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PREVIEW

EVALUATION OF THE UTERINE CAPACITY - VIABLE EMBRYO
MODEL TO EXPLAIN LITTER SIZE IN MICE

by

Edson Luis de Azambuja Ribeiro

A DISSERTATION

Presented to the Faculty of
The Graduate College in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Philosophy

Major: Animal Science

Under the Supervision of Professor Merlyn K. Nielsen

Lincoln, Nebraska

June, 1995

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DISSERTATION TITLE

EVALUATION OF THE UTERINE CAPACITY - VIABLE EMBRYO

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GRADUATE COLLEGE
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EVALUATION OF THE UTERINE CAPACITY - VIABLE EMBRYO

MODEL TO EXPLAIN LITTER SIZE IN MICE

Edson Luis de Azambuja Ribeiro, Ph.D.

University of Nebraska, 1995

Adviser: Merlyn K. Nielsen

A model including ovulation rate, potential embryonic viability, and uterine capacity was used to study litter size in mice. The selection experiment conducted at the University of Nebraska-Lincoln, based on different criteria to improve litter size, was used as the base for this study. The selection criteria were: LS = litter size, IX = an index including ovulation rate and ova success, UT = uterine capacity, and LC = control.

Embryonic survival to 6 d was evaluated in the first study at Generation 36 of this experiment; selection had occurred for 21 generations. Selection for litter size by different criteria increased ovulation rate and embryonic survival to 6 d. Least-squares means for left embryonic survival were .84, .91, .85, and .82, and for right embryonic survival, .91, .90, .86, and .87, respectively for LS, IX, UT and LC.

In another study simulation was used to test the model cited above. Comparisons were made to statistics of the base generation and to responses after 13 generations of selection observed in the cited experiment. Parameters for uterine capacity were generated taking into consideration observed means, standard deviations and correlations between left and

right litter size, as well as responses in number born. The model was able to generate statistics that agreed with observed ones. Simulated mean and heritability for uterine capacity for the base population were 16.30 and .065, respectively. Although ovulation rate was the more limiting component, uterine capacity was important for explaining litter size.

In the last study alternative criteria of selection were compared after 10 generations of simulated selection. Selection indices including ovulation rate and uterine capacity produced greater (23.9 and 14.7%) responses in litter size than direct selection for litter size. Indices including ovulation rate and ova success also produced more response (12.9%) in litter size than direct selection for litter size. Selection for ovulation rate, uterine capacity, or ova success produced responses in litter size that were 4.5, 43.6, and 74.8% smaller than direct selection for litter size. Crossing ovulation rate and uterine capacity lines also produced smaller litter size than direct selection.

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INTRODUCTION

Litter size at birth is one of the most important components of animal production. It influences the availability of animals for replacement, and consequently genetic gains from selection, and costs of production. Tess et al. (1983) reported that an increase in number of pigs born alive will significantly improve the economic efficiency of swine production. In sheep, Bradford (1985) stated that litter size at birth is more important than growth rate in explaining the differences in total weight of lamb weaned.

Improvement of litter size through direct selection has been tested in mice (reviewed by McCarthy, 1982, and Nielsen, 1994), and in livestock species, as in pigs (reviewed by Haley et al., 1988) and sheep (reviewed by Bradford, 1985, and Elsen et al., 1994). In general estimated heritabilities are low, around .10. Selection for the components of litter size alone or for indices have been proposed as more effective ways to improve litter size. Although litter size is a complex trait, it has been generally accepted that its variability is due to two main components, ovulation rate and total prenatal survival (Johnson et al., 1984).

Two selection experiments with mice (Land and Falconer, 1969, and Bradford, 1969, 1979) and one with pigs (Zimmerman and Cunningham, 1975; Cunningham et al., 1979; and Lamberson et al., 1991) showed that direct response is generally observed when selecting for ovulation rate, but a concomitant

correlated response in litter size at birth is in general absent or small. All of them suggested that this lack of or small correlated response in litter size was due to an increase in prenatal mortality. Bradford (1969, 1979) selected two lines of mice for prenatal survival. He observed a decrease in pre- and post-implantation losses, and positive correlated responses in ovulation rate and litter size.

