

Divergent selection for uterine capacity in rabbits. II. Correlated response in litter size and its components estimated with a cryopreserved control population¹

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ABSTRACT: Our objective was to evaluate the correlated responses to selection for litter size and its components after 10 generations of divergent selection for uterine capacity (UC). A total of 294 intact females from the 11th and 12th generations of divergent selection for high and low UC and from a cryopreserved control population was used (139, 112, and 43 females, respectively). Uterine capacity was assessed as litter size in unilaterally ovariectomized females. Traits recorded on females for up to five parities were litter size (LS) and number born alive (NBA). Laparoscopy was performed in all females at d 12 of their second parity, and the

ovulation rate (OR) and number of implanted embryos (IE) were recorded in these females. Embryo survival (ES = IE/OR), fetal survival (FS = LS/IE), and prenatal survival (PS = LS/OR) were computed. Correlated responses in LS and in its components were inferred using Bayesian methods. Correlated responses in LS were asymmetric. The divergence between high and low lines was 2.35 kits, mainly because of a higher correlated response in the low line (1.88 kits). The lower LS in the low line was associated with a lower PS (control – low = 0.14), because of decreases in ES and FS.

Key Words: Litter Size, Ovulation Rate, Prenatal Survival, Rabbits, Uterine Capacity

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Introduction

Experimental selection on uterine capacity (UC) measured as litter size (LS) in unilaterally ovariectomized females (ULO) has been successful in rabbits (Argente et al., 1997; Blasco et al., 2005) and mice (Kirby and Nielsen, 1993). It is unclear, however, whether a larger UC is associated with an increase in LS of intact females, where both uterine horns are functional. In mice, Kirby and Nielsen (1993) found a favorable correlated response in LS when selecting for high UC, but selection for UC was not more effective than direct selection for increased LS.

Selection for increased UC has been proposed as a means to change prenatal survival (PS) (Bennett and Leymaster, 1989). In rabbits, differences in the number of implanted embryos at 6 d of gestation between divergent lines selected by UC were found in intact females (Mocé et al., 2002). In the mice experiment, selection for higher UC increased ovulation rate (OR) (Al-Shorepy et al., 1992; Ribeiro et al., 1996), and embryo survival (ES) until d 6 after mating (Ribeiro et al., 1996) in intact females.

In a companion paper, Blasco et al. (2005) presented results of a divergent selection experiment on UC in rabbits, where the trait was measured as LS in ULO does. The objective of this paper was to evaluate the correlated responses to selection for LS and its components, OR, and ES and fetal survival (FS), and to investigate whether this response was symmetric by contrasting both lines against a cryopreserved control population.

Materials and Methods

Animals

Animals came from a divergent selection experiment on UC described in Blasco et al. (2005). Uterine capacity was assessed as LS in ULO females (Blasco et al., 1994). After 10 generations of selection for UC, selection was

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Table 1. Number of does and litters by generation and line

Generation	Class	High	Low	Control
11	Does	78	64	26
	Litters	254	201	92
12	Does	61	48	17
	Litters	211	165	53

relaxed in Generations 11 and 12. Intact females (in which no ULO was performed) from high and low lines at Generations 11 and 12 were used in the experiment to assess correlated responses. To produce a control population, embryos from the base generation were vitrified and stored in liquid N₂ until transfer. Details of the technique are presented by Vicente and García-Ximénez (1996). After thawing and transferring the embryos, a total of 26 does of the control group was obtained, and these control does were contemporary to the does from the high and the low lines of Generation 11 (Table 1). The control population of Generation 11 was mated to produce the control population of Generation 12. Matings were planned to avoid inbreeding. Animals were housed at the experimental farm of the Universidad Politécnica de Valencia in individual metal cages. Animals were kept under controlled 16-h light:8-h dark photoperiods and fed a commercial diet (barley and wheat as the primary grains, wheat bran, barley straw, and alfalfa hay as the fiber source.). Does were mated first at 18 wk of age and 10 d after each parturition thereafter. Laparoscopies were performed on all does at d 12 of their second gestation, and corpora lutea and implanted embryos (**IE**) were counted. Details of the technique are given by Santacreu et al. (1990).

