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# Feasibility of selection for male contribution to embryo survival as a way of improving male reproductive performance and semen quality in rabbits<sup>1</sup>

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**ABSTRACT:** The objective of this research was assess the male contribution to the number of implanted embryos (IE) and embryo survival (ES) estimated as the rate of variance due to male genetic and permanent environmental effects regarding total variance. In prolific species, the number of embryos and ES at early stages of gestation could be considered as fertility measurements because it indicates the number and rate of fertilized ova which are able to initiate the embryo development. Analyzed traits were ovulation rate (OR) estimated as the number of corpora lutea in both ovaries, IE estimated as the number of implantation sites, and ES calculated as IE/OR. A total of 1477 records from 900 females were used to analyze OR, whereas 1081 records from 855 females and 201 males were used to analyze IE and ES. The number of animals in the pedigree was 1107. The model included the systematic effects of year-season, parity order, lactation, and the random effects of additive

genetics and permanent environmental effects due to the female (for OR) or both sexes (for IE and ES). Gibbs sampling was used to estimate posterior distributions of model parameters. The heritabilities of the male contribution to IE and ES were low (0.05 [0.01, 0.10] and 0.07 [0.02, 0.12]) but these estimates are probably biased downward since laparoscopy was only performed on those does that were pregnant at d 12 of gestation, instead of on all mated does. The genetic correlations between all analyzed traits and also between male and female genetic components of IE and ES were inaccurate, and it was not possible to draw any conclusion about them. The proportion of variation due to the male nonadditive genetic plus permanent environmental effects for IE and ES was almost negligible (0.027 [0.001, 0.058] and 0.031 [0.002, 0.068] for IE and ES, respectively), being the repeatability for male contribution on IE and ES around 8 and 10%, respectively.

**Key words:** embryo survival, genetic parameters, implanted embryos, male, semen quality

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## INTRODUCTION

In recent years, the use of artificial insemination (AI) in rabbit commercial farms has become a common practice. Bucks from paternal lines used for AI not only must have good characteristics of growth and feed efficiency, but also high fertility, seminal production, and quality. Accordingly, traits related to AI efficiency,

including both ejaculate and semen characteristics and male contribution to fertility and prolificacy, have acquired importance.

Increasing the production of fertile doses through improving some of its components (i.e., traits involved in semen production and quality) requires establishing the set of seminal characteristics to be measured and the optimal levels of those traits. In addition, measurement of these laboratorial markers is usually expensive and laborious, which make them inappropriate as selection criteria. As fertility and prolificacy are consequence of both male and female effects plus the interaction between them, a better alternative could be improving male contribution to fertility and prolificacy, since both traits depend strongly on semen quality (Foote,

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2003). Few studies have focused on the feasibility of this kind of selection to improve AI efficiency. First studies performed in rabbit species showed that male contributions to fertility (Piles et al., 2005), or to prolificacy (Piles et al., 2006) were almost null after natural mating. Similar results were found when AI was performed with fresh semen (Tusell et al., 2011). The low male contribution (i.e., male genetic and permanent environmental effects) to prolificacy measured at birth is due mainly to the fact that litter size depends on female contribution and gestational environmental effects. However, failures of fertilization or embryogenesis due to male effect are important before implantation (Saacke et al., 2000), whereas they are masked at later stages of gestation when male is not involved in any biological process. Consequently, male effects on fertility and prolificacy are expected to be better observed at early stages of gestation.

We postulate that male reproductive performance and semen quality could be improved by selecting for male contribution to embryo survival (**ES**), which is defined in rabbit as survival from ovulation to implantation at d 7 of gestation (Mocé et al., 2010). The aim of this research was to assess male contribution to the number of implanted embryos (**IE**) and ES to confirm or reject this hypothesis.

## MATERIALS AND METHODS

### *Animals and Experimental Design*

Ovulation rate (**OR**) was estimated as the number of corpora lutea in both ovaries. Females had two measurements of OR: one by laparoscopy at d 12 of second gestation, and a second postmortem measurement in the last gestation. Number of IE was estimated as the number of implantation sites (Santacreu et al., 1990); the number of implantation sites was counted by laparoscopy at d 12 of second gestation. Embryonic survival was calculated as IE/OR. Five-hundred-and-fifty-three females had a second, postmortem measurement of IE and ES.

