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Identification of Single-Nucleotide Polymorphism in the Progesterone Receptor Gene and Its Association With Reproductive Traits in Rabbits

R. Peiró,*,[1] M. Merchán,[†] M. A. Santacreu,* M. J. Argente,[‡] M. L. García,[‡] J. M. Folch[†] and A. Blasco*

**Instituto de Ciencia y Tecnología Animal, Universidad Politécnica de Valencia, 46071 Valencia, Spain, [†]Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain and [‡]Departamento de Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, 03312 Orihuela, Spain*

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ABSTRACT

A total of 598 F_2 does from a cross between the high and low lines selected divergently for uterine capacity during 10 generations were used in a candidate gene analysis. The presence of major genes affecting the number of implanted embryos and uterine capacity has been suggested in lines divergently selected for uterine capacity. Uterine capacity is a main component of litter size. The progesterone receptor gene was tested as a candidate gene to determine whether polymorphisms explain differences in litter size and its components. Fragments of the promoter region and exons 1–8 were amplified and sequenced. One SNP was found in the promoter region, 2464G>A, three SNPs in the 5′-UTR exon 1, and a silence SNP in exon 7. The first four SNPs were segregated in two haplotypes. The allele G found in the promoter region was found in 75% of the high-line parental animals and in 29% of the low-line parental animals. The GG genotype had 0.5 kits and 0.5 implanted embryos more than the AA genotype. At 48 hr of gestation, the difference in early embryo survival and embryonic stage of development was small. However, at 72 hr of gestation, the GG genotype had 0.36 embryos more than the AA genotype and also had a more advanced embryonic stage of development, showing a lower percentage of compacted morulae and a higher percentage of blastocysts. The difference in litter size between the GG and GA genotypes was similar to the difference found between homozygote genotypes; however, differences in implanted embryos, early embryo survival, and embryo development were not detected between the GG and GA genotypes.

LITTER size is one of the most important traits in rabbit and pig production. Response to direct selection for litter size has been lower than expected (see reviews by Blasco *et al.* 1993 in rabbits and pigs and Rothschild and Bidanel 1998 in pigs). Selection for uterine capacity (UC) has been proposed as an indirect way for improving litter size (Bennett and Leymaster 1989). In rabbits, Blasco *et al.* (1994) proposed litter size of unilaterally ovariectomized (ULO) does as an estimate of UC. Differences in UC and prenatal survival were found after 10 generations of a divergent selection experiment for UC (Blasco *et al.* 2005). The high (H) line showed a higher litter size (2.35 kits in intact females) and also a higher number of embryos before implantation (1.8 embryos in intact females) than the low (L) line (Santacreu *et al.* 2005). This difference was not due to a higher ovulation rate (Mocé *et al.* 2002; Santacreu *et al.* 2005) or fertilization rate (Santacreu *et al.* 1996), both of which were similar in both lines. A difference of 1 embryo between lines was observed at 72 hr of gestation in favor of the H line, using intact does (Mocé *et al.* 2004). The H line also showed a more advanced embryonic stage of development at 48 hr (Peiró *et al.* 2007) and at 72 hr of gestation (Mocé *et al.* 2004).

The difference that appeared in UC was mainly in the first two generations of selection (Blasco *et al.* 2005), suggesting that a major gene may be involved in the genetic determination of the trait. In addition, a complex segregation analysis performed by Argente *et al.* (2003) found evidence of major genes with a moderate effect on UC and a large effect on number of implanted embryos.

Progesterone participates in the release of mature oocytes, facilitation of implantation, and maintenance of pregnancy by promoting uterine growth and suppression of myometrial contractility (Graham and Clarke 1997). Most progesterone functions are exerted through its interaction with a specific nuclear progesterone receptor. These receptors belong to the nuclear receptor superfamily and they are ligand-dependent transcription factors (Evans 1988; Tsai and O'Malley 1994). Progesterone receptor binds its ligand, leading to dimerization of receptor; later, the receptor binds target DNA sequences activating or repressing genes coding

[1]*Corresponding author:* Instituto de Ciencia y Tecnología Animal, Universidad Politécnica de Valencia, P. O. Box 22012, 46071 Valencia, Spain. E-mail: ropeibar@dca.upv.es

other proteins and transcription factors (Tsai and O'Malley 1994). The structure of the rabbit progesterone receptor (*PGR*) gene is unknown but the mRNA sequence is similar to the human one (Loosfelt *et al.* 1986; Misrahi *et al.* 1993).

