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Additional Information

# PROGESTERONE RECEPTOR GENE AS CANDIDATE GENE FOR REPRODUCTIVE TRAITS IN RABBITS: I. LITTER SIZE AND ITS COMPONENTS

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**Short running head:** *PGR* gene and litter size in $F_2$ rabbits

**Key words:** Rabbits, $F_2$, Progesterone receptor gene, Litter size, Gene polymorphisms.

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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 was used in a candidate gene analysis. The presence of major genes affecting 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 candidate gene to determine whether polymorphisms explain differences in litter size and its components. Fragments of the promoter region and exons 1 to 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 the exon 7. The allele G found in the promoter region was found in 75% of the High line animals and in 29% of the Low line animals. The frequency observed in $F_2$ animals was 33% GG, 50% GA and 17% AA. The GG genotype had 0.5 kits and 0.5 implanted embryos more than the AA genotype. The difference in litter size between the GG and GA genotypes was similar to the difference found between homozygote genotypes.

## INTRODUCTION

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 review 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 does (ULO) as an estimate of UC. Differences in UC and prenatal survival were found after 10 generations of a divergent selection experiment for UC. These differences appeared 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. Moreover, a complex segregation analyses 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 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 other proteins and transcription factors (TSAI and O'MALLEY, 1994). The structure of the rabbit progesterone receptor gene (*PGR*) is unknown but the mRNA sequence is similar to the human one (LOOSFELT *et al.,* 1986; MISRAHI *et al.,* 1993).

The objective of the current work is the detection of polymorphisms in the rabbit *PGR* gene to perform an association study with reproductive traits in an $F_2$ population generated from the lines of the above-mentioned experiment of divergent selection for uterine capacity.

## MATERIAL AND METHODS

### Animals

**The High and Low lines:** A total number of 168 animals from $17^{th}$ generation of a divergent selection experiment on uterine capacity (UC) were used. Uterine capacity was estimated as litter size in unilaterally ovariectomized females (ULO) (BLASCO *et al.,* 1994). The High (H) and Low (L) lines were selected for 10 generations (ARGENTE *et al.,* 1997), and then the selection was relaxed until the $17^{th}$ generation. Both lines were derived from a synthetic line (V) selected for 12 generations by litter size at weaning (ESTANY *et al.,* 1989). The V line was founded in 1981 as a synthetic line, crossing animals of four specialized maternal lines of Californian and New Zealand White breeds.

A total of 20 H line does and 18 L line does from $16^{th}$ generation were genotyped to estimate the allele frequency of the *PGR* gene for each line.

At $17^{th}$ generation, the H line had 24 bucks and 61 does and the L line had 20 bucks and 63 does. The left ovary of these does was removed before puberty via midventral incision between 14 and 16 wks of age (BLASCO *et al.,* 1994). Does were mated first at 18 wks of age and at d 10 after each parturition thereafter. Laparoscopies were performed on all does at d 12 of their second gestation, and corpora lutea and implanted embryos were counted. Details of the technique are given by SANTACREU *et al.* (1990).

**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 of divergent selection experiment on uterine capacity. 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 effects (with five levels: nulliparous does, lactating and nonlactating does of second parity, and lactating and nonlactating does with more than two parities). 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. A total of 598 F$_2$ intact does were produced. Mating of close relatives was avoided to reduce inbreeding. Does were mated first at 18 wk of age and at d 10 after each parturition thereafter. Laparoscopies were performed on all does at d 12 of their second gestation and corpora lutea and implanted embryos were counted.

The F$_2$ population was reared at the experimental farm of the Universidad Miguel Hernández, while the H and L lines and the F$_1$ population were reared at the experimental farm of the Polytechnic University of Valencia. All animals were reared in individual cages and were fed a commercial diet. The photoperiod used was 16-h light: 8-h dark.

**Traits**

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 d 12 of the second gestation. Embryo survival (ES) was analyzed as IE fitting OR as a covariate, fetal survival (FS) as litter size (LS) fitting IE as a covariate and prenatal survival (PS) as LS fitting OR as a covariate. Litter size and number of born alive (NBA) were measured in F$_2$ does up to four parities. Uterine capacity, measured as LS in ULO does, and NBA were also measured in H and L lines up to four parities.

**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 samples 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 with the *DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Bucal Swabs* described by Applied Biosystem.

**RNA preparation:** Oviduct tissue of parental does and testis tissue of parental males was 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, Barcelona, Spain).

