GENETIC PARAMETERS FOR SEMEN TRAITS OF RABBIT MALES: II. MOTILITY

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ABSTRACT

The objective of this study was to estimate the genetic parameters of semen motility in a rabbit line selected for increased growth rate in the fattening period. Bucks (n=283) from line R, selected during 25 generations, located in two different insemination stations were used. The animals were weighed at weaning (28 days of age) and at the age of slaughter (63 days) and daily gain (DG, g/d) was computed. The estimations were based on data from 1172 ejaculates. The reproductive rhythm of males were two ejaculates per week, the training period started at five months of age, the semen samples were taken one week after finishing the training period and three months later this period. Sperm motility parameters, assessed using a computer-assisted sperm analysis (CASA) system were: total motile sperm (MOT, %), average path velocity (VAP, µm/s), curvilinear velocity (VCL, µm/s), straight-line velocity (VSL, µm/s), linearity index (LIN, %), amplitude of lateral head displacement (ALH, µm), wobble (WOB, %), straightness (STR, %) and dance (DNC, $\mu m^2/s$). Estimates of heritability, permanent effect and genetic correlation between growth rate and motility characteristic were obtained from the solutions of a bivariate animal model. The parameters of sperm motility studied with CASA methodology had low estimated heritabilities and, in general, they were higher than the estimated permanent effects. Estimates of the heritability were 0.156±0.041 for MOT, 0.171±0.062 for VAP and 0.189±0.062 for LIN, 0.119±0.046 for STR, and 0.186±0.064 for WOB. The majority of traits had a negative and moderate genetic correlation with daily gain, and this correlation was -0.927 for percentage of total motile sperm.

Key words: Genetic parameters, Sperm motility, Growth rate, Paternal line.

INTRODUCTION

The wide use of artificial insemination in the rabbit has raised the interest in improving the quantitative analysis of rabbit semen samples, in order to estimate the potential fertility of seminal doses. Subjective estimation of motility and evaluation of sperm morphology were the two laboratory assays most widely used for rabbit semen evaluation in insemination stations. Although Brun *et al.* (2002) observed that mass motility and the total number of motile spermatozoa per insemination dose were highly correlated with kindling rate in rabbits. Computer-assisted semen analysis methods (CASA) provide more objective motility parameters, and in recent years there has been an increase in the use of these systems to evaluate semen quality in rabbit (Farrell *et al.*, 1993; Lavara *et al.*, 2005).

On the other hand, the bucks used at the artificial insemination stations belong to lines continuously selected for growth rate in order to improve the feed efficiency and to reduce the fattening period. So, from a genetic point of view, it is important to know if the main semen parameters, that could affect fertility rate and litter size in artificial insemination, have genetic variability and are correlated genetically with growth rate.

This work is a companion paper to the one by Lavara *et al.* (2008) presented in this Congress. In this case, the main objective is estimating the genetic parameters for motility sperm in a line selected for growth rate.

MATERIALS AND METHODS

Animals and experimental design

The animals came from a high growth line of rabbits (line R), selected for growth rate (DG, g/d) from weaning to slaughter (28–63 days). Selection methodologies were described by Estany *et al.* (1992). Animals were housed under a photoperiod of 16L:8D, in individual cages, fed with a commercial diet and provided water *ad libitum*, at two insemination stations (89 males in Zarzadilla de Totana, Murcia, and 194 males in Aras de Alpuente, Valencia). Details of the experimental design, semen and sample collection are described in Lavara *et al.* (2008).

Motility evaluation

Semen samples of each ejaculate (10 µl) were diluted 1:20 in an extender Tris-citrate acid-glucose and BSA to prevent the spermatozoa from sticking to the glassware during motility analysis. After, 10µL of the sample were placed into a 10 µm deep Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) for motility analysis using a computer-assisted sperm analysis (CASA) system (Sperm Class Analyzer, S.C.A., Microptic, Barcelona, Spain). This system has a specific setup for rabbit sperm evaluation described in (Lavara *et al.*, 2005). Sperm motility was assessed at 37ºC at 10X using a phase contrast microscope. For each sample four microscopic fields were analyzed and a minimum of 100 sperm evaluated. The percentage of total motile sperm (MOT, %) cells, average path velocity (VAP, µm/s; the average velocity of the smoothed cell path), curvilinear velocity (VCL, µm/s; the average velocity measured over the actual point to point track followed by the cell), straight-line velocity (VSL, µm/s; the average velocity measured in a straight line from the beginning to the end of the track), linearity index (LIN, %; the average value of the ratio VSL/VCL), amplitude of lateral head displacement (ALH, µm; the mean width of the head oscillation as the sperm cells swim), straightness coefficient (STR=(VAP/VCL)x100, %), wobble (WOB= (VAP/VCL)x100, %; a measure of the oscillation of the actual trajectory about its spatial average path) and dance (DNC =(VCL x ALHmed), $\mu m^2/s$) were evaluated.