On the basis of the biological functions of progesterone receptor and previous results of the above-mentioned experiment of divergent selection for uterine capacity in rabbits, it is hypothesized that the *PGR* gene plays an important role in the reproductive traits in rabbits. The objectives of the current study were to identify SNPs in the rabbit *PGR* gene, develop PCR–RFLP methods to detect these SNPs, describe the SNP in two divergently selected lines for UC, and evaluate associations between PGR SNP and reproductive traits in an F_2 population.

MATERIALS AND METHODS

Animals: Does were mated first at 18 weeks of age and at day 10 after each parturition thereafter, and mating of close relatives was avoided to reduce inbreeding. Laparoscopies were performed on all does at day 12 of their second gestation and corpora lutea and implanted embryos were counted. Details of the technique are given by Santacreu *et al.* (1990). All animals were reared in individual cages and were fed a commercial diet. The photoperiod used was 16 hr light:8 hr dark.

The high and low lines: A total number of 168 animals (24 bucks and 61 does from the H line and 20 bucks and 63 does from the L line) from the 17th generation of a divergent selection experiment on UC were used. Uterine capacity was estimated as litter size in ULO females (Blasco *et al.* 1994). The H and L lines were selected for 10 generations (Argente *et al.* 1997), and then the selection was relaxed until the 17th generation. Both lines were derived from a synthetic line (V) selected for 12 generations by litter size at weaning (Estany *et al.* 1989).

The ovariectomy was performed at 14–16 weeks old and all animals were reared at the experimental farm of the Polytechnic University of Valencia.

The F_2 population: The F_1 rabbit population was generated from the reciprocal cross of the H and L lines from the 17-generation divergent selection experiment on UC. Parental animals were selected for UC, using a BLUP procedure on an animal-repeatability model ($h^2 = 0.10$ and $r = 0.15$) with year–season and parity–lactation state (five levels) effects. Three bucks of the H line were crossed with 13 ULO does of the L line and 3 bucks of the L line with 5 ULO does of the H line. Groups of full-sib families were generated by mating 70 F_1 intact does to 10 F_1 bucks, obtaining a total of 598 F_2 intact does, which were crossed to 127 F_2 males.

Three hundred thirty-one F_2 does were mated after their fourth parity when the female was nonlactating, *i.e.*, 28 days after parity. After 48 and 72 hr, a total of 172 and 159 intact does were slaughtered, respectively, by intravenous injection of sodium thiopental in a dose of 50 mg/kg body weight (Tiobarbital; B. Braun Medical S. A., Barcelona, Spain). The total reproductive tract was removed and oviducts and uterine horns were separated and flushed once with 5 and 10 ml of 150 mM ammonium bicarbonate solution, respectively.

The F_2 population was reared at the experimental farm of the Universidad Miguel Hernández de Elche, while the F_1 population was reared at the experimental farm of the Polytechnic University of Valencia.

TABLE 1

Primers used for DNA amplification (a), sequencing (s), and genotyping (g) of the progesterone receptor gene

Primer	Sequence 5′ → 3′	Use
PGRP-F	GAAGCAGGTCATGTCGATTGGAG	a, s, g
PGRP-R	CTGCCCCTCTCTCTAGCACTCTG	a, s
PGRA-F	AGACCAGTGTGGCCCGCTGTAG	a, s
PGRA-R	GGAAGGTCGGGGCCAAACAG	s
PGRB-F	ACAGTGTCCTCGACACGCTCCT	s
PGRB-R	CTTCCCCGGGTCTGGACGAG	a, s
PGRE1-F	CGCAGGTCTACACGCCCTATCTC	a, s
PGRE4-F	AAAAAGTTCAATAAAGTCAGAGTCATG	s
PGRE8-R	TCCTGACCAAAACGAAAGACATACC	a, s
PGR-5′-UTR	CGCCTCTGGTGCCAAGTCTC	g

Traits: Uterine capacity, measured as litter size (LS) in ULO does, and number born alive (NBA) were measured in the H and L lines up to four parities. Litter size and NBA were also measured in F_2 does up to four parities.

