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DIVERGENT SELECTION FOR UTERINE CAPACITY. EARLY EMBRYO MORTALITY

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ABSTRACT

An experiment of divergent selection for uterine capacity was performed during 10 generations and then selection was relaxed until the 18th generation. Early embryo mortality at 25 and 48 hours after mating was studied. A total of 127 intact multiparous females from the 17th generation were slaughtered 25 hours after natural mating, 69 females from the High line and 58 from the Low line. A total of 89 intact nulliparous females from the 18th generation were slaughtered 48 hours after natural mating, 50 females from the High line and 39 from the Low line. Ovulation rate (OR), fertilization rate (FR) and recovery rate (RR) were estimated in both experiments. The embryonic development stage was observed. No differences were found between lines in OR, FR and RR in both experiments. At 25 hours, the percentage of two cell stages *versus* four cell stages was similar in High and Low lines. At 48 hours, a relevant difference of percentage of 8-16 cell *versus* early morulae was found (-9.31%, (P>0)=0.08). Selection for uterine capacity may lead to modification of embryo development at 48 hours after mating, but no differences in embryo survival were found.

Key words: early mortality, rabbit, divergent selection, uterine capacity, Bayesian.

INTRODUCTION

Selection for uterine capacity has been proposed as an indirect method for improving litter size (ARGENTE *et al*., 1997). Uterine capacity is measured as litter size when ovulation rate is not a limiting factor (BLASCO *et al*., 1994). Uterine capacity thus depends on prenatal mortality. This mortality is divided in pre-implantation and post-implantation mortality, usually called embryo and fetal mortality respectively. Embryo mortality is around 10-14% (SANTACREU *et al*., 2000; GARCÍA and BASELGA, 2002). In a divergent selection experiment for uterine capacity, MOCÉ *et al*. (2004) found that the difference between lines in embryo mortality took place most of it before 72 hours of gestation. Furthermore, they observed that the line selected to increase uterine capacity had a higher embryonic development. These results indicate that selection for uterine capacity could affect both early embryo mortality and embryo development. The objective of this experiment is to find when these differences appear studying embryo mortality and development before 72 hours.

MATERIAL AND METHODS

Animals

Animals came from a divergent selection experiment for uterine capacity described by ARGENTE *et al.* (1997). Ten generations of divergent selection were performed, and then the selection was relaxed until the 18th generation. Two different experiments were carried out. A total of 127 intact multiparous females from the 17th generation were slaughtered 25 hours after natural mating, 69 females from the line selected to increase uterine capacity (High line) and 58 from the line selected to decrease uterine capacity (Low line). A total of 89 intact nulliparous females from the 18th generation were slaughtered 48 hours after natural mating, 50 females from the High line and 39 from the Low line.

Embryo recovery and classification: The number of corpora lutea per ovary was scored in both lines. The oviduct and the first one-third of the uterine horn were flushed in order to recover embryos and oocytes with 5 ml of Dulbecco's Phosphate Buffered Saline (®DPBS, Sigma) supplemented with 0.132 g/l calcium chloride, 0.2% Bovine Serum Albumin (®BSA, Sigma) and antibiotic (®Penivet 1, Divasa Farmavic) at room temperature. At 25 hours after mating, all embryos and oocytes recovered were classified as `no cleavage cell´, 2 cell and 4 cell stages. `No cleavage cell´ can be oocytes or one cell stage embryos. In order to differentiate them, they were dyed with Hoestch 33342 (® B-2261, Sigma) with 2-3% citrate sodium and absolute ethanol (® Cod.131086, Panreac) and evaluated by fluorescence microscopy. `No cleavage cells´ were classified as embryos when two dyed pronucleus were observed. At 48 hours after mating, embryos were classified as 8-16 cell stages or as early morulae. In both experiments, embryo classification was carried out by the same operator.