In addition, DNA isolation from 20 and 18 does belonging to the H and L line was performed using ear tissue.

**Amplification:** Two overlapping PCR fragments (700 bp 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 based on 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 s, 65° for 60 s and 72° for 90 s, and a final extension at 72° for 7 min. The 25-µL reaction volume included 50 ng of genomic DNA, 1 x reaction buffer, 0.5 µM of each primer, 200 µM dinucleotide triphosphate, 2 mM $MgCl_2$, and 0.6 U of Taq DNA polymerase (Invitrogen, Barcelona, Spain). The 1859 bp fragment was amplified using PGRA-F and PGRA-R primers and the reaction conditions were similar to those described above, with a hybridization temperature of 68.5° and 1.5mM of $MgCl_2$.

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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 60º and 1.5mM 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, Foster City, CA) and primers indicated in Table 1. Sequences were analyzed using the SeqScape v2.1 software (Applied Biosystems).

In order to identify polymorphisms in the *PGR* gene, the three PCR products described above were resequenced in 25 animals, 22 of which were parental animals of the $F_2$ cross.

**Genotyping:** The implementation of a PCR-RFLP protocol to genotype animals from 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 $MgCl_2$, 1x Taq reaction buffer and 0.6 U 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 s, 66° for 60 s, and 72° for 90 s were performed followed by a final 15 min extension step at 72°. Subsequently, the PCR fragment was digested with the restriction enzyme Eco31I (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 (Figure 1).

**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.

**Reproductive traits for the H and L lines:** Uterine capacity and NBA of animals from ULO does of 17th generation of the H and L lines were analyzed using a Bayesian methodology. A total number of 420 records derived from 124 does were used, with similar amount of records for each line. An animal-repeatability model was used and the pedigree file included 390 animals.

Data are conditionally distributed as:

$\mathbf{y} \mid \mathbf{b}, \mathbf{a}, \mathbf{p}, \sigma^2_e \sim N(\mathbf{Xb}+\mathbf{Za}+\mathbf{Wp}, \mathbf{I}\sigma^2_e)$

where **b** contains the effects of year-season (with four 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) and line (with two levels: H and L). The know incidence matrices are **X**, **Z** and **W** and **I** is the identity matrix. Vectors **a** and **p** were assumed to be *a priori* independent and normally distributed;

$\mathbf{a} \mid \sigma^2_a \sim N(\mathbf{0}, \mathbf{A}\sigma^2_a),$

$\mathbf{p} \mid \sigma^2_p \sim N(\mathbf{0}, \mathbf{I}\sigma^2_p)$

where **A** is the additive genetic relationship matrix. Bounded uniform priors were used for all unknown parameters.

Ovulation rate, IE, ES, FS and PS in ULO does, all measured by laparoscopy in the second gestation, were analyzed using an animal model. A total number of 43 and 48 records per trait for H and L lines respectively were used in these analyses, and the pedigree file included 340 individuals. Data are conditionally distributed as:

$\mathbf{y} \mid \mathbf{b}, \mathbf{a}, \sigma^2_e \sim N(\mathbf{Xb}+\mathbf{Za}, \mathbf{I}\sigma^2_e)$

where **b** contains year-season (with three levels), lactation state (with two levels: lactating and nonlactating does of second parity) and line effects (with two levels: H and L). Vector

**a** was assumed to be *a priori* normally distributed as described before. Bounded uniform priors were used for all unknown parameters.

Features of the marginal posterior distribution of differences between lines were estimated using Gibbs sampling. After some exploratory analyses, we used a chain of 200,000 samples and a burn-in period of 40,000 for each trait. Convergence was tested using the the Geweke´s z-criterion (SORENSEN and GIANOLA, 2002) and Monte Carlo sampling errors were computed using time-series procedures as described by GEYER (1992).

**Reproductive traits for the F₂ population:** To analyze LS and NBA a total number of 2066 records of intact 598 does were used. An animal-repeatability model was used and the pedigree file included 702 animals. Data are conditionally distributed as:

$$\mathbf{y} \mid \mathbf{b}, \mathbf{a}, \mathbf{p}, \sigma^2_a, \sigma^2_p, \sigma^2_e \sim N(\mathbf{Xb}+\mathbf{Za}+\mathbf{Wp}, \mathbf{I}\sigma^2_e)$$

where **b** contains year-season (with 8 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) and *PGR* gene genotype effects (with three levels: GG, GA and AA). The know incidence matrices are **X**, **Z** and **W** and **I** is the identity matrix. Vectors **a** and **p** were assumed to be *a priori* independent and normally distributed as described before. Information from the 14th to 16th generation (774 intact animals), where selection was relaxed, was used to estimate $\sigma^2_a$ and $\sigma^2_p$. For both traits, the heritability was 0.05 and the repeatability was 0.15. Bounded uniform priors were used for all unknown parameters.

