al., 1988), the uniformity of embryonic development, or both. Also, selection decreased the frequency of degenerating embryos on the right side of the uterus. While it is not known from which developmental stage the additional proportion of degenerating embryos in LC were lost, it is possible they reflect greater variability and the loss of delayed embryos within a horn. Variation among replicates was apparent in the average distributions of developmental stage. This may be due to random sampling of the genes that the individual has at each locus. Falconer (1984) points out that continued sampling in several lines through successive generations causes random fluctuation in gene frequency. As a result, random drift exists causing differentiation among replicates. These results demonstrate the importance of replication and the need to include variation among replicates in the estimate of error.

Conclusions

The results provide initial evidence that genetic selection for increased litter size, or for components of litter size, in the mouse appear to have changed the frequencies of allelic forms of genes affecting some characteristics of average stage of embryonic development, and possibly uniformity of development within a uterine horn, at day 3.5 of gestation. Alleles of the (ped) gene which have shown to affect rate of cleavage division (Goldbard et al., 1882) may have been one target of these selection criteria.

Since blastocyst and expanded blastocyst had a greater implantation success and post-implantation survival (Gates, 1965), it is of importance to mention that a greater proportion of embryos in LS, IX and UT were at these two stages. Results also indicate that these selection criteria decrease variability among embryos within the right side horn at day 3.5 of gestation. After 13 generations of selection on these criteria, litter size of LS and IX was significantly greater than LC (Goin et al., 1991). Changes in preimplantation embryonic development have likely contributed to response in litter size.

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VITA 2

Salih Abdu Al-Shorepy

Candidate for the degree of

Master of Science

Thesis: EFFECT OF THREE METHODS OF SELECTION FOR LITTER SIZE IN MICE ON PREIMPLANTATION EMBRYONIC DEVELOPMENT

Major Field: Animal Science

Biographical:

Personal Data: Born in Sharab, Yemen, November 26,1961, the son of Abdu A. and Ward F. Al-Sharabi.

Education: Received the Bachelor of Science from King Faisal University, Ahsa, Saudi Arabia, in June 1985; completed requirements for the Master of Science degree at Oklahoma State University in December, 1991.

Experience: Teaching at Sordod Agricultural School, Hodidah, Yemen, 1985-1987; teaching assistant, Faculty of Agriculture, Sana'a University, 1987-1989; graduate student, Department of Animal Science, Oklahoma State University, 1990-1991.

Professional Organizations: American Society of Animal Science.