GENOTYPE X SPERM DOSAGE INTERACTION ON REPRODUCTIVE PERFORMANCE AFTER ARTIFICIAL INSEMINATION. 1. MALE FERTILITY

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ABSTRACT

Failures in fertilization or embryogenesis have been shown to be in part of semen origin. Fertilization rate depends on the quality and the number of spermatozoa of the dose of artificial insemination. Thus, individual variation in male fertility could be better observed under conditions of low sperm concentration of the AI dose. The aim of this research was to estimate genetic parameters of male fertility after AI with low and high sperm dosage, considered as different traits. The interaction genotype x sperm dosage was estimated to know whether there is individual variation for the effect of sperm dosage on fertility. A total of 6655 AI was performed, involving 2527 crossbred females that were inseminated with homospermic semen doses coming from 250 bucks of the Caldes line. Fertility (defined as success/failure to conception) after AI with doses of 10 x 10^6 (F_{10}) or 40 x 10^6 spermatozoa/ml (F_{40}) was considered as a binary trait. Data were analyzed under a bivariate threshold model. The model for the underlying variables, corresponding to F_{10} and F_{40}, included the systematic effects of: the physiological status of the female, day of insemination-operator, and buck age-building, and the male additive genetic effects, the male non additive genetic plus permanent environmental effects, the female genetic plus permanent environmental effects, the environmental permanent effects of male and day of IA, and a random residual effect. The mean of the marginal posterior distribution for F_{10} minus F_{40} was estimated to be -0.13 (s.d.: 0.02), which indicates a clear effect of the sperm dosage on fertility. However, 40 x 10^6 spermatozoa/ml seems to be not high enough to compensate deficiencies in sperm characteristics precluding sperm access to the ovum and fertilization when homospermic doses are used. It is because F_{40} was still lower than fertility after natural mating (NM). Heritabilities seem to be similar for F_{10} and F_{40} and both of them could be higher than heritability of male fertility after NM. The importance of the genotype x sperm dosage interaction was almost negligible (<12% of the mean of the additive variance), since additive variances were similar for both traits and their genetic correlation was close to 1.

Key words: Artificial insemination, Fertility, Genotype x sperm dosage interaction, Male effects.

INTRODUCTION

Reproductive success, defined by fertility and litter size, greatly determines the efficiency of meat rabbit production. Failures in fertilization or embryogenesis have been shown to be in part of semen origin (Saacke *et al.*, 2000). However, the information in the literature concerning fertility, considered to be a trait of the male or both sexes, is scarce. Piles *et al.* (2005) estimated variance-covariance components of male and female fertility, defined as success or failure to mating, in two populations of rabbits. They demonstrated the existence of genetic and environmental variation for female rabbit fertility that was negligible for male, after natural mating.

When artificial insemination (AI) is practised, fertilization rate depends on the quality and the number of spermatozoa of the dose (Colenbrander *et al*., 2003). Differences in fertility among males which disappear at high sperm dosage are considered "compensable" and are due to semen deficiencies which prevent the sperm access or engagement to the ovum, while differences independent of sperm dosage are considered "non compensable" and are associated with the sperm unable to maintain the fertilization process or subsequent embryogenesis once initiated (Saacke *et al*., 2000). Therefore, individual variation in male fertility could be better observed under limited conditions of AI, such us, low sperm concentration and small or null pre-selection of the ejaculates for any semen quality trait.

Under this hypothesis, the aim of this research was to estimate genetic parameters of male fertility after AI with low and high sperm dosage, considered as different traits, and also the interaction between the genotype and the sperm concentration of the dose of AI, to know if there is individual genetic variation on the effect of sperm dosage on fertility.