To analyze OR, IE and ES a total of 561 records from intact does were used, meanwhile to analyze FS and PS a total of 477 records were used. An animal model was used and the pedigree file included 675 animals. Data are conditionally distributed for all the unknown parameters as:

$$\mathbf{y} \mid \mathbf{b}, \mathbf{a}, \sigma^2_a, \sigma^2_e \sim N(\mathbf{Xb+Za}, \mathbf{I}\sigma^2_e)$$

where **b** contains year-season (with 8 levels), lactation state (with two level: lactating and nonlactating does of second parity) and *PGR* gene genotype effects (with three levels: GG, GA and AA). The vector **a** was assumed to be *a priori* independently and normally distributed as described before. Information from $14^{th}$ to $16^{th}$ generation was used to estimate $\sigma^2_a$. Heritabilities for OR, IE, ES, FS and PS were 0.30, 0.20, 0.10, 0.05 and 0.15 respectively. Bounded uniform priors were used for all unknown parameters.

Marginal posterior distributions conditionally to the additive and permanent variance components of all unknowns were estimated using Gibbs sampling for LS and NBA. For traits measured by laparoscopy in the second gestation, marginal posterior distributions conditionally to the additive variance of all unknowns were estimated using Gibbs sampling. We used a chain of 200,000 samples with a burn-in period of 40,000 for each trait. Convergence was tested using the Z criterion of Geweke and Monte Carlo sampling errors were computed using time-series procedures as described before.

## RESULTS

**Sequence variation analysis**

A 3702 bp sequence of the rabbit *PGR* gene was obtained for 25 animals from the H and L lines (22 were parental animals of the $F_2$ cross). 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 (Table 2). The SNPs of the promoter and exon 1 regions co-segregate in two haplotypes (Table 2). A PCR-RFLP method was developed for genotyping the 2464G>A SNP in the promoter region (Figure 1).

**Allele frequency**

The 2464G>A SNP was not fixed in the H and L lines, the allele G frequency being 0.83 in H line and 0.42 in L line. Table 3 shows the observed genotype frequencies in parental, $F_1$ and $F_2$ animals.

**Reproductive traits for the H and L lines**

Features of the estimated marginal posterior distributions of the differences (D) between the H and L lines in ULO does are presented in Table 4. The H line had higher UC than the L line (P(D>0)=100%) and this difference was 1.32 kits. This difference in UC is associated with differences in IE ($D_{mean}=1.20$) and FS ($D_{mean}=1.28$).

**Reproductive traits for the $F_2$ population**

Table 5 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 LS and NBA in Table 6. 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. Figures 3 and 4 show the marginal posterior distributions of the difference between the GG and AA and between the GG and GA genotypes for LS, OR, EI, ES, FS and PS.

Table 6 also shows what we assume to be a relevant difference (R) among genotypes. In classical statistics, the size of the experiment is usually established for finding a significant difference between two treatments when this difference is considered to be relevant. A relevant value is the quantity below which this difference has no biological or economical meaning. When analyzing traits different from the one used to design the experiment, there is no relationship between the relevance of the difference and its significance. In these cases, we can find non significant differences that are relevant and significant differences that are irrelevant. Besides, discussion is often based on the

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estimate of a contrast and its significance, without considering that the confidence interval may be high and the true value irrelevant. In a Bayesian framework, we can propose a relevant value for each trait and estimate the probability of a difference between treatments being relevant for each case (Pr, Figure 2). Finally, in classical statistics, non-significance does not mean that two treatments are equal. In Bayesian statistics, we can estimate the probability of a difference being, in absolute value, lower than a relevant value; i.e. the probability of both treatments being similar in biological or economical terms (Ps, Figure 2).

Litter size was higher for the GG genotype than for the AA genotype, P(D>0)=98% (Table 6), 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 (data not shown), since these are the values found for $k$ in the confidence interval $[k, \infty)$. Number of born alive showed similar result to LS for homozygote genotypes.

