EFFECT OF OVIDUCTINE (*OVPG1*) GENE FOR EMBRYO SURVIVAL AND DEVELOPMENT IN A F2 RABBIT CROSS

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ABSTRACT

The aim of this study was to analyze the association between the effect of 1413 C>G SNP and the microsatellite located in the promoter region of the *OVPG1* gene and the embryo survival and development at 48 and 72 h of gestation in an F2 population. This population was obtained by crossing two lines divergently selected for high and low uterine capacity. The GG genotype for 1413 C>G SNP and the allele 455 for the microsatellite were more frequent in the low line. A total of 172 and 159 female rabbits were slaughtered at 48 and 72 h of gestation. The ovulation rate, the fertilization rate, early embryo survival and embryonic stage of development were recorded at 48 and 72 h of gestation. At 72 h of gestation, the GG genotype had higher early embryo survival (P(D<0)=86%; D_m=-0.56 embryos; Pr=73%) and percentage of early morulae (P(D<0)=86%; D_m=-10.32%; Pr=60%) than the CC genotype. With respect to the microsatellite located in the promoter region, the -/- genotype had higher ovulation rate than the 455/455 genotype. At 48 h of gestation, we found similar fertilization rate in both homozygote genotypes (Ps=66%) but the -/- genotype had higher early embryo survival and percentage of early morulae than the 455/455 genotype (Pr=85% and 78%). At 72 h of gestation, the -/- genotype had higher fertilization rate, early embryo survival and percentage of early morulae and lower percentage of blastocysts than the 455/455 genotype. The conclusions of this study were that the GG genotype of the SNP and the 455 allele of the microsatellite located in the promoter region of the *OVPG1* gene were associated with a better embryo survival and development at the first stages of gestation.

Key words: Oviductine gene, Embryo survival and development, F_2 population.

INTRODUCTION

A segregation analysis performed by Argente *et al.* (2003) found an evidence for the existence of a major gene with a moderate effect on uterine capacity and a large effect on number of implanted embryos in two lines of rabbits divergently selected for uterine capacity (Argente *et al*., 1997). The mammalian oviduct synthesizes and secretes many proteins, among which are a family of glycoproteins named oviductins. In many species including the rabbit (Oliphant *et al*., 1984), swine (Buhi and Alvarez, 2003), sheep and cattle (Nancarrow and Hill, 1995), oviductins bind to the zona pellucida of postovulatory oocytes during their transit in the oviduct. The sperm treated with oviductins became capacitated (Killian, 2004), and ova pre-treated with the oviductins improved fertilization and embryo development rates (Buhi *et al*., 1993). Merchán *et al.* (2007) reported the full sequence and structure of the rabbit *OVPG1* gene. An 1413 C>G SNP was found in exon 11 of the oviductine gene, determining the amino acidic change Gly/Arg, when oviductine cDNA was amplified and sequenced in 25 animals from two lines selected divergently for uterine capacity (Merchán, 2007). Moreover, a triallelic microsatellite located in the promoter region was reported; the allele $(GT)_{11}T(G)_7$ was named 450, and the alleles $(GT)_{14}(G)_5$ and $(GT)_{15}T(G)_5$ were named 455 and 457, respectively. An association was detected between the lines and the frequency of each mutation: the

allele C and microsatellite 457 were more frequent in the line selected to increase uterine capacity. The objective of this study was to analyze the effect of 1413 C>G SNP and the microsatellite located in the promoter region of the *OVPG1* gene on embryo survival and development at 48 and 72 h of gestation in a F2 population derived from the lines selected by uterine capacity.

