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ANALYSES FOR THE PRESENCE OF A MAJOR GENE AFFECTING UTERINE CAPACITY IN UNILATERALLY OVARIECTOMIZED DOES

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ABSTRACT

The presence of a major gene was investigated for uterine capacity, ovulation rate and number of implanted embryos in a population of rabbit divergently selected for uterine capacity. Bartlett's test, Fain's test and a Bayesian segregation analysis were used to test for the presence of a major gene. All three tests showed that the data appeared consistent with the presence of a major gene affecting the number of implanted embryos. Tests for the segregation of major genes for ovulation rate and uterine capacity were inconclusive. These results suggest that genes affecting the number of implanted embryos could be mapped using this population.
Key Words: Rabbits, uterine capacity, ovulation rate, implanted embryos, major genes

INTRODUCTION

Major genes or quantitative trait loci (QTL) have been detected for litter size (Janss et al., 1997, in pigs) and its components ovulation rate and uterine capacity (Rothschild et al., 1996 and Rohrer et al., 1999, in pigs; Messer et al., 1999, in mice). The inclusion of major gene information could improve efficiency of selection schemes and would improve the understanding of the biology of reproductive traits. In rabbits, we do not know if there are major genes affecting the components of litter size. A divergent selection experiment on uterine capacity showed a large divergence between lines in the first generation of selection (Blasco et al., 2000), indicating the possible existence of a major gene controlling this trait. The objective of this paper is to analyse for the presence of a major gene for uterine capacity in rabbits.

MATERIAL AND METHODS

Animals

Rabbits came from a divergent selection experiment on uterine capacity in unilaterally ovariectomized does (ULO) described in Blasco et al. (2000). Uterine capacity (UC) was estimated as litter size in ULO does (which have only one functional uterine horn). Both lines were derived from a synthetic breed previously selected for litter size for 12 generations.

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Each divergent line had approximately 40 females and 12 males per generation, each female had up to four parities, and data from ten generations of selection were analysed. Data were from 929 does. Table 1 shows the number of records used in the experiment.

Table 1. Number records per generation and per selection line.

	Base Generation			**ULO +**			**ULO -**		
	UC	**OR**	**IE**	**UC**	**OR**	**IE**	**UC**	**OR**	**IE**
G0	196	61	61						
G1				125	35	35	119	34	34
G2				162	44	44	139	36	36
G3				108	28	28	131	37	37
G4				122	31	31	116	33	33
G5				122	35	35	113	30	30
G6				178	46	46	115	30	30
G7				164	31	31	103	18	18
G8				171	36	36	160	30	30
G9				125	25	25	152	33	33
G10				176	38	38	199	44	44

UC:Uterine capacity. OR:Ovulation rate. IE:Number of implanted embryos. ULO+:High line of uterine capacity. ULO-:Low line of uterine capacity.

Surgical Techniques

The left ovary was removed before puberty in ULO does. A laparoscopy was performed on all does at day 12 of their second gestation, and corpora lutea and implanted embryos were counted. The description of the unilateral ovariectomy technique can be found in Blasco et al. (1994). The laparoscopy technique is described in Santacreu et al. (1990).

Traits

OR: total ovulation rate estimated as the number of corpora lutea. IE: total number of implanted embryos estimated as number of implantation sites. UC: total number of rabbits born in ULO does. All the traits were measured in the second parity with the exception of UC, which was measured in up to four parities.

Statistical Analysis

Genetic parameters were estimated by multivariate residual maximum likelihood (REML). UC was analysed with a repeatability animal model, with year-season and lactation as fixed effects. OR and IE were analysed with animal models having year-season and lactation as fixed effects. The VCE statistical package was used for all REML analyses (Groeneveld, 1994).

To test for the presence of major genes for UC, OR and IE, Bartlett's test (Sokal and Rohlf, 1995) was used to test for heterogeneity of within half-sib family variance. The

regression of family variances on family means (Fain, 1978) was performed as well. The regression model was: $V = b_0 + b_1\mu + b_2\mu^2$. Where V is the within family variance and μ is the family mean. A fixed effect of generation was included in the model. The SAS statistical package was employed for these first exploratory analyses (SAS, 1996). For the segregation analysis, a mixed model was used with non-genetic effects of year-season and lactation, genetic effects of background polygenes and the effect of one major gene. Polygenic effects were modelled to be additive. The single major locus was assumed to be autosomal and biallelic with Mendelian transmission probabilities. A possible dominance effect at the single major locus was allowed for. The effects of the single gene were represented by the "a" and "d" for the additive and the dominant effects. The genotypic values of recessive homozygous, heterozygous and dominant homozygous were respectively "–a", "d" and "a". For UC, a repeatability model was used. The MaGGic statistical package was used for these analyses (Janss et al., 1995). Statistical inference was based on a Bayesian approach, with flat prior distributions on [-∞; +∞] for nongenetic effects and the effect at the single locus, on [0; +∞] for the variance components and on [0;1] for the allele frequencies. Gibbs sampling procedure was used to estimate the marginal posterior distributions. A single chain of size 250000 was run. After discarding the first 2000 iterations, samples were saved every 500 iterations, so the total number of samples saved was 497.