MATERIALS AND METHODS

Animals and experimental design

Males belonged to the Caldes line selected for growth rate during the fattening. They were bred and reared on a farm belonging to the IRTA and mated to crossbred does (Prat x V) reared on a commercial farm of two buildings. Does followed a semi-intensive reproductive rhythm: first mating at about 4.5 months of life, with subsequent 42 days reproductive cycles. Bucks were raised with a photoperiod of 16 hours light/day and started the training period at 5 months of age. One ejaculate was collected per male and per week during the first two weeks using artificial vagina. After this period, two ejaculates per male per week were collected, with an interval of 30 minutes between collections. The ejaculates used for this study were collected in three times of the buck's productive life between 5 and 9 months of age. Ejaculates were stored in a dry bath at 35ºC until evaluation but for no more than 15 min after collection. Ejaculates containing urine and calcium carbonate deposits were discarded, and gel plugs were removed. After that, individual motility of ejaculate was measured in aliquots (25µl) under a light microscope (Nikon) at x100 according to a subjective scale from 0 to 5 (Roca *et al.*, 2000). A pre-selection of ejaculates was performed, discarding ejaculates with individual motility lower than 2. After evaluation, ejaculates from one buck were pooled and diluted (1:2) in a commercial saline extender for rabbit semen (CUNIGEL, IMV Technologies) and the cell sperm concentration was measured by using a Nucleocounter SP-100® The pool from each buck was divided in two halves and diluted until 10 x 10^6 and 40 x 10^6 spermatozoa/ml, corresponding the second value to the commercial sperm dosage for this line which produces an average fertility rate about 75-80%, using heterospermic AI doses. Semen doses were stored at 18ºC for 24 hours until AI. Does were treated with subcutaneous application of eCG 12-15 UI for oestrous induction 48 h before A.I. The does were inseminated with 0.5 ml of the pools. The ovulation of does was immediately induced after A.I. by an intramuscular injection of 0.8 mg Busereline acetate.

Fertility (defined as success to conception) after AI with doses of 10 x 10^6 spermatozoa/ml (F_{10}) or fertility after AI with doses of 40 x 10^6 spermatozoa/ml (F_{40}) were considered as different traits which show a binary expression. There were 3,628 records of F_{10} and 3,027 records of F_{40}. Data involved 2,527 females and 250 males. The pedigree, referred to the 250 males, included 733 individuals.

Model and Statistical Analysis

The threshold model postulates that the observed response is related to an underlying normal variable and to a fixed threshold that divides the continuous scale into two intervals that delimit the two response categories (Wright, 1934). Procedures developed by Sorensen *et al*., (1995), based on Markov Chain Monte Carlo methods, allow the analysis of categorical traits using this model. For our study, we assumed a bivariate model for the underlying variables corresponding to F_{10} and F_{40}, which included the systematic effects of: the physiological status of the female (3 levels: 1, for nulliparous

does, 2, for multiparous does in lactation at AI and, 3, for multiparous does not in lactation at AI), a combined effect between the day of insemination and operator (19 levels) and a combined effect between the buck age and the building (9 levels). The model also included the male additive genetic effects (u_{10}, u_{40}), the male non additive genetic plus permanent environmental effects (p_{m10}, p_{m40}), the female genetic plus permanent environmental effects (p_{f10}, p_{f40}), the environmental permanent effects resulting from the combination between male and day of IA (p_{md10}, p_{md40}), and a random residual effect (e_{10}, e_{40}),

A Bayesian approach via the Gibbs sampler was implemented to obtain posterior distributions of the model parameters. The following multivariate normal distributions were assumed *a priori* for random effects:

$$\begin{pmatrix}\mathbf{u}_{10}\\ \mathbf{u}_{40}\end{pmatrix}|\mathbf{G}\sim\begin{pmatrix}\mathbf{0}\\ \mathbf{0}\end{pmatrix},\mathbf{G}\otimes\mathbf{A}\Big);\quad \begin{pmatrix}\mathbf{p}_{m10}\\ \mathbf{p}_{m40}\end{pmatrix}|\mathbf{P}_{m}\sim\begin{pmatrix}\mathbf{0}\\ \mathbf{0}\end{pmatrix},\mathbf{P}_{m}\otimes\mathbf{I}\Big);\quad \begin{pmatrix}\mathbf{p}_{f10}\\ \mathbf{p}_{f40}\end{pmatrix}|\mathbf{P}_{f}\sim\begin{pmatrix}\mathbf{0}\\ \mathbf{0}\end{pmatrix},\mathbf{P}_{f}\otimes\mathbf{I}\Big);\quad \begin{pmatrix}\mathbf{p}_{md10}\\ \mathbf{p}_{md40}\end{pmatrix}|\mathbf{P}_{md}\sim\begin{pmatrix}\mathbf{0}\\ \mathbf{0}\end{pmatrix},\mathbf{P}_{md}\otimes\mathbf{I}\Big);$$

$$\begin{pmatrix}\mathbf{e}_{10}\\ \mathbf{e}_{40}\end{pmatrix}|\mathbf{R}\sim\begin{pmatrix}\mathbf{0}\\ \mathbf{0}\end{pmatrix},\mathbf{R}\otimes\mathbf{I}\Big)$$