The GG genotype had also higher LS and NBA than the GA genotype, $D_{mean}$=0.5 and P(D>0)=99%. These differences were close to the difference found between the GG and AA genotypes. The GA genotype had similar LS and NBA to the AA genotype (Ps=96% and 95% respectively; data not shown), which indicates that the action of this gene on the trait is dominant.

Features of the estimated marginal posterior distributions of the differences among genotypes for OR, IE and survival traits are presented in Tables 7 and 8. As in the previous analyses, all Monte Carlo standard errors were very small, lack of convergence was not detected by the Geweke test and marginal posterior distributions were approximately normal. We considered 0.5 ova per doe as a relevant difference. The GG genotype had similar OR to the AA genotype (Ps=84%). We considered the same relevant value for IE as for LS since there had been no relevant difference in fetal survival between the H and L lines in

previous studies (SANTACREU *et al.,* 2005). The GG genotype had more implanted embryos than the AA genotype, P(D>0)=82% , $D_{mean}$=0.49. We also considered 0.5 embryos as the relevant difference for all 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, $D_{mean}$=0.52 and $D_{mean}$=0.73 respectively, although the probability of finding a relevant difference was 52% and 70% respectively. Conversely, both genotypes had similar FS (Ps=67%). The difference in LS between the GG and AA genotypes therefore seems to be associated with differences in ES.

It was not clear which LS component or components explain the differences found in LS and NBA between the GG and GA genotypes, since Pr were low (Table 8), but it seems that the difference in LS could be due to higher ES, P(D>0)=91%.


## DISCUSSION

After 10 generations of selection for UC, ULO does of the H line had 1 kit more than the L line (MOCÉ *et al.,* 2005), thus the difference in UC obtained in the 17th generation between the H and L lines (Table 4) 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*)*. A genome scanning approach for QTL detection has not been feasible until the recent publication of the first genetic map of the rabbit genome based on microsatellites, comprising 111 markers (CHANTRY-DARMON *et al.,* 2006). Conversely, the candidate gene approach does not rely on genomic maps and has been widely used to identify genes involved in complex characters. Most gene-encoding

hormones, hormone-receptors or factors related to reproductive development and regulation can be considered as physiological candidate genes for reproductive traits. 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 was sequenced in animals from the H and L lines and five SNPs were found: one SNP in the promoter region, three SNPs in the exon 1 located in the 5´UTR region and one silent substitution SNP in the exon 7. 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 sixty-nine percent 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, P(D>0)=98% (Table 4). The GG genotype showed 0.5 kits more than the AA genotype. Difference between homozygote genotypes in LS is 15% of the phenotypic standard deviation of this trait (Table 5) and this difference is 9% 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 NBA shows a similar result

to LS. SANTACREU *et al.* (2005) also found that the difference between the H and L lines in LS was similar to the difference found in NBA.

The difference obtained in LS between the GG and AA genotypes was mainly due to a difference in IE ($D_{mean}$=0.49) and not in OR. This result also agrees with that obtained in the H and L lines, where no relevant difference in OR was found and most of the difference between lines in LS appears before implantation (SANTACREU *et al.,* 2005). The difference found in IE between homozygote genotypes corresponds to 18% of the standard deviation of this trait and to 12% of the difference between the H and L lines (SANTACREU *et al.,* 2005). The GG genotype shows higher survival traits than the AA genotype. The difference of 0.52, 0.34 and 0.73 embryos in ES, FS and PS correspond to survival differences of 4.10%, 2.11% and 4.09%, respectively. These differences between homozygote genotypes represent 17%, 6% and 9% of the differences found by SANTACREU *et al.* (2005) between the H and L lines in ES, FS and PS, respectively. Although the differences in survival traits seem to be small, they correspond to 18%, 10% and 20% of the standard deviation of ES, FS and PS.

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. Moreover, $F_2$ crosses usually complicate the candidate gene analysis due to the extensive linkage disequilibrium created. However, in the present work the H and L parental animals were derived from the synthetic V line and is expected to differ in a reduced number of *loci*. The next step would be the functional and genetic validation of the polymorphisms found in the *PGR* gene. It would be useful to study the *PGR* gene as candidate gene in other commercial lines or breeds to evaluate its effect and its possible use on marked assisted selection or gene assisted selection.