Traits

Traits analyzed in second parity does were OR, estimated as number of corpora lutea; number of IE, estimated as number of implantation sites; LS, estimated as total number of rabbits born; number born alive (**NBA**); PS, calculated as LS/OR; ES, calculated as IE/OR; and FS, calculated as LS/IE. The term embryo denotes fertilized oocytes before and during implantation, and the term fetus will be used from implantation to birth. All traits were measured only in the second parity, with the exception of LS and NBA, which were measured over four parities.

Statistical Analyses

A Bayesian analysis was conducted. Litter size and NBA were analyzed using a linear model including season-year with five levels, parity-lactation state with five levels (nulliparous does, lactating and nonlactating does of second parity, and lactating and nonlactating does with more than two parities), effect of line (high, low, and control), and effect of doe. The model used to analyze the traits of the second parity included the

effects of line, season-year, laparoscopy operator (with two levels), and lactation state (lactating and nonlactating does). Bounded uniform priors were used for all unknowns with the exception of doe effect, which was considered normally distributed with mean 0 and variance $\mathbf{I}\sigma_p^2$, where \mathbf{I} is a unity matrix, and σ_p^2 is the doe effect variance of the trait. Residuals were normally distributed with mean 0 and variance $\mathbf{I}\sigma_e^2$. The priors for the variances also were bounded uniform.

Features of the marginal posterior distribution of differences between line means were estimated using Gibbs sampling. After some exploratory analyses, we used two chains of 400,000 samples each, with a burn-in period of 100,000. Only one of every 60 samples was saved for inferences. Samples of the two chains were used to estimate features of posterior distributions. Convergence was tested for each chain separately using the Z criterion of Geweke (Sorensen and Gianola, 2002). Monte Carlo sampling errors were computed using time-series procedures described in Geyer (1992).

Results and Discussion

Control populations are particularly useful in divergent selection experiments because they allow detection of asymmetric responses. The main problem in the use of control populations is the need for employing experimental facilities that could be used by the lines that are selected. Another issue is that genetic drift can shift the control population from the expected value of the unselected trait. Although they have been rarely used in selection experiments, cryopreserved control populations have advantages, such as a better use of experimental facilities and a decrease in genetic drift. In addition, cryopreserved control populations eliminate effects of unintended selection on related traits that often occur and also decrease effects of natural selection.

Table 2 shows raw means and SD for traits measured in the control line. Results agree with values published in other lines of rabbits (Blasco et al., 1993). Table 3 shows features of the estimated marginal posterior distributions of the differences between lines for LS and NBA. Marginal posterior distributions had very small Monte Carlo SE, and the Geweke test did not detect lack of convergence in any case. Correlated response to selection in LS was not symmetric. A divergence between the high and low lines of 2.35 kits was found, mainly because of a higher correlated response in the low line. Although selection for UC has been proposed as an indirect way of improving LS (Bennett and Leymaster, 1989), the observed increase in LS caused by selection for UC was not greater than the improvement obtained from direct selection for LS in rabbits of approximately 0.1 rabbits per litter per generation (Rochambeau et al., 1998; García and Baselga, 2002a,b), which agrees with the findings of Kirby and Nielsen (1993) in mice. Correlated response in NBA was less than that for LS, and no significant response in the high line was detected. Correlated response in

Table 2. Means, SD, and number of litters for litter size (LS), number born alive (NBA), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS), and prenatal survival (PS) in the control line^a

Item	LS	NBA	OR	IE	ES	FS	PS
Mean	9.2	8.4	14.8	13.1	0.89	0.77	0.68
SD	3.0	3.1	2.9	3.1	0.13	0.17	0.18
No.	145	145	30	30	29	29	28

^aLS was estimated as total number of rabbits born, OR was estimated as number of corpora lutea, IE was the number of implanted embryos measured as number of implantation sites, ES = IE/OR, FS = LS/IE, and PS = LS/OR.

NBA also was asymmetric; however, Blasco et al. (2005) found that direct response to selection for UC was symmetric. Argente et al. (2000) found a large genetic correlation between LS and UC when analyzing data of some intact sibs of ULO does from this divergent selection experiment, which points to the hypothesis of an asymmetric response in UC not detected by Blasco et al. (2005). Nonetheless, Argente et al. (2000) used a small data set, and each doe had only one trait measured (either UC or LS); therefore, their result should be taken with caution. In addition, highest posterior density intervals containing a 95% probability are necessarily wide in this type of experiment, and analyses based on genetic trends as in Blasco et al. (2005) tend to be more informative.