MATERIALS AND METHODS

Animals and experimental design

An F2 rabbit population was generated from the reciprocal cross of High (H) and Low (L) lines of a divergent selection experiment on uterine capacity (Argente *et al*., 1997). Details for the lines, the breeding schemes and the crossbreeding are given by Peiró *et al.* (2007). The F2 animals were reared at the experimental farm of the Universidad Miguel Hernández de Elche. A total of 172 and 159 non-lactating females were mated and slaughtered at 48 or 72 h of their fifth gestation, respectively, by intravenous injection of sodium thiopental in a dose of 50 mg/kg body weight (® Tiobarbital, B. Braun Medical S.A., Barcelona, Spain). All data came from non-lactating females, the range in the interval between the last weaning and the slaughter of female was from 3 to 140 days. The reproductive tract was collected after slaughtering. The number of corpora lutea was recorded on both ovaries (OR). Oviducts and uterine horns were separated and flushed once with 5 and 10 ml of 150 mM ammonium bicarbonate solution at room temperature in order to recover and count the number of cleaved and uncleaved ova. The total number of embryos (TE) and oocytes (OO) were estimated as the number of cleaved and uncleaved ova, respectively. Embryos were classified as normal (NE) or abnormal (AE) according to morphological criteria (Hafez, 2000). At 48 h of gestation, all embryos and oocytes were recovered from oviducts (96% of the total ova shed). The normal embryos were classified as early morulae (EM) or compact morulae (CM). At 72 h of gestation, embryos and oocytes were recovered from oviducts and uterine horns (86% of the total ova shed). The normal embryos were classified as EM, CM and blastocysts (B).

Traits

Variables measured were: ovulation rate (OR), fertilization rate (% FR = 100 x (TE/TE + OO)), percentage of early morulae (% EM = 100 x (EM/NE)), percentage of compacted morulae (% CM = 100 x (CM/NE)) and percentage of blastocysts (% B = 100 x (B/NE)). Early embryo survival (EES) was analyzed as the number of normal embryo recovered fitting ovulation rate as a covariate.

Genotyping of the Rabbit *OVPG1* gene in a F_2 population

Genomic DNA was extracted from blood samples following the protocol of the ABI PRISM™ 6100 Nucleic Acid PrepSation (Applied Biosystems). We designed a pair of primers on exon 11 based on the sequence of the rabbit oviductin cDNA (GenBank accession number AF347052) and a PCR fragment of 174 bp was amplified. Genotyping of the 1413C>G SNP was performed by Pyrosequencing (Pyrosequencing AB) in a PSQ HS 96 instrument. The animals genotyped as G/G_{1413}, C/G_{1413} and C/C_{1413} are named GG, CG and CC, respectively. Genotyping of the microsatellite was preformed with primers OVID-P4-F-FAM (fluorescently labelled with FAM) and OVID-I1-R2. The PCR reaction was performed as describe above for the promoter amplification, in a 15µl final volume and a hybridization temperature of 65ºC. Labelled PCR products were analyzed by capillary electrophoresis in an ABI 3730 DNA Analyzer (Applied Biosystems) and automatically sized relative to an internal standard (CST ROX 70-500, BioVentures, Inc., Murfreesboro, TN) with the GeneMapper™ v4.0 software (Applied Biosystems). For the association study of this microsatellite, the genotypes have been grouped based on previous analyses of the reproductive traits (Merchán, 2007). The genotypes 450/450, 457/457 and 450/457 have been grouped and named -/-, the genotypes 455/450 and 455/457 have been grouped and named 455/-, and the genotype 455/455 have been named 455/455.

Statistical Analysis

Ovulation rate at 48 and 72 h of gestation was analyzed with the following model:

$$y_{ijklmno} = \mu + YS_i + FH_j + I_k + O_l + G_m + S_n + e_{ijklmno}$$

where YS_i was the effect of year-season (with 3 levels), FH_j was the effect of hemorrhagic follicles (with 3 levels: zero, between one to five follicles and six or more), I_k was the effect of interval between weaning of their fourth litter and mating for slaughtering (with 2 levels: until one month or more), O_l is the effect of operator (with 3 levels) and G_m is the effect of *OVPG1* gene genotype (with 3 levels: CC, CG and GG, for SNP 1413C>G, or -/-, 455/- and 455/455 for microsatellite located in the promoter region) and S_n was the effect of the time of gestation (with 2 levels: 48 h and 72 h after mating). Fertilization rate and embryonic stage of development at 48 h of gestation were analyzed using the following model:

$$y_{ijklmn}=\mu + YS_i+FH_j+I_k+O_l+G_m+e_{ijklmn}$$

where the effects are described before.The EES was analyzed including OR as a covariate. Fertilization rate and embryonic stage of development at 72 h of gestation were analyzed using the following model:

$$y_{ijklmno} = \mu + YS_i + FH_j + I_k + O_l + G_m + U_n + e_{ijklmno}$$

where U_n was the effect of presence or absence of embryos in the uterus. The other effects were described before. The EES was analyzed including OR as a covariate. Traits were analyzed using a Bayesian approach. Data are conditionally distributed as:
$y \mid b, \sigma^2_e \sim N(Xb, I\sigma^2_e)$ where b contains the effects to be estimated. The known incidence matrix is X and I is the identity matrix. Bounded uniform priors were used for all unknown parameters. Marginal posterior distributions of all unknowns were estimated using Gibbs sampling. A chain of 120,000 samples with a burn-in period of 20,000 was used. Convergence was tested using Z criterion of Geweke (Sorensen and Gianola, 2002) and Monte Carlo sampling errors were computed using time-series procedures described by Geyer (1992). We calculate the posterior mean of the difference between genotypes (D_m,), the highest posterior density region at 95% ($HPD_{95\%}$) and the probability that the difference between genotypes (P) being higher than zero, when this difference was positive, or lower than zero, when this difference was negative. Also, we assumed a relevant value (R) for each trait, that is a quantity under which this difference has no biological or economical meaning. We also estimate the probability of this difference being lower in absolute value than relevant value (Ps). When the marginal posterior mean of the difference between genotypes is higher than zero, Pr is the probability that the difference is higher than R. However, if the marginal posterior mean of the difference between genotypes is lower than zero, Pr is the probability that the difference being lower than –R.

RESULTS AND DISCUSSION

Table 1 shows the number of females by genotype and time of slaughter. Features of the estimated marginal posterior distributions of the difference (D) between the CC and the GG genotypes for the 1413C>G SNP of the *OVPG1* gene for OR, FR, EES and embryo development are presented in Table 2.

Table 1: Number of females by genotype, time of slaughtered and trait

	1413C>G SNP				Microsatellite			
	Genotype				Genotype			
	CC	CG	GG	Total	(-/-)	(455/-)	(455/455)	Total
Ovulation Rate	121	163	47	331	174	235	21	330
Traits at 48 h	40	58	21	119	62	48	10	120
Traits at 72 h	50	52	10	112	60	46	7	113

The CC and GG genotypes showed similar OR (D_m=0.39 ova). At 48 h of gestation, there was no relevant differences between the homozygote genotypes in FR (Ps=90%). At this time of gestation, we did not find relevant differences in EES and %EM (0.03 embryos and -6.16%, respectively) between

both homozygote genotypes, although both precisions were low. At 72 h of gestation, we consider that both genotypes had similar FR because the difference was irrelevant (D_m=-3.02%). The GG genotype had higher EES (P(D<0)=86%; D_m=-0.56 embryos; Pr=73%) and % EM (P(D<0)=86%; D_m=-10.32%; Pr=60%) than the CC genotype. These results agree with previous results obtained in the F2 population, where the GG genotype (more frequent in the L line) presented higher number of implanted embryos and litter size (Merchán *et al*., 2006) than the CC genotype.

Table 2: Features of the estimated marginal posterior distributions of the differences (D) between the CC and GG genotypes for 1413C>G SNP of the *OVPG1* gene in ovulation rate (OR), fertilization rate (%FR), early embryo survival (EES) and percentage of early morulae (%EM), of compacted morulae (%CM) and of blastocysts (%B) in the F_2 population at 48 and 72 h of gestation

	D_m	$HPD_{95\%}$	P (%)	R	Ps (%)	Pr (%)	MCse	Z
OR	0.39	-0.37 , 1.24	83	0.5	59	40	0.005	-0.54
48 h of gestation								
% FR	-1.77	-4.31 , 0.83	91	3.5	90	10	0.015	-0.12
EES	0.03	-0.64 , 0.70	54	0.25	53	26	0.004	-1.11
% EM	-6.16	-21.97 , 7.08	79	8	57	40	0.084	-0.10
72 h of gestation								
% FR	-3.02	-7.34 , 1.45	91	3.5	58	42	0.025	-0.15
EES	-0.56	-1.72 , 0.38	86	0.25	21	73	0.006	1.11
% EM	-10.32	-28.92 , 7.32	86	8	38	60	0.106	-0.89
% CM	2.48	-19.72 , 23.55	58	8	51	31	0.126	-0.89
% B	7.60	-7.65 , 22.07	83	8	52	48	0.087	-0.89

