# GENOTYPE X SPERM DOSAGE INTERACTION ON REPRODUCTIVE PERFORMANCE AFTER ARTIFICIAL INSEMINATION. 2. MALE LITTER SIZE

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## ABSTRACT

Failures in fertilization or embryogenesis have been shown to be in part of semen origin. When artificial insemination (AI) is practised in prolific species, under conditions of low sperm concentration of the AI dose, differences among males in number of kits born per litter (TB) could be better observed than when natural mating (NM) is practised, due to the effect of the individual variation of semen characteristics with an effect on this trait that can be compensated increasing the number of spermatozoa. This research aimed to estimate genetic parameters of male on TB after AI with low ($10x10^6$ spermatozoa/ml, $TB_{10}$) and high ($40x10^6$ spermatozoa/ml, $TB_{40}$) sperm dosage, considered as different traits, and also the interaction between the genotype and the sperm dosage, to know whether there is individual genetic variation on the effect of sperm dosage on TB. The number of records was 1650 and 1856 for $TB_{10}$ and $TB_{40}$, respectively, corresponding to 1129 females and 202 males for $TB_{10}$ and 1188 females and 209 males for $TB_{40}$. The pedigree, referred to the males, included 733 individuals. The mean of the estimated marginal posterior distribution (EMPD) for $TB_{10}$ minus $TB_{40}$ was -1.25 kits (s.d.: 0.22), which supposed an increase of 17.3% in TB over the mean of the EMPD for $TB_{10}$. Heritability of male TB after AI is higher than the corresponding value after NM. The additive genetic variances for $TB_{10}$ and $TB_{40}$ were no significantly different and the genetic correlation between both traits can be considered as high. Consequently, the estimated value of the genotype x sperm dosage interaction was almost negligible (mode (mean) <1.5%, <16% of the average of the means of the EMPD for the additive variances) and therefore, no individual genetic variation was observed for the effect of sperm dosage on the total number of kits born. Estimates of genetic parameters suggest that most of the genes affecting $TB_{10}$ are also affecting $TB_{40}$ and thus, within the range of sperm dosage studied, selection to improve male TB after AI could be performed at any sperm dosage, and could have a higher response to selection than selection for male TB after NM.

**Key words**: Artificial insemination, Litter size, Genotype x sperm dosage interaction, Male effects.

## INTRODUCTION

Reproductive success, defined by fertility and litter size, greatly determines the efficiency of meat rabbit production. Litter size may be determined by a male component, since failures in fertilization or embryogenesis have been shown to be in part of semen origin (Saacke *et al*., 2000). The only information in the literature about the magnitude of the buck component associated with these traits was given by Piles *et al*. (2006) who found a very small effect of the buck on litter size after natural mating (NM). However, when artificial insemination (AI) is practised using doses with a smaller number of spermatozoa than in the ejaculate, differences among males in fertilization rate could be better observed due to the effect of the individual variation of semen characteristics with an effect on fertility that can be compensated with high sperm dosage, since it associated with semen deficiencies which prevent the sperm access or engagement to the ovum. Variation on fertility rate due to these characteristics could be expressed in a higher magnitude at low concentration of the dose for AI (Piles

*et al*., 2008). The same could happen at the level of the number of fertilized ova and developed embryos of prolific species. Thus, variation due to the male in litter size could be very small at high sperm dosage but could be better observed under poor AI conditions, such us, low sperm concentration and no or small pre-selection of the ejaculates for any semen quality trait.

In order to check this hypothesis, the aim of this job was to estimate variance components of the total number of kits born per litter after AI with low and high sperm dosage, considered to be different traits of the male, and to estimate the variance of 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 litter size.

## MATERIALS AND METHODS

### Animals and experimental design

The males came from 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-mo of life, with subsequent 42-d reproductive cycles. Bucks were raised with a photoperiod of 16 hours light/day and started the training period at 5 mo 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 mo 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 (25 µl) under a light microscope (Nikon) at x100 according to a subjective scale from 0 to 5 (Roca *et al.*, 2000). Ejaculates with individual motility lower than 2 were discarded. After evaluation, ejaculates from one buck were pooled and diluted (1:2) in a commercial saline extender for rabbit semen (CUNIGEL) 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 $10 \times 10^6$ and $40 \times 10^6$ 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.