The greater correlated response in the low line than in the high line might be due to a major gene affecting UC and LS segregating in the population. Argente et al. (2003) performed a complex segregation analysis with data from 10 generations of selection, finding evidence of a QTL with large effects on UC and number of IE. The estimated frequency of the favorable allele for UC was 0.7 in the base population of our divergent selection experiment. The high frequency of the favorable allele could be due to both divergent lines originating from a population previously selected for LS at weaning for 12 generations. If so, selection for UC would have been more efficient in the low line than in the high line. Complex segregation analysis is imprecise, but its results agree with the high divergence found by

Blasco et al. (2005) in the first and second generation of selection. Further, if UC is highly correlated with LS, as Argente et al. (2000) suggested, an asymmetric response in LS should occur. In mice, Falconer (1960) found asymmetric response to selection for LS and suggested that selection to decrease LS would lead to an increase of frequencies of alleles with deleterious effects. More research is needed to clarify this point, and an F2 experiment from high and low lines is being conducted to search for QTL related to LS, UC, and their components.

Tables 4 and 5 show features of the marginal posterior distributions of components of LS. As before, Monte Carlo SE were small, and the Geweke test did not detect lack of convergence in any case. A divergence between high and low lines of 1.79 IE was found. This agrees with results found at 72 h of gestation in intact and ULO does of Generations 13, 14, and 15 (Mocé et al., 2004). Differences in IE and LS between high and low lines were mainly due to differences in PS.

No clear correlated responses were found in the high vs. control lines for OR, IE, and ES (Tables 4 and 5). The correlated response in LS of the high line seems to be due to a correlated response in FS (Table 5), with P (high – control) > 0 being 0.88. In Bayesian analysis, a probability of 0.88 is the actual probability of the difference between both lines, neither a significant risk nor a P -value of classical analysis; thus, a researcher should evaluate whether this represents sufficient evidence. In the mice experiment, the line selected for high

Table 3. Features of the marginal posterior distributions of the differences between high and control line (H – C), control and low line (C – L), and high and low line (H – L) in litter size (LS) and number born alive (NBA)

Trait	Contrast	Mean	HPD _{95%} ^a	k ^b	$P > 0$ ^c	MC _{SE} ^d	Z ^e
LS	H – C	0.47	–0.28 to 1.20	–0.14	0.90	0.005	–0.23
	C – L	1.88	1.12 to 2.64	1.26	1.00	0.005	0.68
	H – L	2.35	1.81 to 2.89	1.91	1.00	0.003	0.53
NBA	H – C	0.15	–0.63 to 0.93	–0.49	0.65	0.005	–0.8
	C – L	1.69	0.89 to 2.49	1.04	1.00	0.005	0.68
	H – L	1.84	1.27 to 2.39	1.37	1.00	0.003	0.04

^aHighest posterior density region of 95% probability.

^bLimit of the interval [k, +∞) containing a probability of 95%.

^cProbability of the difference being larger than zero.

^dMonte Carlo SE.

^eZ of the Geweke criterion.

Table 4. Features of the marginal posterior distributions of the differences between high and control line (H – C), control and low line (C – L), and high and low line (H – L) in ovulation rate (OR) and number of implanted embryos (IE)

Trait	Contrast	Mean	HPD _{95%} ^a	k ^b	P > 0 ^c	MC _{SE} ^d	Z ^e
OR	H – C	-0.32	-1.40 to 0.76	-1.21	0.28	0.007	0.34
	C – L	0.75	-0.37 to 1.87	-0.16	0.91	0.007	0.01
	H – L	0.43	-0.27 to 1.13	-0.14	0.90	0.005	0.54
IE	H – C	-0.28	-1.54 to 0.98	-1.31	0.32	0.008	0.01
	C – L	2.07	0.79 to 3.35	1.03	0.99	0.008	0.27
	H – L	1.79	0.95 to 2.63	1.12	1.00	0.006	0.48

^aHighest posterior density region of 95% probability.

^bLimit of the interval [k, +∞) containing a probability of 95%.

^cProbability of the difference being larger than zero.

^dMonte Carlo SE.

^eZ of the Geweke criterion.