A total of 1477 records from 900 females were used to analyze OR, whereas 1081 records from 855 females and 201 males were used to analyze IE and ES. The number of animals in the pedigree was 1107; some animals in the pedigree did not have records. Animals belonged to a selection experiment for OR described by Laborda et al. (2011).

### *Statistical Analysis*

Data were analyzed under a bayesian approach by using a bivariate Gaussian mixed model. The analysis

was performed using the Gibbs2f90 developed by Misztal (2010). The model for OR was:

$$y_{OR} = X_{OR}\beta_{OR} + Z_{1,OR}u_{f,OR} + Z_{2,OR}p_{f,OR} + e_{OR}$$

Where  $\beta_{OR}$  is the vector of systematic effects,  $u_{f,OR}$  is the vector of additive genetic effects of the female,  $p_{f,OR}$  is the vector of maternal permanent environmental effects, and  $e_{OR}$  is the random residual vector. Incidence matrices  $X_{OR}$ ,  $Z_{1,OR}$ , and  $Z_{2,OR}$  relate data to the corresponding systematic, genetic, and permanent environmental effects. The systematic effects included in the model were: year-season (31 levels), parity order (4 levels: 2, 3, 4,  $\geq 5$ ) and lactation (2 levels: 1 = lactating, 2 = nonlactating).

The model assumed for IE and ES was:

$$y_{IE} = X_{IE}\beta_{IE} + Z_{1,IE}u_{f,IE} + Z_{2,IE}u_{m,IE} + Z_{3,IE}p_{f,OR} + z_{4,IE}u_{m,IE} + e_{IE}$$

Where  $\beta_{IE}$  is the vector of systematic effects,  $u_{f,IE}$  and  $u_{m,IE}$  are the vectors of female and male additive genetic effects, respectively;  $p_{f,IE}$  and  $p_{m,IE}$  are the vectors of female and male nonadditive genetic plus permanent environmental effects, respectively; and  $e_{IE}$  is the vector of residuals. Terms  $X_{IE}$ ,  $Z_{1,IE}$ ,  $Z_{2,IE}$ ,  $Z_{3,IE}$ , and  $Z_{4,IE}$  are incidence matrices that relate data to the corresponding systematic, genetic, and permanent environmental effects. The systematic effects included in the model were the same as for OR.

The following multivariate normal distributions were assumed a priori for random effects:

$$P(\beta) \sim k; P(u | G) \sim (0, G \otimes A);$$

$$P(p_{m,IE} | \sigma_{p_{m,IE}}^2) \sim N(0, \sigma_{p_{m,IE}}^2 \otimes I);$$

$$P(p_f | P_f) \sim N(0, P_f \otimes I);$$

$$P(e | R) \sim N(0, R \otimes I)$$

Where  $A$  is the relationship matrix, and  $k$  is a constant.