A total of 6,655 IA were performed in a commercial farm of two buildings, involving 2,527 crossbred females that were inseminated with homoespermic semen doses from 250 bucks of the Caldes line. The total number of kits born per litter after AI with doses of $10 \times 10^6$ spermatozoa/ml ($TB_{10}$) and the total number of kits born alive after AI with doses of $40 \times 10^6$ of spermatozoa/ml ($TB_{40}$) were considered as different traits. The number of records was 1,650 and 1,856 for $TB_{10}$ and $TB_{40}$, respectively, corresponding to 1,129 females and 202 males for $TB_{10}$ and 1,188 females and 209 males for $TB_{40}$. The pedigree, referred to the males, included 733 individuals.

### Model and Statistical Analysis

Markov Chain Monte Carlo methods were used for inference. We assumed a bivariate model 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 combination effect between the day of insemination and inseminator (19 levels) and a combination 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 ($e_{10}$, $e_{40}$).

The analysis was performed via the Gibbs sampler. Marginal posterior distributions (MPD) for all model parameters were obtained. The following multivariate normal distributions were assumed *a priori* for random effects:

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

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

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 ($\sigma^2_{GxE}$) was estimated from the samples of genetic variances following Mathur (2002): $\sigma^2_{GxE} = 0.5(\sigma_{g10} - \sigma_{g40})^2 + \sigma_{g10}\sigma_{g40}(1 - r_g)$

## RESULTS AND DISCUSSION

The mean (s.d.) of the estimated marginal posterior distribution (EMPD) for $TB_{10}$ and $TB_{40}$ were 7.2 (0.3) and 8.5 (0.2), respectively. The mean of the EMPD for $TB_{10}$ minus $TB_{40}$ was -1.25 kits (s.d.: 0.22), which supposed an increase of 17.3 % in TB over the mean of the EMPD for $TB_{10}$ (5.8% in TB per increase of $10x10^6$ spermatozoa/ml in the AI dose). Thus, it seems that the effect of sperm dosage is also expressed at the level of the number of fertilized ova and developed embryos.

The estimated MPD of variance components for $TB_{10}$ and $TB_{40}$ are summarized in Table 1. They are not very accurate but it seems that heritability of male litter size after AI is higher than the corresponding value after NM (Piles *et al*., 2006).

**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 the female effects ($perm_f$) and due to the environmental effects of male and day of AI ($perm_{md}$), and phenotypic variance ($\sigma^2$) for $TB_{10}$ and $TB_{40}$

| | $TB_{10}$ | | | | $TB_{40}$ | | | |
|---|---|---|---|---|---|---|---|---|
| parameter | PM[1] | PSD[2] | HPD95%[3] | MCse[4] | PM[1] | PSD[2] | HPD95%[3] | MCse[4] |
| $h^2$ | 0.087 | 0.035 | 0.0219, 0.16 | 0.0013 | 0.059 | 0.027 | 0.0083, 0.11 | 0.0010 |
| $perm_m$ | 0.037 | 0.027 | 0.00037, 0.087 | 0.0010 | 0.040 | 0.023 | 0.0014, 0.085 | 0.0008 |
| $perm_f$ | 0.053 | 0.029 | 0.0011, 0.10 | 0.0011 | 0.059 | 0.022 | 0.0186, 0.10 | 0.0008 |
| $perm_{md}$ | 0.111 | 0.030 | 0.056, 0.17 | 0.0008 | 0.083 | 0.027 | 0.034, 0.14 | 0.0007 |
| $\sigma^2$ | 10.0 | 0.477 | 9.15, 10.99 | 0.012 | 8.02 | 0.332 | 7.41, 8.71 | 0.0074 |

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

The additive genetic variances for $TB_{10}$ and $TB_{40}$ were no significantly different (but the probability of the genetic additive variance for TB10 > mean of the EMPD of the genetic additive variance for TB40 was higher than 87%) and the genetic correlation between both traits was near to 1 (Figure 1). Thus, the genotype x sperm dosage interaction was almost negligible (mode (mean) <1.5% (<16%) of the average of the means of the EMPD for the additive variances) so there was not observed individual genetic variation on the effect of sperm dosage on the total number of kits born. Estimates of genetic parameters also suggest that, like fertility, most of the genes affecting $TB_{10}$ are also affecting $TB_{40}$ and thus, responses to selection for increased TB due to male effects, which could be obtained after AI -

within this range of sperm dosage-, would be the same, no matter the semen dosage used. Moreover, the 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 males for TB after AI could be performed at any sperm dosage, and could have a higher response than selection of males to improve TB after NM.

