CANDIDATE GENES FOR REPRODUCTIVE TRAITS IN RABBITS: II. PROGESTERONE RECEPTOR GENE

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SUMMARY

One SNP (G/ A_{2464}) was found in the promotor region of the progesterone receptor gene (PRG) in two divergent lines selected to increase (H) and decrease uterine capacity (L). The allele G was the predominant in the H line. A total of 494 F2 does, generated from the cross of H and L lines, were genotyped for the SNP G/A_{2464} in the promoter region to examine the effect of the progesterone receptor genotypes on litter size and its components. The three PGR genotypes are named GG, AG and AA. In the F2 population were found a total of 171 animals GG, 247 AG and 78 AA. The GG genotype had a higher number of implanted embryos and litter size than CC genotype (0.62 and 0.76, respectively). Thus, the SNP G/A_{2464} could explain a large part of the difference between H and L lines.

INTRODUCTION

A divergent selection experiment for uterine capacity was performed along ten generations in rabbits (Blasco *et al*., 2005). The line selected to increase uterine capacity (H) showed 1.8 implanted embryos and 2.4 kits more than the line selected to decrease uterine capacity (L) in intact females (Santacreu *et al.*, 2005). Some results suggest the presence of a major gene affecting uterine capacity and the number of implanted embryos (Argente *et al.* (2003); Blasco *et al*., 2005).

Progesterone receptor (*PRG*) was tested as candidate gene due to its role in the reproductive functions in rabbits (Gutierrez-Sagal *et al*., 1993). The protein encoded by this gene is a nuclear receptor that binds specifically to progesterone and mediates its biological actions. Progesterone is a key component in the complex regulation of the reproductive events. The progesterone participates in release of mature oocytes, facilitation of implantation, and maintenance of pregnancy, by promotion of uterine growth and suppression of myometrial contractility (Graham and Clarke, 1997). Studies performed with *PGR* knockout mice provided evidence of the physiological role for PGR. Female mice lacking PGR exhibit impaired sexual behaviour and neuroendocrine gonadotrophin regulation, anovulation, uterine dysfunction and alterations in the mammary gland (Li *et al*., 2004).

The progesterone receptor gene had been partially amplified and sequenced in 25 animals from H and L lines (Merchán *et al.*, 2005). One SNP (G/A_{2464}) was found in the *PRG* promoter region. Significant association was detected between the allele frequencies of this SNP and lines, being the allele G the predominant in the H line.

The objective of the current study was to genotype the SNP G/A_{2464} in a population of 494 F2 females to examine the effect of progesterone receptor genotype on litter size and its components.

MATERIAL AND METHODS

Animals. A F1 population was generated from the cross of 3 males of H line (selected to increased uterine capacity) with 12 females of L line (selected to decrease uterine capacity) and from the cross of 3 males of L line with 5 females of H line. The cross of 10 F1 males with 70 F1 females produced 494 F2 females. Does were reared at the experimental farm of the Universidad Miguel Hernández in individual cages. Animals were fed with a commercial diet and the photoperiod used was 16-h light: 8-h dark.

Genotyping of the progesterone receptor gene. Genomic DNA was extracted from blood samples following the protocol of the ABI PRISM^{TM} 6100 Nucleic Acid PrepSation (Applied Biosystems). Genotyping of the SNP G/A_{2464} was performed by PCR-RFLP: a pair of primers were designed based on the promoter sequence of the rabbit PGR (GenBank accession number X06623) and a PCR fragment of 558 bp was amplified and then digested with the *Eco*31I restriction endonuclease (Fermentas). The animals genotyped as G/G_{2464}, A/G_{2464} and A/A_{2464} are named GG, AG and AA, respectively.

Traits. The following traits were recorded: total number of kits born (LS) and number of born alive (NBA) measured up to four parities; ovulation rate (OR), estimated as the number of corpora lutea, and number of implanted embryos (EI), estimated as the number of implantation site, at day 12 of the second gestation by laparoscopy. Traits calculated were: embryo survival (ES=(EI/OR)x100), foetal survival (FS=(LS/EI)x100) and prenatal survival (PS=(LS/OR)x100).

Statistical analyses. The analysis was based on Bayesian methods. Bounded uniform priors were used for all unknowns and data were assumed to be normally distributed. The effects included in the analyses were: lactation (with three levels: nulliparous, lactating and nonlactating), year-season (with six levels) and genotype (with three levels: homozygous GG, heterozigous AG and homozygous AA). The effect of a permanent environment was included in the model for litter size and it was considered to be normal distributed a priori. Marginal posterior distributions of all unknowns were estimated by Gibbs Sampling (Sorensen and Gianola, 2002). After some exploratory analyses we used one chain of 100,000 samples discarding the first 20,000 and saving every 20. Convergence was tested using the Z criterion of Geweke.