$$\beta' = (\beta'_{OR}, \beta'_{IE}),$$

$$u' = (u'_{f,OR}, u'_{f,IE}, u'_{m,IE}),$$

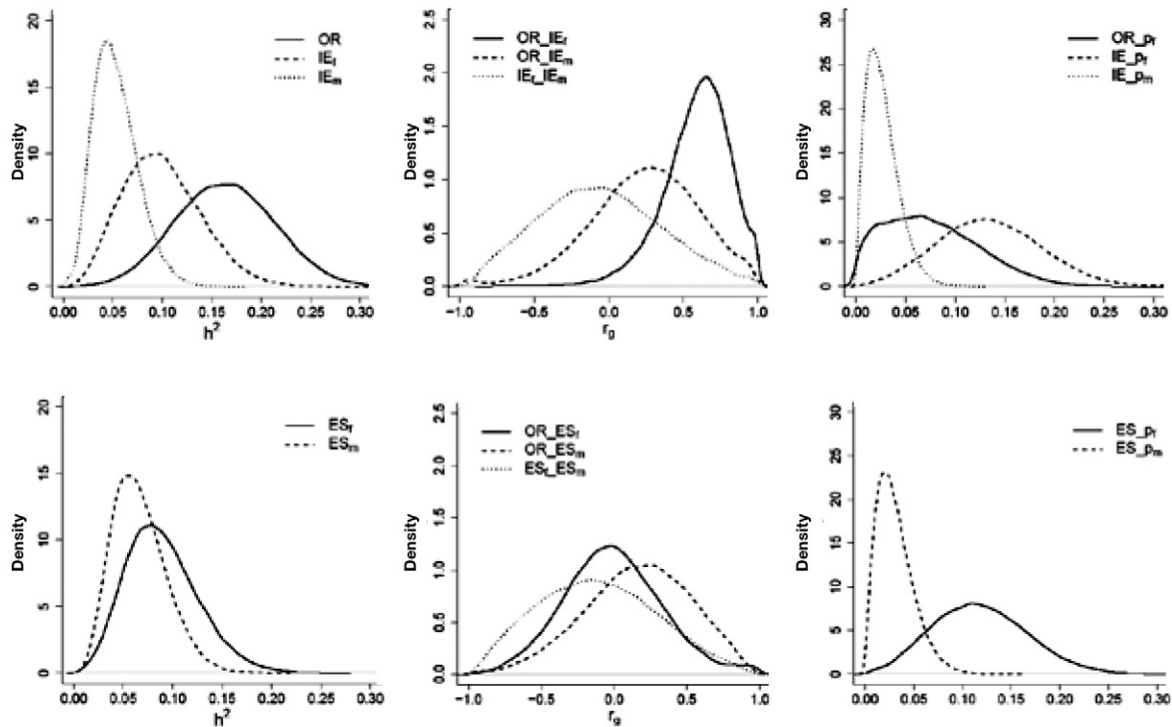
$$G = \begin{bmatrix} \sigma_{u_{f,OR}}^2 & \sigma_{u_{f,OR}, u_{f,IE}} & \sigma_{u_{f,OR}, u_{m,IE}} \\ \sigma_{u_{f,IE}, u_{f,OR}} & \sigma_{u_{f,IE}}^2 & \sigma_{u_{f,IE}, u_{m,IE}} \\ \sigma_{u_{m,IE}, u_{f,OR}} & \sigma_{u_{m,IE}, u_{f,IE}} & \sigma_{u_{m,IE}}^2 \end{bmatrix},$$

$$P_f = (P_{f,OR}, P_{f,IE}),$$

$$P_f = \begin{bmatrix} \sigma_{P_{f,OR}}^2 & \sigma_{P_{f,OR}, P_{f,IE}} \\ \sigma_{P_{f,IE}, P_{f,OR}} & \sigma_{P_{f,IE}}^2 \end{bmatrix},$$

and

$$R = \begin{bmatrix} \sigma_{e_{OR}}^2 & \sigma_{e_{OR}, e_{IE}} \\ \sigma_{e_{IE}, e_{OR}} & \sigma_{e_{IE}}^2 \end{bmatrix}.$$



**Figure 1.** Ratios of variance components for ovulation rate (OR) and female and male contributions to number of implanted embryos ( $IE_f$  and  $IE_m$ , respectively) and embryo survival ( $ES_f$  and  $ES_m$ , respectively);  $h^2$ , heritability;  $r_g$ , genetic correlation;  $p_f$ , female permanent environmental effects; and  $p_m$ , male permanent environmental effects.

Bounded uniform priors were assumed for the systematic effects and the (co)variance components ( $\mathbf{G}$ ,  $\mathbf{P}_f$ ,  $\sigma_{p_{m,IE}}^2$  and  $\mathbf{R}$ ). A single chain of 2000,000 iterations was run. The first 200,000 iterations of each chain were discarded, and samples of the parameters of interest were saved every 20 iterations.

## RESULTS AND DISCUSSION

The means for OR, IE, and ES were 15.8 ova, 12.5, and 0.78 IE per ova, respectively. The posterior mean (**PM**) and the interval of 95% of the highest posterior density [**HPD95%**] of the phenotypic variance for OR, IE, and ES were 6.05 [5.56, 6.54], 14.38 [13.07, 15.75], and 0.047 [0.043, 0.052], respectively. These results agree with those estimates reported in other maternal lines (Brun et al., 1992; García and Baselga, 2002; Santacreu et al., 2005).