The following traits were recorded in the second gestation: ovulation rate (OR), estimated as the number of corpora lutea, and number of implanted embryos (IE), estimated as the number of implantation sites at day 12 of the second gestation. Embryo survival (ES) was analyzed as IE fitting OR as a covariate, fetal survival (FS) as LS fitting IE as a covariate, and prenatal survival (PS) as LS fitting OR as a covariate.

The females were slaughtered during the fifth gestation, and the following traits were recorded: OR, the total number of embryos (TE), the number of normal embryos (NE), and the number of oocytes (OO) recovered. Embryos were classified according to morphological criteria (Hafez 2000), using a binocular stereoscopic microscope, Leica MZ75-200×. The traits analyzed were as follows: fertilization rate (FR) (% FR = TE/(TE + OO) × 100), percentage of normal embryos (% NE = NE/TE × 100), percentage of early morulae (EM) (% EM = EM/NE × 100), percentage of compacted morulae (CM) (% CM = CM/NE × 100), and percentage of blastocysts (B) (% B = B/NE × 100). Early embryo survival (EES) was analyzed as NE fitting OR as a covariate.

Amplification and sequencing of the rabbit *PGR* gene: *DNA isolation:* Ear tissue was recovered from 6 H and 12 L animals, parental animals. Venous blood was collected in EDTA sample tubes of 80 F_1 and 598 F_2 animals. Genomic DNA from 300 mg ear tissue was purified by standard procedures, using proteinase K digestion followed by phenol/chloroform extraction and precipitation with ethanol. Genomic DNA from 80 μl venous blood collected in EDTA sample tubes was purified as described by Applied Biosystems (Foster City, CA).

RNA preparation: Oviduct tissue of parental does and testis tissue of parental males were collected. Total RNA was extracted from 100 mg of each tissue, using Trizol reagent (Invitrogen, Barcelona, Spain). Synthesis of cDNA was performed with the Thermoscript RT–PCR kit (Invitrogen). In addition, DNA isolation from 20 and 18 does belonging to the H and L lines at generation 16 was performed using ear tissue.

Amplification: Two overlapping PCR fragments (700 and 1859 bp) comprising the promoter region and part of exon 1 of the rabbit *PGR* gene were amplified using genomic DNA from parental animals. Primers (Table 1) were designed on the basis of rabbit *PGR* gene sequence (GenBank accession no. X06623). The 700-bp fragment was amplified with primers PGRP-F and PGRP-R and the PCR conditions were 95° for 5 min, followed by 35 cycles of 95° for 30 sec, 65° for 60 sec, and 72° for 90 sec, and a final extension at 72° for 7 min. The 25-μl

reaction volume included 50 ng of genomic DNA, 1× reaction buffer, 0.5 μM of each primer, 200 μM dinucleotide triphosphate, 2 mM $MgCl_2$, and 0.6 unit of Taq DNA polymerase (Invitrogen). The 1859-bp fragment was amplified using PGRA-F and PGRB-R primers and the reaction conditions were similar to those described above, with a hybridization temperature of 68.5° and 1.5 mM of $MgCl_2$.

A 1245-bp fragment spanning from exon 1 to exon 8 was amplified by RT–PCR, using primers PGRE1-F and PGRE8-R designed from the rabbit mRNA sequence (GenBank accession no. M14547). PCR conditions were as described above, with a hybridization temperature of 60° and 1.5 mM of $MgCl_2$.