RESULTS AND DISCUSSION

In the first generation of this divergent selection experiment, a high difference between lines for uterine capacity was found (Blasco et al., 2000). This difference increased slowly until the end of the experiment. After ten generations of divergent selection on uterine capacity, intact does of this experiment were compared with a control population. The ULO─ line had a litter size (with both uterine horns being functional) of 2.1 less kids than the control, whereas ULO+ had 0.6 more kids than the control line (Santacreu et al., 2000). Since litter size has a high genetic correlation with UC (Argente et al., 2000), this suggests that selection on UC has been highly effective to reduce UC in the low line, but much less effective to improve UC in the high line. Moreover, heritability of UC and IE in the high line were lower that in the low line (table 2). These results are consistent with the segregation of a major gene of large effect for UC and IE in the ULO- line.

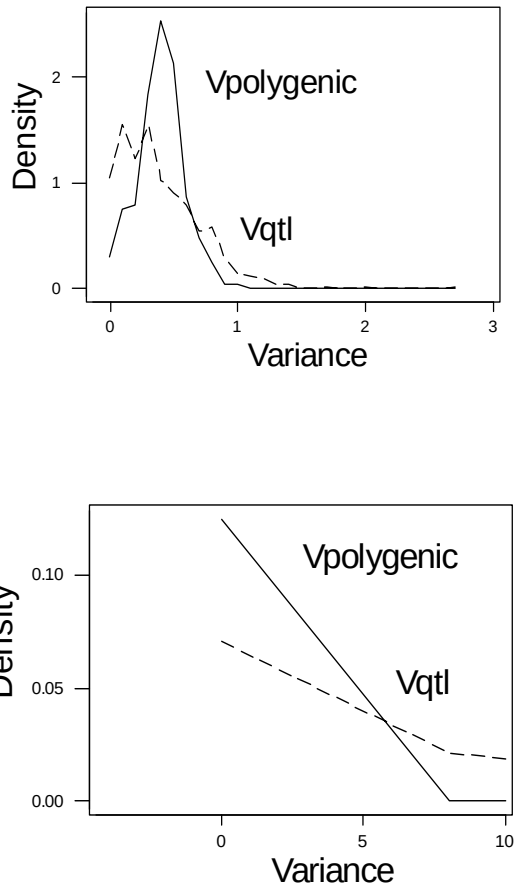
Table 2. Genetic parameters for uterine capacity (UC), ovulation rate (OR) and number of implanted embryos (IE) from REML analyses.

	h^2_{UC}	Per_{UC}	h^2_{OR}	h^2_{IE}
ULO+	0.06 ± 0.03	0.12 ± 0.03	0.39±0.07	0.23±0.05
ULO─	0.16 ± 0.04	0.08± 0.03	0.34±0.05	0.49±0.05

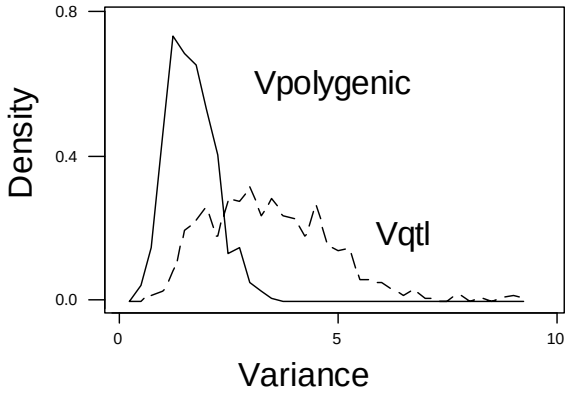
h^2:Heritability. per_{UC}:permanent environmental effect. ULO+:High line of uterine capacity. ULO─:Low line of uterine capacity.

Within-family variances were heterogeneous (Bartlett's test) for UC ($P<0.001$) and IE ($P<0.05$). This is consistent with the presence of a major gene segregating for these traits but not for OR. Fain's test showed that linear (b_1) and quadratic (b_2) regression coefficients were not significantly different from zero for UC but they were different from zero for OR (b_1, $P< 0.05$; b_2, $P<0.05$) and IE (b_1, $P< 0.02$; b_2, $P<0.06$).

The Bayesian approach for segregation analysis allowed the estimating of marginal posterior distributions for the polygenic variance ($V_{polygenic}$) and single-gene variance (V_{qtl}) in a mixed inheritance model (figure 1). Statistical inferences are based on the shapes of the estimated posterior distribution of variance components. Presence of a single-gene segregating would show a posterior distribution with a global mode for $V_{qtl}>0$, which may still be accompanied by a local mode or nonzero density at $V_{qtl}=0$. In this case, the major gene variance was concluded to be different from zero when the global mode had a density 20 times larger than density at $V_{qtl}=0$, corresponding to a 5% significance level (Janss et al., 1995). As can be see in figure 1, UC showed polygenic variance but a low effect of a single gene, OR showed a poor convergence and for IE the presence of a major gene was detected in addition to polygenic variance. Messer et al. (1999) have mapped QTL for OR and UC.