UC showed a correlated response in LS caused by a greater OR (Al-Shorepy et al., 1992; Ribeiro et al., 1996), and a greater ES until d 6 after mating (Ribeiro et al., 1996). In rabbits, there are no other results relating to correlated response from lines selected to improve UC. Experiments of selection on LS have been conducted with rabbits, with variable results in the correlated response for the components of LS. In studies of Rochambeau et al. (1994; two selected lines) and García and Baselga (2002a; one selected line), selection for LS increased OR without any change in PS; however, García and Baselga (2002b) found that a correlated response in FS in another line selected for LS. In selection experiments for prolificacy in mice, increased LS has been essentially due to increased OR (Falconer, 1960; Joakimsen and Baker, 1977; Bakker et al., 1978; Ribeiro et al., 1996). Bakker et al. (1978) and Ribeiro et al. (1996) also observed an increase in PS. In pigs, it seems that an increase of LS in a line selected for this trait was due to an increase in OR maintaining the same PS (Bolet et al., 1989).

The correlated response in LS in the low line might be partially due to a lower OR (Table 4), but it was

clearly associated with a lower PS (at least 7%; see the [k, +∞) interval with a 95% probability; Table 5), as well as lower ES and FS. Differences in ES may be due to differences in fertilization rate, embryo viability, or factors related to the oviduct or/and uterine physiology of the doe. Fertilization rate seems to be very high, and no differences have been found in fertilization rate between high and low lines in both intact and ULO does (Santacreu et al., 1996). Mocé et al. (2004), in intact and ULO does of our experiment, found that most of the differences in number of embryos before implantation between high and low lines were evident at 72 h of gestation, when embryos are still in the oviduct, whereas no differences were found from 72 h of gestation to 7 d of gestation, when implantation takes place in rabbits. Mocé et al. (2004) also found differences between high and low lines in FS. Fetal survival was affected by the recipient line, independent from the embryo donor line (high or low), suggesting that FS is a maternal trait. Current research is focused on a better assessment of the time of gestation at which embryo and fetal mortality occur and on the physiological aspects related to genetic analysis of these traits.

Table 5. Features of the marginal posterior distributions of the differences between high and control line (H – C), control and low line (C – L), and high and low line (H – L) in embryo survival (ES), fetal survival (FS), and prenatal survival (PS)

Trait	Contrast	Mean	HPD _{95%} ^a	k ^b	P > 0 ^c	MC _{SE} ^d	Z ^e
ES	H – C	0.00	-0.07 to 0.07	-0.05	0.55	0.0004	0.20
	C – L	0.10	0.03 to 0.17	0.04	0.99	0.0005	-0.42
	H – L	0.10	0.06 to 0.14	0.06	1.00	0.0003	-0.38
FS	H – C	0.04	-0.04 to 0.12	-0.02	0.88	0.0005	1.10
	C – L	0.08	0.00 to 0.16	0.02	0.98	0.0005	-0.20
	H – L	0.13	0.07 to 0.19	0.08	1.00	0.0003	1.49
PS	H – C	0.05	-0.03 to 0.13	-0.02	0.87	0.0005	0.62
	C – L	0.14	0.06 to 0.22	0.07	1.00	0.0005	-0.05
	H – L	0.19	0.13 to 0.25	0.14	1.00	0.0003	0.91

^aHighest posterior density region of 95% probability.

^bLimit of the interval [k, +∞) containing a probability of 95%.

^cProbability of the difference being larger than zero.

^dMonte Carlo SE.

^eZ of the Geweke criterion.

Implications

Selection for high uterine capacity in rabbits was not more effective at increasing prolificacy than direct selection for litter size. Genes controlling litter size act at different stages of gestation, and correlated response in litter size depends on both embryo and fetal survival.