Residual variances were set to 1. Bounded uniform priors were assumed for the systematic effects and the (co)variance components (G, P_m, P_f, P_{md} and R). A single chain of 230,000 iterations was run. The first 30,000 iterations of each chain were discarded, and samples of the parameters of interest were saved for each of 10 iterations. The sampling variance of the chains was obtained by computing Monte Carlo standard errors (Geyer, 1992). Burn-in was determined using the procedure of Raftery and Lewis (1992). The posterior distribution of the interaction variance (σ^2_{GxE}) was estimated from the samples of genetic variances following Mathur (2002): $\sigma^2_{GxE}=0.5\left(\sigma_{g10}-\sigma_{g40}\right)^2+\sigma_{g10}\sigma_{g40}\left(1-r_g\right)$

RESULTS AND DISCUSSION

The crude mean for F_{10} and F_{40} were 0.46 and 0.59, respectively. The mean of the estimated marginal posterior distribution (EMPD) for F_{10} minus F_{40} was estimated to be -0.13 (s.d.: 0.02). This result indicates a clear effect of the sperm dosage on fertility (about 31% of the mean for F10). The sperm concentration of the ejaculate in this line was estimated to be $252x10^6$ spermatozoa/ml (García-Tomás *et al*., 2006) and fertility rate after natural mating (NM) with purebred females of the same line was 80.5% (Piles *et al*., 2005). Therefore, if no differences in fertility among crossbred and purebred females are assumed, an increase of 30 x 10^6 spermatozoa/ml seems to be not high enough to compensate deficiencies in sperm characteristics precluding sperm access to the ovum or the ability to engage the ovum sufficiently to initiate fertilization and to block polyspermy, when homospermic doses are used. The effect of sperm dosage could be non linear and moreover, there could be effects of other factors related to the AI process (different from the semen characteristics), which could explain differences in fertility rate after AI with high sperm dosage and the same ratio after NM. Individual differences in factors with an effect that can not be compensated with a high number of spermatozoa of the AI dose and in the sperm concentration of the ejaculate, would explain the small variation due to the male in fertility after NM.

The EMPD of variance components for F_{10} and F_{40} are summarized in Table 1. Although they are inaccurate heritabilities seem to be similar for F_{10} and F_{40} and both of them could be higher than the corresponding value to male fertility after NM (0.013, Piles *et al*., 2005), being the probability of a value higher than 0.02 equal to 84% and 94% for F_{10} and F_{40}, respectively.

This suggests that genetic variance after NM could be due mainly to individual genetic variation in semen characteristics with an effect that can not be compensated with high sperm dosage since it associated with the sperm unable to maintain the fertilization process or subsequent embryogenesis once initiated, whereas genetic variance after AI would be due to any kind of semen characteristics. Variance components also showed that the importance of the genotype x sperm dosage interaction was

almost negligible (<12% of the mean of the additive variance). This is because the genetic variances seems to be relatively close for both traits and their genetic correlation seems to be near to 1 (Figure 1).

Table 1: Summary statistics of marginal posterior distributions of heritability (h^2), ratio of variation due to the male non additive genetic plus environmental effects ($perm_m$), due to female effects ($perm_f$), and due to male and day of AI environmental effects ($perm_{md}$), and phenotypic variance (σ^2) for F_{10} and F_{40}

	F_{10}				F_{40}			
parameter	PM[1]	PSD[2]	HPD95%[3]	MCse[4]	PM[1]	PSD[2]	HPD95%[3]	MCse[4]
h^2	0.076	0.057	0.00056, 0.19	0.0023	0.090	0.053	0.0058, 0.19	0.0021
$perm_m$	0.161	0.066	0.017, 0.28	0.0025	0.114	0.052	0.0062, 0.20	0.0019
$perm_f$	0.104	0.027	0.053, 0.16	0.00098	0.066	0.027	0.0062, 0.11	0.0011
$perm_{md}$	0.293	0.047	0.204, 0.383	0.0014	0.300	0.045	0.217, 0.392	0.0013
σ^2	2.76	0.28	2.26, 3.33	0.00093	2.35	0.21	1.96, 2.78	0.0072