Traits

The following traits were analyzed. OR: Ovulation rate per ovary, estimated as number of corpora lutea. FR: Fertilization rate, estimated per horn as the ratio number of embryos recovered / total recovered (embryos + oocytes). RR: Recovery rate, estimated as the ratio total recovered / OR. Development stage: 2 cell (%): percentage of 2 cell stages per side (number of 2 cell embryos / all cleavage cell recovered), 4 cell (%): percentage of 4 cell stages (number of 4 cell embryos / all cleavage cell recovered), 8-16 cell (%): percentage of 8-16 cell stages (number of 8-16 cell embryos / total number of embryos recovered) and early morulae (%): percentage of early morulae (number of early morulae / total number of embryos recovered).

Statistical Analysis

The analysis was based on Bayesian methods. A repeatability animal model for all traits was fitted. Table 1 shows the environmental effects and their levels at 25 and 48 hours. At 48 hours, lactation and recovery effects were not considered because all females were nulliparous and the recoveries were in plate. Bounded uniform priors were used for all unknowns. Data were assumed to be normally distributed. Marginal posterior

distributions of all unknowns were estimated by using Gibbs Sampling. A chain of 20,000 samples with a burning period of 2,000 was used. Only one sample each 20 was kept for analysis. Converge was tested for each chain using the Z criterion of Geweke (see SORENSEN and GIANOLA, 2002).

Table 1. Models fitted for ovulation rate (OR), recovery rate (RR), fertilization rate (FR) and development stage.

Trait	Line [A]	Side [B]	Lactation [C]	Recovery [D]
OR	+	+	+	
RR	+	+	+	+
FR	+			
Development stage	+			

[A] Line: High and Low lines; [B] Side: Right and Left; [C] Lactation: Lactating or Non-lactating doe; [D] Recovery: Tube or Plate; [C,D] At 48 hours, these effects were not considered**.**

RESULTS AND DISCUSSION

Table 2 shows the raw means of all variables. In the experiment at 25 hours, the average of ovulation rate is similar to the ones obtained by other authors in maternal lines (13-14 ova per female) (review by BLASCO, 1993). Recovery rate and fertilization rate were high. Similar results were obtained by other authors in lines selected for reproductive traits (TORRES *et al.* 1987; GARCÍA-XIMÉNEZ and VICENTE (1992); BOLET and THEAU-CLEMENT, 1994). At 25 hours after mating, the majority of embryos were in two cell stages (86.6%), which agrees with other authors as MÉNÉZO and RENARD (1991) and GARCÍA-XIMÉNEZ and VICENTE (1992).

Table 2. Raw means and coefficients of variation (within brackets) by ovary and uterine horn for traits at 25 and 48 hours after mating.

	OR	RR	FR	Development stage (%)			
				2 cell	4 cell	8-16 cell	Early morulae
25 h.	7.22	0.87	0.98	86.6	13.4	-	-
	(0.31)	(0.19)	(0.09)	(0.27)	(0.30)		
48 h.	6.20	0.96	0.98	-	-	24.1	75.9
	(0.37)	(0.09)	(0.11)			(1.25)	(0.80)

OR: ovulation rate; RR: recovery rate; FR: fertilization rate.

Table 3 shows features of the estimated marginal posterior distributions of the differences between High and Low lines at 25 hours after mating. MCse were small and lack of convergence was not detected by Geweke test. Marginal posterior distributions were apparently normal, thus mode, mean and median were coincident. No difference between lines for ovulation rate was found. An advantage of the Bayesian approach through MCMC procedures is the possibility of easy construction of all kind of confidence intervals and probability computation. We can calculate not only the probability of difference being higher than zero, but also the probability of these differences being

economically or biologically relevant (P_r). Considering that 0.5 ova per ovary is a relevant difference, the probability of the difference in absolute value being higher than 0.5 is very small between lines (0.13). SANTACREU *et al.* (2000) and MOCÉ *et al*. (2004) did not find differences between both lines for ovulation rate either. No differences for fertilization rate and recovery rate were found, being (P>0)<0.95. A difference of less than 0.5 embryos or oocytes recovered by uterine horn is considered irrelevant. This leads to a difference of 7% in fertilization rate, recovery rate and percentage of development stage. The probability of relevant difference in fertilization rate and recovery rate is very small. Besides, there was no difference of percentage of 2 cell stages between lines.