Literature Cited

- Al-Shorepy, S. A., A. C. Clutter, R. M. Blair, and M. K. Nielsen. 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., A. Blasco, J. A. Ortega, C. S. Haley, and P. M. Visscher. 2003. Analyses for the presence of a major gene affecting uterine capacity in unilaterally ovariectomized rabbits. *Genetics* 163:1061–1068.
- Argente, M. J., M. A. Santacreu, A. Climent, and A. Blasco. 2000. Genetic correlations between litter size and uterine capacity. *Proc. 7th World Rabbit Cong., Valencia, Spain A:333–338.*
- Argente, M. J., M. A. Santacreu, A. Climent, G. Bolet, and A. Blasco. 1997. Divergent selection for uterine capacity in rabbits. *J. Anim. Sci.* 75:2350–2354.
- Bakker, H., J. H. Wallinga, and R. D. Politiek. 1978. Reproduction and body weight of mice after long-term selection for large litter size. *J. Anim. Sci.* 46:1572–1580.
- Bennett, G. L., and K. A. Leymaster. 1989. Integration of ovulation rate, potential embryonic viability and uterine capacity into a model of litter size in swine. *J. Anim. Sci.* 67:1230–1241.
- Blasco, A., M. J. Argente, C. S. Haley, and M. A. Santacreu. 1994. Relationships between components of litter size in unilaterally ovariectomized and intact rabbit does. *J. Anim. Sci.* 72:3066–3072.
- Blasco, A., J. P. Bidanel, G. Bolet, C. S. Haley, and M. A. Santacreu. 1993. Genetic variability in prenatal survival of polytocous species. A review. *Livest. Prod. Sci.* 37:1–21.
- Blasco, A., J. A. Ortega, M. A. Santacreu, and A. Climent. 2005. Divergent selection for uterine capacity in rabbits. I. Genetic parameters and response to selection. *J. Anim. Sci.* 83:2297–2302.
- Bolet, G., L. Ollivier, and P. Dando. 1989. Sélection sur la prolificité chez le porc. I. Résultats d'une expérience de sélection sur onze générations. *Genet. Sel. Evol.* 21:93–106.
- Falconer, D. S. 1960. The genetics of litter size in mice. *J. Cell. Comp. Physiol.* 56(Suppl. 1):153–167.
- García, M. L., and M. Baselga. 2002a. Estimation of genetic response to selection in litter size of rabbits using a cryopreserved control population. *Livest. Prod. Sci.* 74:45–53.
- García, M. L., and M. Baselga. 2002b. Genetic response to selection for reproductive performance in a maternal line of rabbits. *World Rabbit Sci.* 10(2):71–76.
- Geyer, C. M. 1992. Practical Markov Chain Monte Carlo (with discussion). *Statist. Sci.* 7:467–511.
- Joakimsen, O., and R. L. Baker. 1977. Selection for litter size in mice. *Acta Agric. Scand.* 27:301–318.
- Kirby, Y. L., and M. K. Nielsen. 1993. Alternative methods of selection for litter size in mice. III. Response to 21 generations of selection. *J. Anim. Sci.* 71:571–578.
- Mocé, M. L., M. A. Santacreu, and A. Climent. 2002. Effect of divergent selection for uterine capacity on progesterone, estradiol and cholesterol levels around implantation time in rabbits. *World Rabbit Sci.* 10(3):89–97.
- Mocé, M. L., M. A. Santacreu, A. Climent, and A. Blasco. 2004. The effect of divergent selection for uterine capacity on prenatal survival in rabbits: Maternal and embryonic genetic effects. *J. Anim. Sci.* 82:68–73.
- Ribeiro, E. L. A., M. A. van Engelen, and M. K. Nielsen. 1996. Embryonal survival to 6 days in mice selected on different criteria for litter size. *J. Anim. Sci.* 74:610–615.
- Rochambeau, H., G. Bolet, and F. Tudela. 1994. Long-term selection. Comparison of two rabbit strains. *Proc. 5th World Cong. Genet. Appl. Livest. Prod., Guelph, ON, Canada* 19:257–260.
- Rochambeau, H., R. Duzert, and F. Tudela. 1998. Long-term selection experiment in rabbit. Estimation of genetic progress on litter size at weaning. *Proc. 6th World Cong. Genet. Appl. Livest. Prod., Armidale, Australia* 26:112–115.
- Santacreu, M. A., A. Climent, M. Gallego, L. Fayos, and A. Blasco. 1996. Fertilization rate and early embryo development in two rabbit lines selected on uterine capacity. *Proc. 6th World Rabbit Cong., Toulouse, France* 2:355–357.
- Santacreu, M. A., M. P. Viudes, and A. Blasco. 1990. Evaluation par coelioscopie des corps jaunes et des embryons. Influence sur la taille de portée chez la lapine. *Reprod. Nutr. Dev.* 30:583–588.
- Sorensen, D., and D. Gianola. 2002. *Likelihood, Bayesian and MCMC Methods in Quantitative Genetics.* Springer, New York, NY.
- Vicente, J. S., and F. García-Ximénez. 1996. Direct transfer of vitrified rabbit embryos. *Theriogenology* 45:811–815.