(a) UC (b) OR



(c) IE

Figure 1. Estimated marginal posterior distributions for polygenic variance ($V_{polygenic}$) and single-gene variance (V_{qtl}) in mixed inheritance model for (a) uterine capacity, (b) ovulation rate and (c) the number of implanted embryos.

Table 3 shows the different genetic parameters for IE estimated from the segregation analysis. A major gene was found to be segregating and the estimated frequency of the allele which reduces IE was 0.29± 0.07. This result could explain that selection on uterine capacity was more effective in decreasing the litter size in ULO does than increasing it. The additive effect (a) was estimated to be 3 kids, which would make a difference between the two homozygous of 6 kids. This difference is high when compared to the phenotypic difference between lines. Janss et al. (1997) found a difference between homozygous genotypes of 6 pigs for litter size, but in their case phenotypic difference was higher. The gene was found to be completely dominant (a=d). The estimate of the polygenic heritability (Vpolygenic/Vtotal) was similar in both models. The proportion of total variance due to the major gene (using the additive variance only) was approximately 15%.

Table 3. Estimated means and standard deviation (SD) of posterior densities for genetic parameter for the number of implanted embryos (IE).

	a ± SD	d ± SD	q ± SD	h^2 ± SD	h^{2*} ± SD	h_{qtl}^2 ± SD
IE	3.16 ± 0.33	3.48 ± 0.45	0.29 ± 0.07	0.21 ± 0.07	0.19 ± 0.06	0.15 ± 0.08

a: Additive effect. d: Dominant effect. q: Frequency of unfavourable allele. h^2: Heritability without major gene in the model (Vpolygenic/Vtotal). h^{2*}: Heritability with major gene in the model (Vpolygenic/Vtotal). h_{qtl}^2: Heritability of the major gene (Va/Vtotal; with Va= the additive variance due to the major gene).

Methods to detect the segregation of genes of large effects on quantitative traits from phenotypic data only are notoriously difficult and usually not robust to violations of assumptions such as normality of residuals and homogeneity of variances. Therefore, results from variance analysis should be treated with caution, and conclusive evidence for the segregation of QTL of large effects should be obtained from collecting genotypic (marker) data. Nevertheless, in summary, all our results suggest that there may be genes of large effects on the number of implanted embryos segregating in this population. There is inconclusive evidence for the segregation of major genes for ovulation rate and uterine capacity.

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REFERENCES

Argente, M.J., Santacreu, M.A., Climent, A., Blasco, A. 2000. Genetic correlations between litter size an uterine capacity. 7th World Rabbit Congress. Valencia. 4-7 July 2000.

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. Anim. Sci. 72:3066-3072.

Blasco, A., Ortega, J.A., Santacreu, M.A. 2000.Selection for uterine capacity: I Genetic trends and correlated response in components of litter size. 7th World Rabbit Congress. Valencia 4-7 July 2000

Fain, P.R. 1978. Characteristics of simple sibship variance tests for the detection of major loci and application to height, weight and spatial performance. Ann. Hum. Genet. 42: 109-120.

Groeneveld, E. 1994. VCE-A multivariate multimodel REML (co)variance component estimation package. 5th World Congress on Genetics Applied to Animal Production, Guelph, 1994. Vol 22:47.

Janss, L.L.G., Thompson, R., Van Arendonk, J.A.M. 1995. Application of Gibbs sampling for inference in a mixed major gene-polygenic inheritance model in animal population. Theor. Appl. Genet. 91: 1137-1147.

Janss, L.L.G., Johan, A. M., Van Arendonk, J.A.M., Brascamp, E.W.P. 1997. Segregation analysis for presence of major genes affecting growth, backfat, and litter size in Dutch Meishan crossbreds. J. Anim. Sci. 75: 2864-2876.

Messer, L., Mousel, M., Nielsen, M., Pomp, D. 1999. Identification of quantitative trait loci (QTL) affecting components of litter size in unilaterally ovariectomized mice. Plant & Animal Genome VII conference. 340.

Rohrer, G.A., Ford, J.J., Wise, T.H., Vallet, J.L., Christenson, R.K. 1999. Identification of quantitative trait loci affecting females reprodutive traits in a multigeneration Meishan-White composite swine population. J. Anim. Sci. 77:1385-1391.

Rothschild, M., Jacobson, C., Vaske, D., Tuggle, C., Wang, L., Short, T., Eckardt, G., Sasaki, S., Vincent, A., McLaren., D., Southwood, O., Van der Steen, H., Mileham, A., Plastow, G. 1996. The estrogen receptor locus is associated with a major gene influencing litter size in pigs. Proc. Natl. Acad. Sci. 93:201-205.

S.A.S.1996. SAS/STAT User's Guide (Release 6.12). SAS. Inst. Inc., Cary, NC.

Santacreu, M.A., Viudes, M.P., Blasco, A. 1990. Evaluation par coelioscopie des corps jaunes et des embryons . Influence sur la taille de protee chez la lapine. Reproduc. Nutr. Dev. 30:583-588.

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. Valencia 4-7 July 2000

Sokal, R.R., Rohlf, F.J. 1995. Biometry. 2nd Ed.W.H. Freeman and Co., NY.