RESULTS AND DISCUSSION

Means and coefficients of variation for the traits measured in F2 population are presented in table 1. Results agree with values published in other lines of rabbits (Blasco *et al.*, 1993).

A total of 171 animals GG, 247 AG and 78 AA were found in the F2 population. Features of the estimated marginal posterior distributions of the differences (D) between GG and AA genotypes are presented in table 2. All Monte Carlo standard error (MCse) were very small and lack of convergence was not detected by the Geweke test. Marginal posterior distributions were approximately normal, so only the posterior mean of the difference is given in the tables.

Table 1. Raw means and standard deviations (within brackets) and number of records (n) for ovulation rate (OR), number of implanted embryos (IE), total number of kits born (LS), total number of kits born alive (NBA), embryo survival (ES), foetal survival (FS) and prenatal survival (PS) in the F2 population.

OR	IE	LS	NBA	ES	FS	PS
14.70(2.80)	11.14(3.68)	7.95(3.28)	7.03(3.25)	75.62(21.29)	70.95(23.18)	55.49(22.23)
n = 494	494	1497	1497	494	381	381

Considering that 1 ova per female is a relevant difference (table 2, b=1), the estimated difference between genotypes GG and AA in OR was not considered to be relevant because the probability of OR being between 1 and -1 is 99%, which means that the probability of OR being higher or lower than 1 is very low (1%). This result agrees with Mocé *et al.* (2004), not relevant differences were found between H and L lines.

Table 2. Features of the estimated marginal posterior distributions of the differences between GG and AA genotypes of receptor of progesterone for ovulation rate (OR), number of implanted embryos (IE), total number of kits born (LS), total number of kits born alive (NBA), embryo survival (ES), foetal survival (FS) and prenatal survival (PS).

	OR	IE	LS	NBA	ES	FS	PS
D	0.30	0.62	0.76	0.71	3.04	3.07	4.59
$HPD_{95\%}$	-0.43,1.08	-0.35,1.63	0.14,1.36	0.06,1.32	-3.22,9.18	-5.87,13.26	-4.97,14.17
P(D>0) (%)	79	89	99	99	84	73	83
B	1	0.5	0.5	0.5	4	4	4
Pr (%)	4	60	79	74	39	50	59
P(D>b) (%)	4	60	79	74	39	43	55
MCse	0.007	0.008	0.005	0.005	0.04	0.05	0.05
Z	-0.29	0.74	0.92	-0.84	-0.20	0.68	-0.12

D: posterior mean of the difference between GG and AA genotype; $HPD_{95\%}$: highest posterior density region at 95%; b: assumed relevant difference; Pr: probability of relevance (probability of D being higher than b in absolute value); P(D>b): probability of D being relevant in favour of GG genotype; MCse: Monte Carlo standard error; Z: Z-score of the Geweke test.

We assume a difference of 0.5 implanted embryos to be a relevant difference for IE, because it represents 25% of the difference between H and L lines for this trait (Santacreu *et al.,* 2005). The GG genotype had around 0.6 implanted embryos (relevant difference) more than AA genotype. Although $HPD_{95\%}$ included zero, GG genotype had a higher number of implanted embryos with a high probability (P(D>0) is 89%) and the probability of a relevant difference was moderate (P(D>b) is 60%) and always in favour of GG genotype. We assumed a difference of 0.5 kits could be considered as a relevant difference for LS and NBA. The GG genotype had a higher litter size (P(D>0) is 99%). This difference was around 0.7 kits, and the probability of a relevant difference was high (P(D>b) is 79%) and always in favour of GG genotype. Similar results were obtained for NBA. We deduced relevant differences for survival rates (ES, FS and PS) from the former relevant difference of 0.5 embryos (4%). The GG genotype had a higher ES and FS than AA genotype, P(D>0%) is 84% and 73%, respectively. Although these differences between genotypes are not relevant, around 3% in each survival trait, the probability of a relevant difference in favour of GG genotype were 39 and 43% respectively (table 2). The GG genotype had a higher PS (D=4.6%); P(D>0) is 83%) Although $HPD_{95\%}$ included zero, the probability of a relevant difference was moderate (P(D>b) is 59%) and the probability of relevant difference in favour of GG genotype was 55%. The genotype of the receptor of progesterone represent the 35% and 33% of the difference between H and L line found by Santacreu et al. (2005) for implanted embryos and number of total born.

CONCLUSION

The polymorphisms analyzed in progesterone receptor that are associated to H and L lines could explain a large part of the difference found for implanted embryos (EI), total number of kits born (LS) and number of kits born alive (NBA). It would be interesting to know if this SNP is the causal mutation or if it is only a DNA marker in linkage disequilibrium with the causal mutation.

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