Johnson et al. (1984) suggested that selection for an index with optimum weights for ovulation rate and embryonic survival would provide greater response in litter size than direct selection for litter size (a natural index) in pigs. Results of an experiment with mice showed that response in total number born per generation was slightly greater, through Generation 13, for an index including ovulation rate and ova success (prenatal survival) than direct selection for litter size, .17 and .15, respectively (Gion et al., 1990). However, after 21 generations, direct selection for litter size was slightly better than the index selection, .16 and .14, respectively (Kirby and Nielsen, 1993). The component weightings in the index were not changed during the 21 generations of selection which may explain the poorer effectiveness of the index selection in later generations.

Bennett and Leymaster (1989) proposed a new model to explain litter size. By their model litter size is the result of the interaction of ovulation rate, potential embryonic viability, and uterine capacity. This model differs from the

one including ovulation rate and total prenatal mortality by accounting for the two phases where losses occur: embryonic mortality, related to the ovum/embryo, and fetal mortality, related to the uterine resources of the dam. Simulated selection showed that indices including ovulation rate and uterine capacity were superior to an index including ovulation rate and prenatal survival, and both kind of indices were superior to direct selection for litter size (Bennett and Leymaster, 1990b).

The objectives of this study were to test the model suggested by Bennett and Leymaster (1989) using data from mice at the University of Nebraska-Lincoln, selected on alternative criteria to improve litter size, and then to devise new strategies and indices of selection for a population of mice. To achieve our objectives, the work was divided in three parts. In PART I embryonic survival to 6 d was evaluated in these populations of mice. Embryonic survival was required to estimate potentially viable embryos. In PART II the model was developed and implemented. Means and variability for uterine capacity were generated and responses to simulated selection were compared to observed responses. In PART III new selection indices, not used in these populations, including ovulation rate and ova success or uterine capacity were derived, and responses to 10 generations of simulated selection were compared.

LITERATURE REVIEW

Selection for Litter Size

Litter size at birth is a major component in animal production (Tess et al., 1983; Bradford, 1985; Haley et al., 1988; Elsen et al., 1994). Although this trait has a low heritability, it has a large genetic variability between and within breeds, and this fact can be used in breeding programs, as described for sheep by Elsen et al. (1994).

Direct responses to selection in mice have, in general, been positive. However, differences in rates of response among experiments occurred. These differences are probably due to differences in animal populations, intensities of selection, methods of selection, and environmental effects, e.g. hormonal treatments. The selection experiment by Bradford (1968) studied several of these points. He reported realized heritabilities of .13 and .22 after 10 generations of mass selection in lines derived from 4- and 8-way cross stocks, respectively. When selection was practiced intra-litter the response obtained was over half the response observed in mass selection. When selection was practiced in a superovulated line the estimated realized heritability was negative. Results of this same experiment after 51 generations of selection were reported by Bradford (1979). The line selected for large litter size presented a continuous response for 30 generations, then a plateau for about 14 generations, and new response after that. In an experiment using within-family

selection (Falconer et al., 1960), response to selection ceased after Generation 20, when a difference of 1.6 mice was observed between the selected and the control line. The realized heritability was 8.3%.

In other mass selection experiments with mice a decrease in response after 20 (Joakimsen and Baker, 1977), 29 (Bakker et al., 1978), and 12 (Eisen, 1978) generations of practiced selection did not occur. The estimated realized heritabilities for high selected lines, cited in the same order of the experiments, were .18, .11, and .19. The results of the selection experiment conducted at the University of Nebraska-Lincoln agreed with these experiments. After 21 generations of selection the realized heritability was .10 (Kirby and Nielsen, 1993).

Even after selection has stopped, differences between lines are maintained. Lubritz et al. (1991) observed a higher litter size in the high litter size line (L+) than in the control line, 17.31 vs 12.06, at Generations 60 and 61 of the experiment started by Eisen (1978). Selection occurred for only 23 generations.

Responses in litter size in these selection experiments were realized through changes in its components. Bakker et al. (1978) cited that the higher litter size of the selected line, compared to the control, was mainly observed due to a higher ovulation rate, however lower pre- and post-implantation losses also contributed to the response. Ovulation rate is

also identified as the main trait that changed in the other experiments (Falconer, 1960; Bradford, 1969, 1979; Joakimsen and Baker, 1977; Durrant et al., 1980; Spruill and Eisen, 1985; Gion et al., 1990). However, changes in embryonic and fetal losses, or in total prenatal survival, varied. While Falconer (1960) observed a decrease in prenatal survival, Bradford (1969) stated that pre- and post-implantation losses did not change; however, Bradford (1979) reported that at late generations an increased survival rate was important for explaining response in litter size. In the experiment started by Eisen (1978), Durrant et al. (1980) observed no differences in pre-implantation embryonic survival between the selected (L+) and the control line; but post-implantation losses were smaller in the selected line. In the same experiment, Spruill and Eisen (1985) reported that prenatal survival was about 10% higher in the selected line; the major difference occurring during the pre-implantation period.