The amplified products were sequenced using the BigDye Terminator v3.1 ready reaction cycle sequencing kit in an ABI PRISM 3100 Avant sequencer (Applied Biosystems) and primers indicated in Table 1. Sequences were analyzed using the SeqScape v2.1 software (Applied Biosystems).

To identify polymorphisms in the *PGR* gene, the three PCR products described above were resequenced in 25 animals.

Genotyping: The implementation of a PCR–RFLP protocol to genotype animals from the 16th generation, F_1 and F_2, were carried out for the 2464G>A SNP. Primers PGRP-F and PGR-5′-UTR (Table 1) were designed to amplify a 558-bp promoter fragment, including the polymorphic position. The PCR conditions were the following: 50 ng of genomic DNA, 0.5 μM of each primer, 200 μM dinucleotide triphosphate, 1.5 mM $MgCl_2$, 1× Taq reaction buffer, and 0.6 unit AmpliTaq Gold (Applied Biosystems) in a final volume of 25 μl. After denaturation at 95° for 10 min, 35 amplification cycles comprising 95° for 30 sec, 66° for 60 sec, and 72° for 90 sec were performed followed by a final 15-min extension step at 72°. Subsequently, the PCR fragment was digested with the restriction enzyme *Eco*31I (Fermentas) and the restriction fragments were examined by electrophoresis on 2% agarose gels. The PCR–RFLP assay yielded two bands of 416 and 142 bp (genotype GG), a single 558-bp band (genotype AA), and all three bands for the genotype GA.

Statistical analysis: *Allele frequency:* A chi-square test with the Fisher correction was used to test the association between the frequency of alleles and the H and L lines, and also the parental animals.

Reproductive traits for the H and L lines: Uterine capacity and NBA of animals from ULO does of the 17th generation of the H and L lines were analyzed using Bayesian methodology. A total number of 420 records derived from 124 does were used, with a similar amount of records for each line. The animal-repeatability model

$$y_{ijklm} = \mathrm{YS}_i + \mathrm{PL}_j + \mathrm{LI}_k + u_{ijkl} + p_{ikjl} + e_{ijklm}$$

was used, where YS was the effect of year–season (four levels), PL was the effect of parity–lactation state of the doe (five levels), LI was the line (two levels: H and L), u was the breeding value of the animal, and p was the permanent environmental effects.

Ovulation rate, IE, ES, FS, and PS in ULO does, all measured by laparoscopy in the second gestation, were analyzed using an animal model including YS, PL, and LI as before. Total numbers of 43 and 48 records per trait for H and L lines, respectively, were used in these analyses.

Reproductive traits for the F_2 population: To analyze LS and NBA, a total of 2066 records of 598 intact does were used. The animal-repeatability model

$$y_{ijklm} = \mathrm{YS}_i + \mathrm{PL}_j + G_k + u_{ijkl} + p_{ikjl} + e_{ijklm}$$

was used, where G was the effect of the *PGR* gene genotype (three levels: GG, GA, and AA).

A total of 561 records from intact does were used to analyze OR, IE, and ES, while a total of 477 records were used to analyze FS and PS. An animal model was used including YS, PL, and G as before.

For all univariant analysis, bounded uniform priors were used for all unknown parameters. Marginal posterior distributions conditional to the variance components of all unknowns were estimated using Gibbs sampling. A chain of 200,000 samples with a burn-in period of 40,000 for each trait was used. Convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures as described by Geyer (1992).

TABLE 2

Polymorphisms detected in the rabbit progesterone receptor gene

Region	Position[a]	Ref[b]	Pol[c]	Haplotype A[d]	Haplotype B
Promoter	2464	G	A	G	A
Exon 1	2866	G	T	G	T
(5′-UTR)	2906	A	G	A	G
	2974	G	C	G	C

[a] Position using as reference the GenBank sequence X06623 (promoter and exon 1).
[b] Nucleotide in the reference sequence.
[c] Polymorphisms found.
[d] The SNPs are coincident with the GenBank sequence.