To sum up, the diallelic 2464G>A SNP located in the promoter region of the *PGR* gene may explain part of the difference found in litter size between the H and L lines. The difference in LS found between the GG and AA genotypes was mainly due to a difference in embryo survival. The GA and AA genotypes had similar LS, thus a negative dominance effect was also found for this trait. The *PGR* gene could be related to early embryo survival and development, since a higher early embryo survival and development is related to higher IE and LS in the H line (MOCÉ *et al.,* 2004; PEIRÓ *et al.,* 2007) and we have found that the *PGR* gene explain part of the difference in LS between the H and L lines.

## ACKNOWLEDGEMENTS

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Figure 1: The PCR-RFLP assay for analyzing the 2464G>A SNP of the rabbit progesterone receptor gene. Lanes 2 and 6: digestion of homozygous GG. Lane 5: digestion of homozygous AA. Lanes 1, 3 and 4: digestion of heterozygous GA. Lane M: molecular weight marker (HaeIII).

Figure 2: Examples of results of the marginal posterior distribution of the difference between two treatments in two different characters. (a) The difference between T1 and T2 is relevant for character 1 with high probability (Pr). (b) There is no relevant difference between T1 and T2 for character 2. $R_1$, relevant value for character 1; $R_2$, relevant value for character 2; Ps, probability of similarity; Pr, probability of relevance; T1, treatment 1; T2, treatment 2.

Figure 3: Marginal posterior distributions of the difference between the GG and AA genotypes of progesterone receptor gene for litter size (LS), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS). R represents the relevant difference proposed for each trait.