Table 3. Features of the estimated marginal posterior distributions of the differences between High and Low lines at 25 hours after mating.

	OR	RR	FR	2 cells (%)
Mean	0.08	0.030	0.012	1.24
$HPD_{95\%}$	-0.60,0.64	-0.022,0.077	-0.020,0.042	-7.53,10.87
P>0	0.61	0.88	0.74	0.60
P_r	0.13	0.06	0.00	0.09
MCse	0.003	0.0003	0.0002	0.047
Z	0.56	-0.85	-0.12	-0.72

OR: ovulation rate; RR: recovery rate; FR: fertilization rate; $HPD_{95\%}$: highest posterior density region at 95%; P_r: Probability of relevant differences between lines. Absolute values for relevant differences: 0.5 for OR, and 7% for RR, FR and 2 cells (%); MCse: Monte Carlo standard error; Z: Z-score of the Geweke test.

In the experiment at 48 hours, females were nulliparous and the ovulation rate was lower than the experiment at 25 hours (Table 2). HULOT and MATHERON (1980) obtained the same difference when comparing ovulation rate between nulliparous and multiparous females. Recovery rate and fertilization rate were high, like in the experiment at 25 hours. Around 75% of embryos recovered were early morulae, as in MÉNÉZO and RENARD (1991) and GARCÍA-XIMÉNEZ and VICENTE (1992).

Table 4 shows features of the estimated marginal posterior distributions of the differences between High and Low lines at 48 hours after mating. MCse were small and lack of convergence was not detected by Geweke test. Marginal posterior distributions were apparently normal. The assumed values for relevant differences were the same. No differences for ovulation rate, fertilization rate and recovery rate between lines were found as in the experiment at 25 hours. High line seems to have a lower percentage of 8-16 cell (versus early morulae) than Low line, being (P>0)=0.08 and thus (P<0)=0.92. The difference between lines was apparently relevant (-9.31%), although due to the high highest posterior density region at 95**%** we could not state whether this difference was indeed relevant since P_r=0.58. MOCÉ *et al*. (2004) found that the High line showed a more advanced embryonic stage of development at 72 hours (18% more blastocysts). In other species like mice or pigs, some authors also observed an advancement of average

embryonic stage in lines select for reproductive traits (AL-SHOREPY *et al.,* 1992; HERRLER *et al.,* 1998).

Table 4. Features of the estimated marginal posterior distributions of the differences between High and Low lines at 48 hours after mating.

	OR	RR	FR	8-16 cells (%)
Mean	0.32	-0.027	0.007	-9.31
$HPD_{95\%}$	-0.55,1.03	-0.062,0.007	-0.024,0.039	-21.40,4.46
P>0	0.80	0.07	0.67	0.08
P_r	0.38	0.01	0.00	0.58
MCse	0.004	0.0002	0.0002	0.0652
Z	0.34	0.44	-0.82	-0.53

OR: ovulation rate; RR: recovery rate; FR: fertilization rate; $HPD_{95\%}$: highest posterior density region at 95%; P_r: Probability of relevant differences between lines. Absolute values for relevant differences: 0.5 for OR, and 7% for RR, FR and 8-16 cells (%); MCse: Monte Carlo standard error; Z: Z-score of the Geweke test.