RESULTS

Sequence variation analysis: A 3702-bp sequence of the rabbit *PGR* gene was obtained for 25 animals from the H and L lines. The sequenced fragment comprises the promoter, the 5′-UTR, almost all the coding region, and the 3′-UTR. Five polymorphic positions were identified: one SNP in the promoter, three SNPs in exon 1 (5′-UTR), and one silent SNP in exon 7. The SNPs of the promoter and exon 1 regions cosegregate in two haplotypes (Table 2). In addition, the last SNP was not associated to the H and L lines. A PCR–RFLP method was developed for genotyping the 2464G>A SNP in the promoter region.

Allele frequency: The 2464G>A SNP was not fixed in the H and L lines, the allele G frequency being 0.83 in the H line and 0.42 in the L line ($P < 0.05$) and the allele G frequency being 0.75 in H parental animals and 0.29 in L parental animals (Table 3). Table 3 also shows the observed genotype frequencies in the F_1 and F_2 animals.

Reproductive traits for the H and L lines: Features of the estimated marginal posterior distributions of the differences between the H and L lines in ULO does showed the H line had higher UC than the L line and this difference was 1.32 kits. This difference in UC is associated with differences in IE (1.20) and FS (1.28).

Reproductive traits for the F_2 population: Table 4 shows raw means and standard deviations for LS, NBA, OR, and IE in the F_2 population. Features of the estimated marginal posterior distributions of the differences (D) between the GG and AA genotypes and between the GG and GA genotypes are presented for

TABLE 3

Frequency distribution of the 2464G>A SNP located in the promoter region of the progesterone receptor gene in the F_2 experiment

		Genotypes			Allelic frequency	
	N	GG	GA	AA	G	A
Parental						
H	6	4	1	1	0.75	0.25
L	12	2	3	7	0.29	0.71
F_1	80	23	52	5	0.61	0.39
F_2	598	197	299	102	0.58	0.42

N, number of genotyped animals; H, high line; L, low line.

TABLE 4

Mean, standard deviation (SD), and number (*N*) of does and litters (within parentheses) for total number of kits born (LS), number born alive (NBA), ovulation rate (OR), number of implanted embryos (IE), fertilization rate (% FR), number of normal embryos (NE), percentage of early morulae (% EM), percentage of compacted morulae (% CM), and percentage of blastocysts (% B) in the F_2 population

	Trait	Mean	SD	*N*
	LS	8.16	3.22	598 (2066)
	NBA	7.28	3.51	598 (2066)
	OR	14.7	2.8	561
	IE	11.1	3.8	561
48 hr gestation	% FR	98.1	4.9	121
	NE	11.9	2.3	121
	% EM	13.3	25.7	121
	% CM	86.7	25.7	121
72 hr gestation	% FR	97.6	6.3	125
	NE	11.8	2.7	125
	% EM	12.2	25.8	125
	% CM	72.9	24.3	125
	% B	14.9	24.7	125

LS and NBA in Table 5. The $P(D > 0)$ is more practical than a classical hypothesis test because actual probabilities are used instead of significance levels. In addition, highest posterior density at 95% ($HPD_{95\%}$) intervals are equivalent to classical confidence intervals. All Monte Carlo standard errors (MCSE) were very small and lack of convergence was not detected by the Geweke test. Marginal posterior distributions were approximately normal.

Table 5 also shows what we assume to be a relevant difference (*R*) among genotypes, the probability of this difference being relevant for each case (P_r), and the probability of a difference being, in absolute value, lower than a relevant value, *i.e.*, the probability of both genotypes being similar in biological or economical terms (P_s). This last probability allows us to distinguish the case in which both treatments have equal effects and the case in which we do not find differences between treatments because of low precision.