The proportion of the phenotypic variance due to male non additive and environmental permanent effects were the same for $TB_{10}$ and $TB_{40}$, and the correlation between these effects was estimated to be high (Figure 1). The magnitude of these effects was almost negligible. The mean of the EMPD was about 4% for both traits (lower than the corresponding value of heritability for $TB_{10}$). Estimates of genetic plus environmental variation attributable to the doe were small. This could be explained assuming that the variance of permanent environmental effects due to the female was small due to the heterosis effect on this trait.

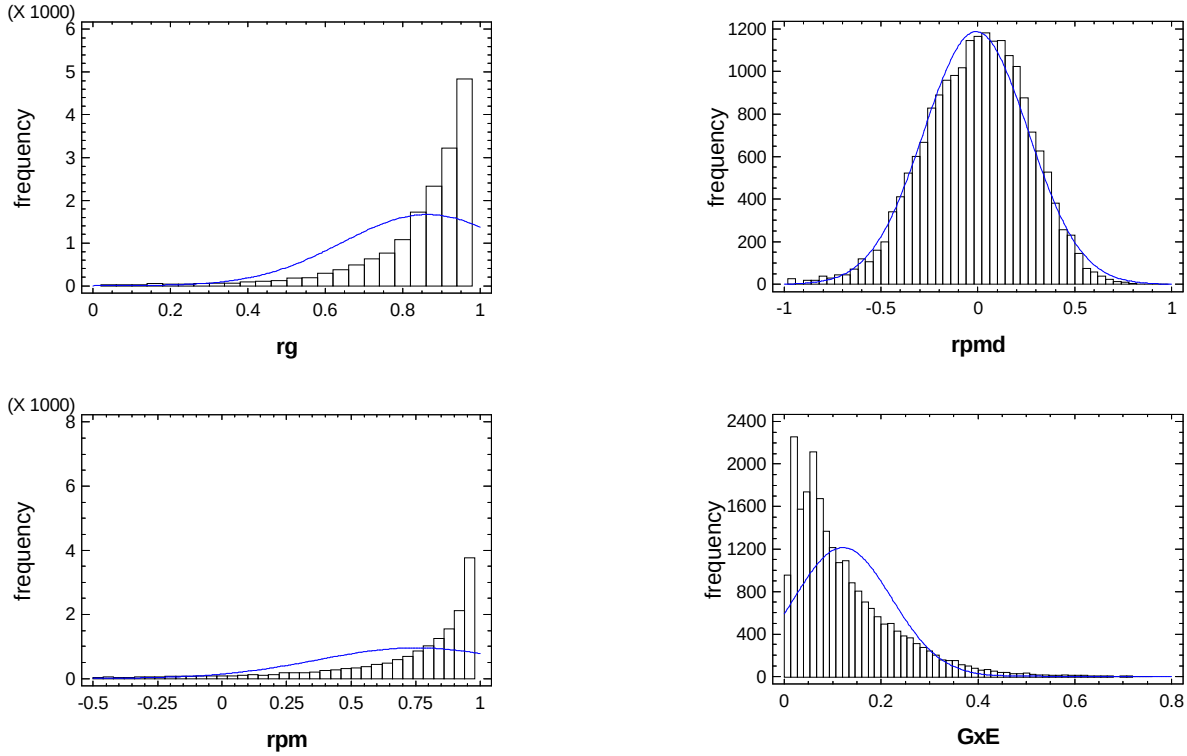


**Figure 1:** Histogram of frequencies and estimated marginal posterior distribution of genetic correlation (rg), correlation among environmental male effects (rpm), and correlation between environmental effects of male and day of AI (rpmd) for $TB_{10}$ and $TB_{40}$, and variance of the interaction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

## CONCLUSIONS

Additive genetic variance of male total number of kits born was higher after AI than the same value after NM. Sperm dosage has an important effect on this trait but there is no individual variation on it. The expected response to selection for increase this trait, through the selection of the AI males, would be the same independently of the sperm concentration of the dose of IA, at least within the range studied. Differences between males in the number of sperm required to reach a fixed TB would be probably due to individual differences in semen characteristics but not to individual differences in the effect of sperm dosage.

## ACKNOWLEDGEMENTS

Research was supported by INIA-RTA2005-0008-C02 project and an INIA grant for LL. Tusell. The authors are grateful to Daniel Mozer Schonborn and Sinela Mozer Schonborn for their contribution to the experimental work.

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