Figure 4: Marginal posterior distributions of the difference between the GG and GA genotypes of progesterone receptor gene for litter size (LS), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS). R represents the relevant value proposed for each trait.
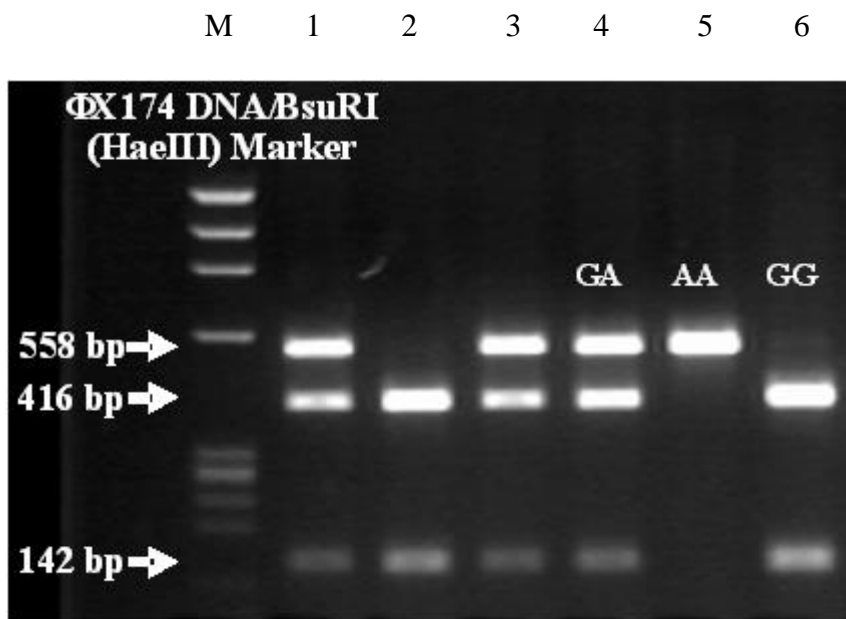
Figure 1
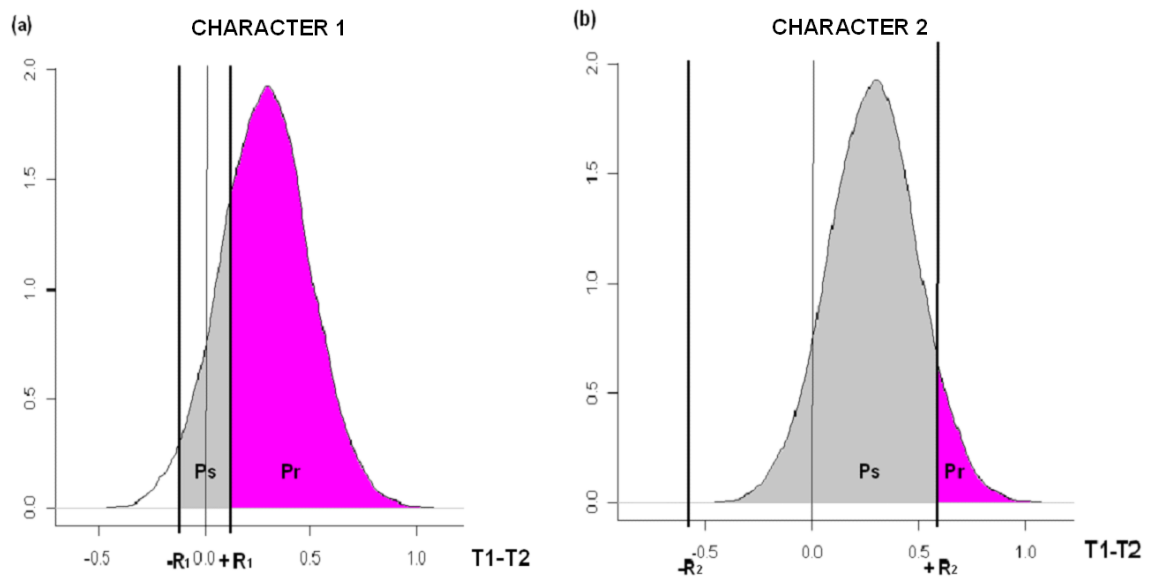
Figure 2
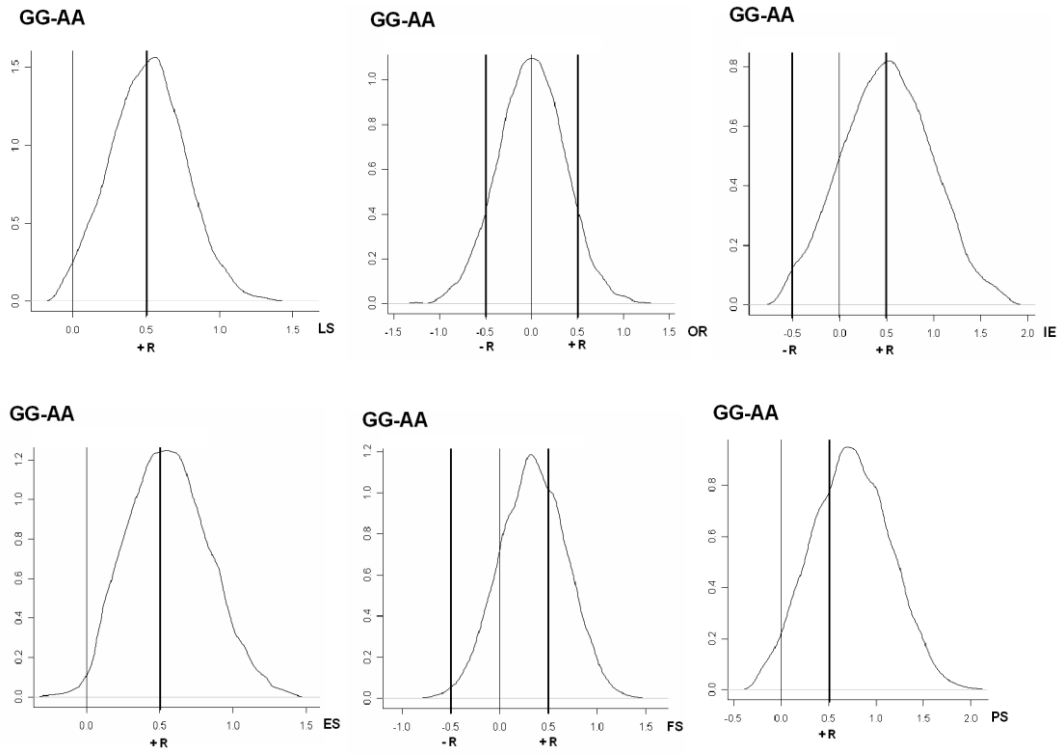
Figure 3
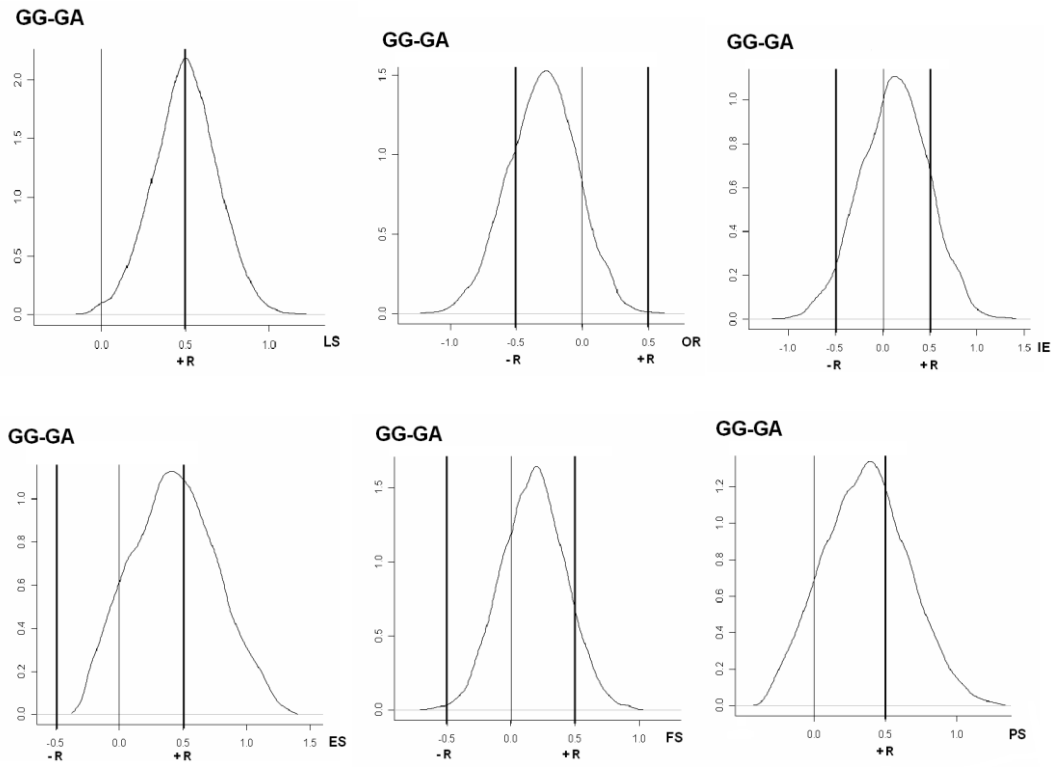
Figure 4