Litter size was higher for the GG genotype than for the AA genotype, $P(D > 0) = 98\%$ (Table 5), and the difference was 0.5 kits. This difference was at least 0.29, 0.17, and 0.07 kits with a probability of 80, 85, and 95% respectively. For these genotypes, NBA showed a similar result to LS. Features of the estimated marginal posterior distributions of the differences (*D*) among genotypes for OR, IE, and survival traits are also presented in Table 5. We considered 0.5 ova per doe as a relevant difference, the same difference as for LS. The GG genotype had similar OR to the AA genotype ($P_s = 84\%$). We also considered 0.5 embryos as the relevant difference for IE and all survival traits. The GG genotype had 0.49 implanted embryos more than the AA genotype, $P(D > 0) = 82\%$. Regarding survival traits, the GG genotype had higher ES and PS than the AA genotype, $P(D > 0) = 95\%$ and $P(D > 0) = 96\%$, respectively. In both cases, the estimated differences were relevant, 0.52 and 0.73, respectively, although the probabilities of finding a relevant difference were 52 and 70%, respectively. Conversely, both genotypes had similar FS ($P_s = 67\%$). Thus, the difference in LS between the GG and AA genotypes therefore seems to be associated with differences in ES. Regarding embryo survival and development, we consider for fertilization rate a relevant difference of 3.5%, which corresponds to the previous relevant value of 0.5 embryos (Table 6). There were no relevant differences in fertilization rate at both 48 and 72 hr of gestation between homozygote genotypes, $P_s = 100\%$. We consider 0.25 embryos as a relevant value for EES since we found half of the difference in the number of implanted embryos between the H and L lines at 72 hr of gestation (Mocé *et al.* 2004; Santacreu *et al.* 2005). We did not find a relevant difference between the GG and AA genotypes for EES at 48 hr of gestation, but the precision of the estimation was low. The relevant value for all the embryonic stages of development was established as one-third of the phenotypic standard deviation of the trait, 8%, due to the difficulties of ascribing economical significance to these traits. At 48 hr of gestation, both homozygote genotypes also showed a similar embryonic stage of development ($P_s = 73\%$). At 72 hr of gestation, the GG genotype had higher EES than the AA genotype, $P(D > 0) = 80\%$, and the difference was relevant (0.36 embryos recovered, $P_r = 60\%$). The GG genotype had also a more advanced embryonic stage of development, showing a similar percentage of early morulae ($P_s = 70\%$), a lower percentage of compacted morulae [$P(D < 0) = 79\%$], and a higher percentage of blastocysts [9.26%, $P(D > 0) = 93\%$, $P_r = 64\%$].

The assumed relevant values for the differences between the GG and GA genotypes were the same as before. The difference between the GG and GA genotypes in LS was similar to the difference found between homozygote genotypes [0.5, $P(D > 0) = 99\%$; Table 5].

TABLE 5

Features of the estimated marginal posterior distributions of the differences between different genotypes of the 2464G>A SNP for the promoter region of the progesterone receptor gene 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 F_2 population

		D	$HPD_{95\%}$	P (%)	R	P_s (%)	P_r (%)
GG–AA	LS	0.51	0.01, 1.04	98	0.5	48	52
	NBA	0.49	−0.09, 1.00	96	0.5	51	49
	OR	0.01	−0.70, 0.73	50	0.5	84	8
	IE	0.49	−0.55, 1.40	82	0.5	50	47
	ES	0.52	−0.08, 1.13	95	0.5	48	52
	FS	0.34	−0.33, 0.97	84	0.5	67	33
	PS	0.73	−0.09, 1.52	96	0.5	30	70
GG–GA	LS	0.50	0.06, 0.92	99	0.5	65	35
	NBA	0.49	0.10, 0.91	99	0.5	52	48
	OR	−0.30	−0.79, 0.24	88	0.5	78	22
	IE	0.13	−0.55, 0.86	65	0.5	81	15
	ES	0.42	−0.15, 1.00	91	0.5	61	39
	FS	0.17	−0.30, 0.65	76	0.5	90	9
	PS	0.36	−0.25, 0.94	87	0.5	68	32

D, posterior mean of the difference; $HPD_{95\%}$, highest posterior density region at 95%; P, $P(D > 0)$ when $D > 0$ and $P(D < 0)$ when $D < 0$; R, assumed relevant difference between genotypes; P_s, probability of similarity (probability of the absolute value of D being lower than R); P_r, probability of relevance [$P(D > R)$ when $D > 0$ and $P(D < R)$ when $D < 0$].