D_m, posterior mean of the difference between the CC and GG genotypes; $HPD_{95\%}$, highest posterior density region at 95%; P, P(D>0) when D>0 and P(D<0) when D<0; R, assumed relevant difference; Ps, probability of similarity (probability of D absolute value being lower than R); Pr, P(D>R) when D>0 and P(D<R) when D<0, MCse, Monte Carlo standard error; Z, Z-score of the Geweke test

Table 3 shows the results of the estimated marginal posterior distributions of the differences between the -/- and 455/455 genotype for the microsatellite located in the promoter region of the *OVPG1* gene. These results should be taken with caution due to the low number of data recorded for the 455/455 genotype (Table 1) and then, the low precision of the estimations. The -/- genotype had a higher OR than the 455/455 genotype. This result was not expected because previous studies in the H and L lines indicated that both lines had similar OR (Mocé *et al*., 2004). At 48 h of gestation, both genotypes showed similar FR (D_m=-2.17%) but the -/- genotype had higher EES than the 455/455 genotype (Pr=85%) and lower embryonic stage of development since the -/- genotypes had a higher % EM (D_m=27.52%; Pr=78%), the lower embryonic stage of gestation at this stage of gestation.

Table 3: Features of the estimated marginal posterior distributions of the differences (D) between the -/- and 455/455 genotypes for the microsatellite located in the promoter region of the *OVPG1* gene in ovulation rate (OR), fertilization rate (%FR), early embryo survival (EES) and percentage of early morulae (%EM), of compacted morulae (%CM) and of blastocysts (%B) in the F_2 population at 48 and 72 h of gestation

	D_m	$HPD_{95\%}$	P (%)	R	Ps (%)	Pr (%)	MCse	Z
OR	1.47	-0.19 , 3.18	96	0.5	12	87	0.010	0.18
48 h of gestation								
% FR	2.1	-3.72 , 7.45	78	3.5	66	31	0.032	0.03
EES	0.94	-0.56 , 2.23	90	0.25	10	85	0.010	-0.52
% EM	27.52	-30.10, 74.16	85	8	13	78	0.296	-0.19
72 h of gestation								
% FR	7.24	-0.64, 15.02	96	3.5	17	82	0.046	-0.03
EES	1.54	-0.49 , -3.41	94	0.25	6	90	0.011	1.19
% EM	9.13	-25.12 ; 42.27	71	8	31	53	0.195	0.71
% CM	2.48	-19.72 ,23.55	58	8	51	31	0.126	-0.89
% B	-20.35	- 46.54 ; 8.07	93	8	15	83	0.22	-0.17

D_m, posterior mean of the difference between -/- and 455/455 genotypes; $HPD_{95\%}$, highest posterior density region at 95%; P, P(D>0) when D>0 and P(D<0) when D<0; R, assumed relevant difference; Ps, probability of similarity (probability of D absolute value being lower than R); Pr, P(D>R) when D>0 and P(D<R) when D<0, MCse, Monte Carlo standard error; Z, Z-score of the Geweke test

At 72 h of gestation, the -/- genotype had higher FR and EES than the 455/455 genotype, showing 7.24% and 1.54 embryos, respectively. Moreover, the probability of relevance in both cases was at least 82%. Regarding the embryonic stage of development, the -/- genotype showed a lower embryonic stage of development having higher % EM (D_m=9.13%) and lower % B (D_m=-20.35% and Pr=83%).

CONCLUSIONS

The GG genotype of the SNP of the exon 11 and the 455 allele of the microsatellite located in the promoter region of the *OVPG1* gene were associated with a difference in the embryo survival and development in the first stages of gestation. Nevertheless, these alleles are more frequents in the low line selected for uterine capacity.

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