When selection was for smaller litter size, reduced embryonic viability was the main factor explaining response to selection (Falconer, 1960; Bradford, 1979). However, Joakimsen and Baker (1977) stated that the decrease in litter size in the low line was due to a decline in ovulation rate and an increase in embryonic losses.

Responses of selection experiments with livestock species in general agree with mice experiments. In a literature review, Haley et al. (1988) reported that heritability for

litter size in pigs is around .10. However, results of a selection experiment conducted for 11 generations, based on total number of piglets born in the first two litters of a sow, showed a non-significant response of $.024 \pm .077$ piglet per generation (Bolet et al., 1989). The realized heritability was $.04 \pm .13$. According to the authors, the low intensity of selection was the main cause for the non-success of this experiment. A significant correlated response in ovulation rate and no change in embryonic survival at d 30 were observed.

In another experiment with swine response to selection occurred. Lamberson et al. (1991) reported response of 1.06 pigs per litter in a line selected 8 generations for litter size. This same line was previously selected for 9 generations on ovulation rate. The realized heritability of LS was .15. Gama and Johnson (1993) observed that the response in the selection line was due to increased ovulation rate and uterine capacity; uterine capacity was estimated as twice the number of fully formed pigs in one uterine horn, measured after unilateral hysterectomy-ovariectomy. A reduction in the number of mummified pigs at birth was also observed.

Results of selection experiments with sheep (Bradford, 1985; Schoenian and Burfening, 1990) and rabbits (Rochambeau et al., 1994) agree with the ones reported for mice and pigs. Bradford (1985), based on literature review, reported a mean heritability of .10 for litter size in sheep, and Rochambeau

et al (1994) estimated heritabilities of $.06 \pm .02$ and $.07 \pm .02$, respectively, for litter size at birth and weaning in rabbits.

Selection for Components of Litter Size

As mentioned before, litter size at birth is a complex trait and is the result of the interaction of several components. Leymaster and Johnson (1994) commented that a conceptual model for litter size includes ovulation rate, and embryonic and fetal mortality, where fetal mortality is a result of the interaction of ovulation rate and potential embryonic viability with uterine capacity.

Selection for these components has been practiced to explore possible higher heritabilities and to study their genetic variation. Walkley and Smith (1980) stated that with high heritabilities and high genetic correlation with litter size, responses may be doubled or tripled by indirect selection.

Ovulation rate. Land and Falconer (1969) worked with five lines of mice for twelve generations: two lines selected for high ovulation rate (natural and induced), two lines selected for low ovulation rate (natural and induced), and a control line. They started the experiment with mass selection, but subsequently within family selection was practiced to increase effective population size and to eliminate maternal effects.

Ovulation rates at Generation 12 were 21, 16 and 14, respectively for the high, control and low natural ovulation lines. The heritability of natural ovulation rate estimated from the correlation between paternal half-sibs was $.22 \pm .19$. Response to selection in natural or induced ovulation was effective in both directions. Realized heritabilities were 31% ($.18 \pm .013$ for within litters) and 22% ($.11 \pm .038$ for within litters), respectively, for natural and induced ovulation lines. Selection for high natural ovulation produced no effect on litter size, possibly due to an increase in prenatal mortality. The low natural ovulation line had a reduction in litter size in accordance to the reduction in ovulation rate. However, selection for induced ovulation produced changes in the same direction in litter size.

Bradford (1969) reported results of another selection experiment with mice based on several selection criteria. Two of the lines were selected based on ovulation rate, one with natural ovulation and the other superovulated. Females and males were selected based on an index combining information from full and half sisters. Direct response to selection was positive in the natural ovulated line, but the same did not occur in the superovulated one. The realized heritability was slightly less than .10 in the naturally ovulated line. Neither of the lines presented a significantly higher litter size relative to the control after 10 generations of selection. In the naturally ovulated line there was an apparent increase in

post-implantation embryonic mortality. Bradford (1979) continued the selection experiment cited above for another four or five generations. After that lines were maintained with relaxed selection. After Generation 10, direct response for ovulation rate was still observed. Litter size increased a little, both during the period of selection for ovulation rate, and during 15 generations of random selection within litters. The author stated that "natural selection among embryos after implantation resulted in a significant improvement in genetic potential for prenatal survival" as a possible cause for this increase in litter size.