Therefore, the GA genotype had similar LS and NBA to the AA genotype ($P_s = 96$ and 95%, respectively), which indicates that the action of this gene on the trait is dominant. It was not clear which LS component or components explain the differences found in LS and NBA between the GG and GA genotypes, since P_r's were low, but it seems that the difference in LS could be due to higher ES, $P(D > 0) = 91\%$. There were no relevant differences in fertilization rate at both stages of gestation between the GG and GA genotypes (Table 6). We

TABLE 6

Features of the estimated marginal posterior distributions of the differences between different genotypes of the 2464G>A SNP for the promoter region of the progesterone receptor gene for fertilization rate (% FR), early embryo survival (EES), percentage of early morulae (% EM), percentage of compacted morulae (% CM), and percentage of blastocysts (% B) at 48 and 72 hr of gestation in the F_2 population

			D	$HPD_{95\%}$	P (%)	R	P_s (%)	P_r (%)
GG–AA	48 hr	% FR	−0.29	−2.85, 2.16	57	3.5	100	0
		EES	0.14	−0.57, 0.82	65	0.25	56	32
		% EM	−0.40	−15.38, 14.80	52	8	73	15
	72 hr	% FR	0.34	−3.07, 3.41	57	3.5	100	0
		EES	0.36	−0.53, 1.18	80	0.25	33	60
		% EM	−2.15	−16.34, 11.61	61	8	70	21
		% CM	−7.09	−25.14, 11.26	79	8	49	47
		% B	9.26	−3.63, 22.62	93	8	36	64
GG–GA	48 hr	% FR	1.06	−0.91, 3.21	85	3.5	100	0
		EES	0.17	−0.39, 0.68	74	0.25	55	38
		% EM	−2.97	−14.07, 8.21	69	8	77	19
	72 hr	% FR	1.14	−1.18, 3.43	86	3.5	100	0
		EES	0.13	−0.54, 0.79	64	0.25	51	36
		% EM	−2.52	−13.26, 9.06	33	8	80	16
		% CM	1.61	−11.73, 15.38	59	8	76	16
		% B	0.93	−12.84, 14.38	53	8	77	12

D, posterior mean of the difference; $HPD_{95\%}$, highest posterior density region at 95%; P, $P(D > 0)$ when $D > 0$ and $P(D < 0)$ when $D < 0$; R, assumed relevant difference between genotypes; P_s, probability of similarity (probability of the absolute value of D being lower than R); P_r, probability of relevance [$P(D > R)$ when $D > 0$ and $P(D < R)$ when $D < 0$].

did not find any relevant difference between the GG and GA genotypes in EES at both stages of gestation, although the estimation had low precision. A similar pattern in the embryonic stage of development was also observed between the GG and GA genotypes (P_s was >75% in all the embryonic stages of development).

DISCUSSION

After 10 generations of selection for UC, ULO does of the H line had 1 kit more than does of the L line (Mocé *et al.* 2005); thus the difference in UC obtained in the 17th generation between the H and L lines agrees with previous results. This difference was approximately half of the difference found using intact does (2.35 kits; Santacreu *et al.* 2005).

Previous results suggested the presence of a major gene affecting UC and IE in ULO does segregating in the base population, with different allele frequencies (Argente *et al.* 2003; Blasco *et al.* 2005). The recent publication of the first genetic map of the rabbit genome was based on microsatellites, comprising 111 markers (Chantry-Darmon *et al.* 2006), but they are insufficient to perform a genome-scanning approach for QTL detection. The *PGR* gene, which has not been previously analyzed in association studies in livestock species, was chosen as a candidate gene for litter size and its components. The promoter and almost all the coding region of the rabbit *PGR* gene were sequenced in animals from the H and L lines and five SNPs were found. The first four SNPs segregated in two haplotypes. The polymorphism that most likely explains differences found between the H and L lines is the SNP 2464G>A found in the promoter region. This 2464G>A SNP is not fixed in the lines. The allelic frequencies in the H line agree with the frequency estimated by a complex segregation analysis by Argente *et al.* (2003) in the base population, who estimated a 69% favorable allele frequency. Most of the difference in UC between the H and L lines seems to be due to the response in the L line (Mocé *et al.* 2005); thus selection to decrease UC may have reduced the favorable allele frequency for UC.