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 |

Table 2: Polymorphisms detected in the rabbit progesterone receptor gene.

| Region | Position[1] | Ref[2] | Pol[3] | Haplotype A[4] | Haplotype B |
|---|---|---|---|---|---|
| Promoter | 2464 | G | A | G | A |
| Exon 1 (5´UTR) | 2866 | G | T | G | T |
| | 2906 | A | G | A | G |
| | 2974 | G | C | G | C |
| Exon 7 | 2732 | G* | A | - | - |

[1] Position using as reference the GenBank sequences X06623 (promoter and exon 1) and M14547 (exon 7); [2] Nucleotide in the reference sequence; [3] Polymorphisms found; [4] The SNPs are coincident with the GenBank sequence; * Silence SNP.

Table 3: Frequency distribution of the 2464G>A SNP located in the promoter region of the progesterone receptor gene in the $F_2$ experiment.

| | | N | Genotypes | | | Allelic frequency | |
|---|---|---|---|---|---|---|---|
| | | | 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: Features of the marginal posterior distributions of the differences (D) between the H and L lines for uterine capacity (UC), number of born alive (NBA), ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS) in unilaterally ovariectomized (ULO) does.

|  | $D_{mean}$ | $HPD_{95\%}$ | P (%) |
|---|---|---|---|
| UC | 1.32 | 0.70 , 1.92 | 100 |
| $NBA_{ULO}$ | 1.31 | 0.68 , 2.00 | 100 |
| $OR_{ULO}$ | 0.42 | -0.71 , 1.53 | 75 |
| $IE_{ULO}$ | 1.20 | -0.12 , 2.78 | 95 |
| $ES_{ULO}$ | 0.80 | -0.68 , 2.11 | 82 |
| $FS_{ULO}$ | 1.28 | 0.31 , 2.33 | 99 |
| $PS_{ULO}$ | 1.52 | 0.19 , 2.95 | 99 |

$D_{mean}$, posterior mean of the difference between the H and L lines; $HPD_{95\%}$, highest posterior density region at 95%; P, $P(D>0)$ when $D>0$ and $P(D<0)$ when $D<0$.