[1]PM: posterior mean. [2]PSD: posterior standard deviation. [3]HPD95%: High posterior density interval at 95%. [4]MCse: Monte Carlo standard error

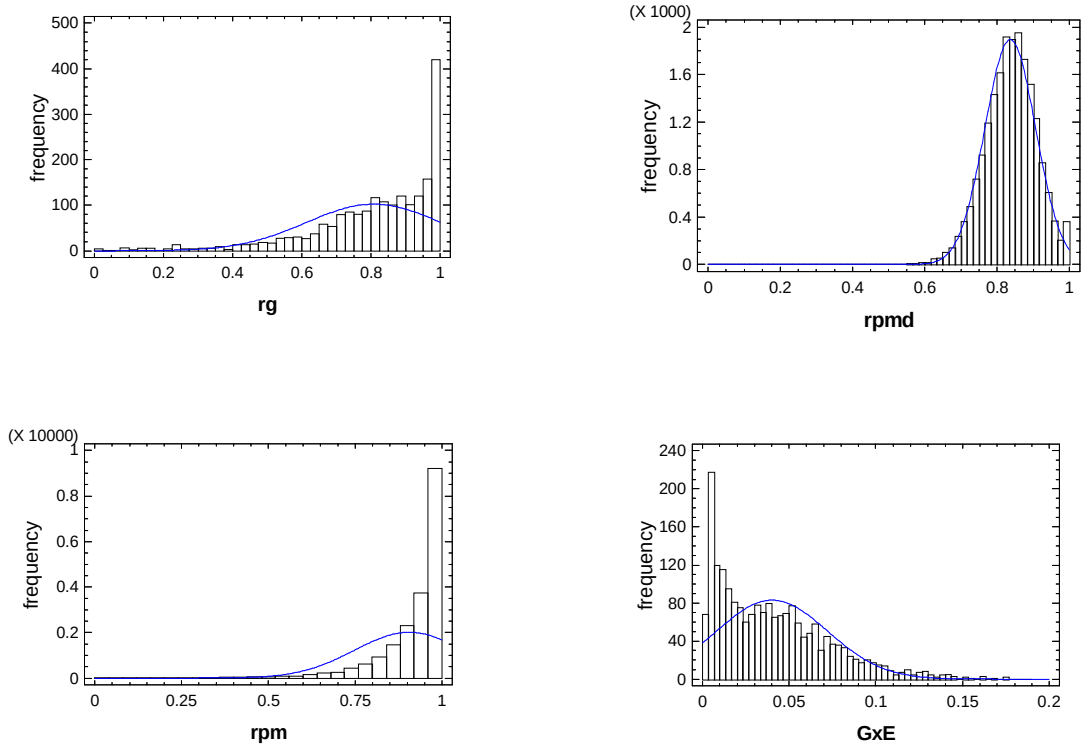

Figure 1: Histogram of frequencies and estimated marginal posterior distribution of genetic correlation (rg), correlation between male and day of IA environmental effects (rpmd) and, correlation between environmental male effects (rpm) for F_{10} and $F_{40,}$ and variance of the interaction between the genotype and the sperm dosage (GxE)

This means that, probably, the same genes are affecting F_{10} and F_{40} and, thus, the responses to selection for increased male fertility that could be obtained after AI – within this range of sperm dosage – would be the same, and also that the proportion of response selecting for one trait, that could be expected for the other trait – as a correlated response – would be high. Thus, within the range of sperm dosage studied, selection to improve male fertility after AI could be performed at any sperm dosage, and could have a higher response to selection than selection for male fertility after NM. On the other hand, although there is an effect of sperm dosage on male fertility, there is negligible individual genetic variation of this effect and therefore, probably there is no individual genetic variation on the effect of "compensable" semen characteristics as a whole on fertility after AI. Thus, if the objective is

to improve male fertility with non limiting sperm dosage through indirect selection for semen quality traits, the selection criteria should be "non compensable" traits, but if the objective was to optimize the use of the ejaculates to obtain a higher number of doses of AI, the selection criteria should be "compensable" semen characteristics or both.

CONCLUSIONS

Sperm dosage has an important effect on male fertility but there is no individual genetic variation on this effect. The expected response to selection would be the same independently of the sperm concentration of the dose of AI at least, within the range studied. Differences between males in the number of spermatozoa required to reach a fixed fertilization rate are probably due to individual differences in semen characteristics but not to individual differences in the effect of sperm dosage.

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