Mass selection for ovulation rate (corpora lutea) was practiced in a multibreed synthetic line of swine at the University of Nebraska-Lincoln. Results of the first five generations of selection were reported by Zimmerman and Cunningham (1975), and after nine generations of selection by Cunningham et al. (1979). Realized heritabilities after five and nine generations of selection were $.40 \pm .08$ and $.42 \pm .06$, respectively. Lamberson et al. (1991) reported results for this same line after nine generations of selection, same as Cunningham et al. (1979) did, plus 10 generations of relaxed selection. Response to selection for ovulation rate was about 3.7 eggs. Differences between selected and control lines were maintained during relaxed selection. Response in litter size per generation to selection for ovulation rate at Generation 9 was $.089 \pm .058$ pigs. Cunningham et al. (1979)

reported a value of $.06 \pm .07$. The difference was explained by the introduction of more data in Generation 9, and the evaluation was made from Generation -1 to 9, instead of from Generation 0 to 9, as done by Cunningham et al. (1979). Only 20% of the average increase in ovulation rate was realized as more pigs at birth (Lamberson et al., 1991). Johnson et al. (1984) reported that most of the embryonic loss occurred prior to d 30 of gestation in the control line, but additional embryonic loss occurred to d 70 in the high ovulation rate line.

Responses in ovulation rate are realized through changes in the physiology of the animal. Land and Falconer (1969) observed that the increase in ovulation rate after selection for high natural ovulation was due to an increase in FSH activity, but the decrease in ovulation rate in the low natural selection line was due to changes in ovarian sensitivity. Changes in ovulation rate following selection for low and high induced ovulation were due basically to changes in ovarian sensitivity. The increase in FSH activity in the high natural ovulation line agrees with the suggestion made by McLaren (1963), that the duration of FSH stimulation of the ovary is important for an increased ovulation rate. However, Durrant et al. (1980) cited that enhanced ovarian sensitivity to gonadotrophin is also important to achieve higher ovulation rate. Durrant et al. (1980) observed a positive correlation ($.30$, $P < .01$) between ovarian weight at d 2 after mating and

ovulation rate. They also found that selection for large litter size, a line with high ovulation rate, increased ovarian weights independently of body weight. These results agree with the results reported by Wiebold and Becker (1987), that dam weight had an important effect on ovulation rate. They also speculated that greater ovulation rate in the right ovary compared to the left ovary, could possibly be explained by greater amounts of gonadotrophin receptor.

Conversely, Lubritz et al. (1991) working with mice sampled from later generations of the lines started by Eisen (1978) and used by Durrant et al. (1980), observed higher responses to superovulation treatment in lines with lower natural ovulation rate. The explanation given by the authors was that the line with high ovulation rate, the line selected for high litter size, already had amounts of endogenous FSH and LH, or GnRH needed for high ovulation rates. So, treatment for superovulation with PMSG, which has FSH and LH activity, and with hCG, which has LH activity, had less supplemental value on increasing number of eggs shed. On the other hand, lines with lower ovulation rate, as the control line and a line selected for low litter size and greater body weight, probably did not have sufficient endogenous hormone levels for maximum ovulation rate and thus responded to superovulation. In these lines supplemental exogenous hormones, PMSG and hCG, were beneficial in increasing ovulation rate.

Embryonic survival (or embryonic mortality). By definition embryonic mortality refers to losses of embryos that occur before implantation; however, in the literature sometimes it includes fetal (post-implantation) mortality. Here we are going to use the terms embryonic and fetal mortality to losses occurring pre- and post-implantation, respectively. When both are combined, we are going to use the term total prenatal mortality.

There is one selection experiment with mice that used prenatal survival as the criterion of selection. Bradford (1969) reported results of two selected lines. One of the lines was selected on naturally ovulated females and the other line had superovulated females. Prenatal survival was expressed as the ratio of number of normal fetuses at 16 d of gestation to number of corpora lutea, the ratio was weighted by number of normal fetuses. This procedure was used in order to not select against number of corpora lutea at the same time as selecting for prenatal survival. Selection was based on full- and half-sib information. Selection in untreated (normally ovulated) females was successful to increase prenatal survival. Positive correlated responses to ovulation rate and litter size were observed. After 10 generations litter size in this line was more than 25% larger than in the control. Selection on superovulated females also increased prenatal survival and litter size, but not ovulation rate. Bradford (1979) reported results in subsequent generations of