Table 5: Mean, standard deviation (SD) and number (N) of does and litters (within brackets) for total number of kits born (LS), number of born alive (NBA), ovulation rate (OR) and number of implanted embryos (IE) in the $F_2$ population.

|      | LS         | NBA        | OR   | IE   |
|------|------------|------------|------|------|
| Mean | 8.16       | 7.28       | 14.7 | 11.1 |
| SD   | 3.22       | 3.51       | 2.8  | 3.8  |
| N    | 598 (2066) | 598 (2066) | 561  | 561  |

Table 6: Features of the estimated marginal posterior distributions of the differences (D) between different genotypes of 2464G>A SNP for the promoter region of the progesterone receptor gene for total number of kits born (LS) and total number of kits born alive (NBA) in the $F_2$ population.

| | GG-AA | | GG-GA | |
|---|---|---|---|---|
| | LS | NBA | LS | NBA |
| $D_{mean}$ | 0.51 | 0.49 | 0.50 | 0.49 |
| $HPD_{95\%}$ | 0.01 , 1.04 | -0.09 , 1.00 | 0.06 , 0.92 | 0.10 , 0.91 |
| P (%) | 98 | 96 | 99 | 99 |
| R | 0.5 | 0.5 | 0.5 | 0.5 |
| Ps (%) | 48 | 51 | 65 | 52 |
| Pr (%) | 52 | 49 | 35 | 48 |
| MCse | 0.003 | 0.003 | 0.002 | 0.002 |
| Z | 0.76 | 0.49 | -0.82 | 1.69 |

$D_{mean}$, posterior mean of the difference between the GG and GA or AA 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 between genotypes; Ps, probability of similarity (probability of the absolute value of D being lower than R); Pr, probability of relevance (P(D>b) when D>0 and P(D<b) when D<0); MCse, Monte Carlo standard error; Z, Z-score of the Geweke test.

Table 7: Features of the estimated marginal posterior distributions of the differences (D) between the GG and AA genotypes of progesterone receptor gene for ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS) in the $F_2$ population.

| | OR | IE | ES | FS | PS |
|---|---|---|---|---|---|
| $D_{mean}$ | 0.01 | 0.49 | 0.52 | 0.34 | 0.73 |
| $HPD_{95\%}$ | -0.70 , 0.73 | -0.55 , 1.40 | -0.08 , 1.13 | -0.33 , 0.97 | -0.09 , 1.52 |
| P (%) | 50 | 82 | 95 | 84 | 96 |
| R | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Ps (%) | 84 | 50 | 48 | 67 | 30 |
| Pr (%) | 8 | 47 | 52 | 33 | 70 |
| MCse | 0.003 | 0.005 | 0.005 | 0.005 | 0.005 |
| Z | -1.30 | -0.88 | 0.21 | -0.36 | 0.06 |

$D_{mean}$, posterior mean of the difference between the GG and AA 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 between genotypes; Ps, probability of similarity (probability of the absolute value of D being lower than R); Pr, probability of relevance (P(D>b) when D>0 and P(D<b) when D<0); MCse, Monte Carlo standard error; Z, Z-score of the Geweke test.

Table 8: Features of the estimated marginal posterior distributions of the differences (D) between the GG and GA genotypes of progesterone receptor gene (*PGR*) for ovulation rate (OR), number of implanted embryos (IE), embryo survival (ES), fetal survival (FS) and prenatal survival (PS) in the $F_2$ population.

| | OR | IE | ES | FS | PS |
|---|---|---|---|---|---|
| $D_{mean}$ | -0.30 | 0.13 | 0.42 | 0.17 | 0.36 |
| $HPD_{95\%}$ | -0.79 , 0.24 | -0.55 , 0.86 | -0.15 , 1.00 | -0.30 , 0.65 | -0.25 , 0.94 |
| P (%) | 88 | 65 | 91 | 76 | 87 |
| R | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Ps (%) | 78 | 81 | 61 | 90 | 68 |
| Pr (%) | 22 | 15 | 39 | 9 | 32 |
| MCse | 0.003 | 0.004 | 0.004 | 0.004 | 0.004 |
| Z | -0.30 | -1.35 | 0.28 | 0.17 | -0.77 |

$D_{mean}$, posterior mean of the difference between the GG and GA 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 between genotypes; Ps, probability of similarity (probability of the absolute value of D being lower than R); Pr, probability of relevance (P(D>b) when D>0 and P(D<b) when D<0); MCse, Monte Carlo standard error; Z, Z-score of the Geweke test.