Recovery rate was the same at 25 and 48 hours after mating in High and Low lines. However, MOCÉ *et al.* (2004) found that High line showed a higher recovery rate at 72 hours. Our results and the results of MOCÉ *et al.* (2004) indicate than these differences between lines appear between 25 and 72 hours after mating. Selection for uterine capacity may lead to a modification of the development stage between 25 and 48 hours. In this period of gestation, the formation of the zygote and the activation of the embryonic genome occur. The transition from maternal to zygotic control had been described at the 8-16 cell stages rabbit embryo (around 40 hours after mating) (KANKA, 2003). At this stage, all cells are transcriptionally active and synthesize both mRNA and rRNA. Furthermore, the transcriptionally active nucleoli appear during early morulae.

CONCLUSIONS

Selection by uterine capacity does not modify embryo mortality at 48 hours after mating but may modify the embryo development stage.

REFERENCES

AL-SHOREPY S.A., CLUTTER A.C., BLAIR R.M., NIELSEN M.K. 1992. Effects of three methods of selection for litter size in mice on pre-implantation embryonic development. *Biol. Reprod.* **46**:958-963.

ARGENTE M.J., Santacreu M.A., Climent A., Blasco A., Bolet G. 1997. Divergent selection for uterine effciency in rabbits. *J. of Anim. Sci.* **75**:2350-2354.

BOLET G., THEAU-CLEMENT M. 1994. Fertilization rate and preimplantation embryonic development in two rabbiy strains of different fecundity, in purebreeding and crossbreeding. *Anim. Reprod. Sci.* **36**:153-162.

BLASCO A., BIDANEL J.P., BOLET G., HALEY C.S. 1993. The gentics od prenatal survival of pigs and rabbits: a review. *Livestock Prod. Sci.* **37**:1-21.

BLASCO A., ARGENTE, M.J. HALEY C.S., SANTACREU M.A. 1994. Relationships between components of litter size in unilaterally ovariectomized and intact rabbit does. *J. of Anim. Sci.* **72**:3066-3072.

GARCIA M.L., BASELGA M. 2002. Estimation genetic response to selection in litter size rabbit using a cryopreserved control population. *Livestock Prod. Sci.* **61**:905-921.

GARCIA-XIMENEZ F., VICENTE J.V. 1992. Effect of ovarian cystic or haemorrhagic follicles on embryo recovery and survival after transfer in hCG ovulated rabbits. *Nutr. Reprod. Dev.* **32**:143-149.

HERRLER A., FRUSCHE A.C., BEIER H.M. 1998. Insulin and insuline-like growth factor-I promote rabbit blastocyst development and prevent apoptosis. *Biol. Reprod.* **59**:1302-1310.

HULOT F., MATHERON G. 1980. Comparaison de la reproduction de lapins de deux genotypes effects de l´age et de la saison. *2th World Rabbit Congress* **A***:* 293-302.

KANKA J. 2003. Gene expression and chromatin structure in the preimplantation embryo. *Theriogenology* **59**: 3-19.

MENEZO Y., RENARD J.P. 1991. La vie de l´œuf avant l´implantation. In: *La reproduction chez les mammifères et l´home.* (Edit. Thibault M.C.) INRA, pp. 339-358.

MOCE M.L., SANTACREU M.A., CLIMENT A., BLASCO A. 2004. The effect of divergent selection for uterine capacity on prenatal survival in rabbits: Maternal and embryonic genetic effects. *J.of Anim. Sci*. **82**:68-73.

SANTACREU M.A., ARGENTE M.J., MOCÉ M.L., BLASCO A. 2000. Selection for uterine capacity. II Response to selection estimated with a cryopreserved control population. *7th World Rabbit Congress* **A***: 491-496*.

SORENSEN D., GIANOLA D. 2002. Likelihood, Bayesian, and MCMC Methods in Quantitative Genetics. Springer. New York.

TORRES S., HULOT F., MEUNIER M., SEVELLEC C. 1987. Comparative study of preimplantation development and embryonic loss in two rabbit strains. *Reprod. Nutr. Dev* **27**:707-714.