Uterine capacity is closely related to LS. A high genetic correlation between UC and LS was obtained by Argente *et al.* (2000). Moreover, correlated responses in LS and its components after 10 generations of divergent selection for UC were obtained by Santacreu *et al.* (2005). The association study performed in the F_2 rabbit population showed that the GG genotype, the most frequent genotype in the H line, had higher LS than the AA genotype (0.5 kits). The difference between homozygote genotypes in LS is 15% of the phenotypic standard deviation of this trait and it is ~10% of the difference between the H and L intact does (Santacreu *et al.* 2005). The difference obtained between genotypes was not due to the number of born dead, as was also found by Santacreu *et al.* (2005). The difference obtained in LS between the GG and AA genotypes was mainly due to a difference in IE and not in OR. This result also agrees with the result obtained in the H and L lines in intact does (Santacreu *et al.* 2005). The difference found in IE between homozygote genotypes is 18% of the standard deviation of this trait and 12% of the difference between the H and L lines (Santacreu *et al.* 2005). Regarding ES, FS, and PS, the GG genotype showed higher survival rates than the AA genotype. These differences are 17, 6, and 9% of the differences found by Santacreu *et al.* (2005) between the H and L lines in these traits, respectively. Thus, the analyzed *PGR* polymorphisms explain only a substantial part of the difference found between both the H and L lines in LS. The difference between homozygote genotypes in LS was mainly obtained before implantation, as it occurred in the H and L lines (Santacreu *et al.* 2005).

Differences in early embryo survival in the first stages of gestation could explain the difference found in IE. Progesterone plays an important role in the first stages of gestation, being its physiological effects mediated by the progesterone receptor. During the first 4 days of gestation, Anzaldúa *et al.* (2007) found *PGR* mRNA and protein expression in the rabbit oviduct and uterus; thus this gene is expressed in the first days of gestation. Thus, these polymorphisms could also explain at least part of the difference in EES and development found between the H and L lines in the first stages of gestation. The *PGR* gene does not seem to have an important effect on the EES at 48 hr of gestation. In concordance with this result, Peiró *et al.* (2007) obtained no relevant difference in EES at this stage of gestation when comparing the H and L lines. At 72 hr of gestation, the GG genotype had higher EES and also a higher embryonic stage of development. These results agree with previous results obtained in the H and L lines at this stage of gestation (Peiró *et al.* 2007). The differences found between homozygote genotypes are 13 and 38% of the phenotypic standard deviation of EES and of the percentage of blastocysts, respectively. Differences in early embryo survival in the first stages of gestation can explain the difference found in IE.

Comparing the GG and GA genotypes at 72 hr of gestation, the GA genotype shows both EES and embryonic stage of development similar to that of the GG genotype and higher than that of the AA genotype. At implantation, the GA genotype showed a similar number of implanted embryos to that of the AA genotype and a lower number than that of the GG genotype. Similar results were obtained in LS.

Using the candidate gene approach to identify an SNP effect is insufficient to conclude that this SNP is the causal mutation of differences in the traits. Relevant differences might also be obtained if the genotyped SNP is in linkage disequilibrium with the causal mutation. However, in the present work the H and L parental animals were derived from the synthetic V line and it is

expected to differ in a reduced number of *loci*. The next step would be the functional and genetic validations of the polymorphisms found in the *PGR* gene since it is unknown whether the SNPs found in the *PGR* gene modify their expression and how they could affect early embryo survival and development. It would also be useful to study the *PGR* gene as a candidate gene in other commercial lines or breeds to evaluate its effect and its possible use.

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