The PM [**HPD95%**] of the heritability ( $h^2$ ) for OR was somewhat moderate, being 0.16 [0.07, 0.26] as shown in Fig. 1. This result also agrees with previous estimates of this parameter in other maternal lines of rabbits (Blasco et al., 1993; Bolet et al., 1994) and with those estimates obtained from the same set of data with different models (Laborda et al., 2011). Estimated  $h^2$  for the female contribution to IE and ES was lower than for OR, 0.10 [0.03, 0.18] and 0.10 [0.02, 0.16], respectively, as reported by Laborda et al. (2012).

Estimates of  $h^2$  of the male contribution to IE and ES were low, being 0.05 [0.01, 0.10] and 0.07 [0.02, 0.12], respectively; however, they were greater than the corresponding value for male contribution to prolificacy at kindling probably because male effect at early stages of gestation is less masked by the effect of the female and the environmental factors through gestation. Piles et al. (2006) found that male contribution to prolificacy at kindling (genetic plus permanent environmental effects) was only around 1% in three maternal lines of rabbit. Differences among males for their genetic effect on ES would be due to variations in seminal deficiencies which can prevent the fertilization process or the subsequent embryogenesis once initiated (Den Daas et al., 1998; Saacke et al., 2000). Thus, it could be possible to improve semen quality with genetic selection by ES, although response to selection could be low. This type of selection could be performed without the use of laparoscopy, through the slaughter of *tester females* which could be different from the ones of the selection line. In fact, the optimal design would be to use crossbred females used for production, because in this case, selection would consider the possible interaction effect between the male and the female really used for production (i.e., the environment).

Male contribution to the number of IE and ES could be greater than the one estimated in the present analysis. First, after natural mating, sperm dosage (i.e., the total number of sperm in the ejaculate) excess the

threshold needed to fertilize a large rate of ova and only differences among males in seminal traits that are independent of sperm dosage can be observed (Tusell et al., 2010); therefore, individual variation among males for IE and ES could be better observed from data obtained from does artificially inseminated under limited AI conditions, such as low sperm concentration, small or null preselection of the ejaculates for any semen quality trait, or long storage period of the AI doses. Second, laparoscopy was performed only on those females that were pregnant at d 12 of gestation. Therefore, individual variation for male and female contributions to the number of IE and ES could be partly biased downward, because infertile or subfertile males and females are not contributing to the variance leading to low estimates of these parameters.

As expected, the proportion of variation due to the male nonadditive genetic plus permanent environmental effects was almost negligible for IE (0.026 [0.001, 0.058]) and for ES (0.031 [0.002, 0.067]), that is, the repeatability for male contribution to these traits were around 8 and 10%, respectively. The PM [HPD95%] of the rate of the variation due to the female genetic nonadditive plus permanent environmental effects for IE and ES were estimated to be 0.13 [0.03, 0.23] and 0.12 [0.03, 0.21], respectively, with the repeatability of the female contribution to these traits around 23%.

The genetic correlation between the female contributions to OR and IE was moderate and favorable (0.59 [0.18, 1.00]), and therefore, a correlated response in IE was observed as stated by Laborda et al. (2012). On the other side, the genetic correlations between OR and female contribution to ES, between OR and male contribution to IE and ES, and between female and male contributions to IE and ES were all estimated with great imprecision due to the limited amount of records, and it is not possible to draw any conclusion about them. There are no estimates published for these parameters in rabbits or in other prolific species.

The posterior mean of the correlation between permanent environmental effects due to the female for the different traits were very imprecise, and it is not possible to draw a reliable conclusion either. Finally, the residual correlation between the OR and IE was positive and moderate (PM [HPD95%]: 0.40 [0.32, 0.48]), whereas it was low and negative for OR and ES (−0.12 [−0.21, −0.02]).

### Conclusions

This study shows the possible existence of genetic determinism of male contribution to the number of IE and ES after natural mating. The heritabilities for these traits were small, but greater than the heritability of male

contribution to litter size; however, response to selection to improve contribution to reproductive performance after natural mating would be still probably low. Further research is needed to determine whether there might be an interaction between male genotype and mating conditions (natural mating or AI, sperm dosage, duration of dose storage, etc.) such that it would be possible to find the best conditions to get the maximum genetic progress in male contribution to the number of IE and ES, and thus in semen characteristics.

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