Statistical Analysis

Variance and covariance components were estimated using the derivate-free multiple traits restricted maximum likelihood (REML) procedure (Neumaier and Groeneveld, 1998). A bivariate animal model was used for DG and seminal traits. The mixed model used for DG was:

$$y_{ijklm} = \mu + b*LS + YS_i + OP_j + a_k + c_l + e_{ijklm}$$

where y_{ijklm} is DG, μ is the general mean, LS is the covariate litter size at birth, YS_i is the fixed effect of year-season with 9 levels, OP_j is the fixed effect of parity order in which the animal was born with 3 levels (1, 2, >2 parities), a_k is the additive genetic value of the animal, c_l is the random effect of the litter in which the animal *k* was born, and e_{ijklm} is the residual.

The mixed model used for seminal traits was:

$$y_{ijhokmn} = \mu + YS_i + N_j + L_h + P_o + a_k + p_m + e_{ijhokmn}$$

where $y_{ijhokmn}$ is seminal traits, μ is the general mean, YS_i is the fixed-effect of year season with 7 levels, N_j is the fixed effect order of ejaculate with 2 levels (first or the second ejaculated obtained one day), L_h is the fixed effect location with 2 levels (Zarzadilla de Totona or Aras de Alpuente), P_o is the fixed effect of period (A, B), a_k is the additive genetic value of the male, p_m is the permanent environmental effect of the male *k* over all its ejaculates, and $e_{ijhokmn}$ is the residual.

RESULTS AND DISCUSSION

The summary statistics for the overall motility descriptors are shown in Table 1 and 2. A total of 1257 ejaculates were evaluated using the CASA analysis. The average of MOT was 61.7%, the minimum value was 0.0% and the maximum was 100%. Similar values have been obtained by Quintero-Moreno *et al.* (2007) using pooled semen samples and the CASA analysis system but Lavara *et al.* (2005) observed a motility equal to 80.5% as a consequence of the pre-selection of the ejaculates used in their study, prior to the inclusion in the final semen pool that was used to inseminate the females. The average of VCL, VSL, VAP, LIN, STR, WOB, ALH and DNC was 84.3 μm/s, 48.5 μm/s, 58.1 μm/s, 59.5%, 80.7%, 69.9%, 3.2% and 320.7 $\mu m^2/s$, respectively. Young and Bodt (1994) indicated higher means when they used rabbit non-hyperactivated spermatozoa evaluated with CASA analysis, nevertheless the means obtained by Lavara *et al.* (2005) were lower than in this experiment.

Table 1: Summary statistics of the percentage of total motile sperm (MOT) cells, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity index (LIN)

	MOT (%)	VCL (μm/s)	VSL (μm/s)	VAP (μm/s)	LIN (%)
N	1257	1172	1172	1172	1172
Mean	61.7	84.3	48.5	58.1	59.5
Range	0.0-100.0	23.5-84.5	9.3-133.4	14.9-152.8	9.1-97.1
S.D.	27.0	19.6	16.1	16.1	15.8
CV	43.8	23.3	33.2	27.7	26.6

N: number of observation, S.D.: standard deviation, CV: coefficient of variation

Table 2: Summary statistics of straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH), and dance (DNC)

	STR (%)	WOB (%)	ALH (μ)	DNC ($\mu m^2/s$)
N	1172	1172	1172	1172
Mean	80.7	69.9	3.2	320.7
Range	19.9-99.5	7.3-97.6	0.1-9.9	7.1-3846.8
S.D.	11.2	12.7	1.1	202.3
CV	13.9	18.2	34.4	63.1

N: number of observation, S.D.: standard deviation, CV: coefficient of variation

The trait that showed more variation was DNC (CV=63.1%), followed by MOT, VSL and ALH, and the trait that sowed minor variation was STR (CV=13.9%).

Estimated ratio variance components for motility traits are shown in Tables 3 and 4. Estimates of the heritability were 0.156±0.041 for MOT, 0.171±0.062 for VAP, 0.189±0.062 for LIN, 0.119±0.046 for STR, and 0.176±0.064 for WOB. The estimated heritability was not different of zero for VCL, ALH and DNC. In boars, some estimated heritability for progressive motion of spermatozoa evaluated microscopically was 0.38±0.036 (Smital *et al.*, 2005) or 0.44 (Oh *et al.*, 2003). When the motility was studied in two rabbit lines divergently selected for 63-d body weight, the Low line presented higher LIN, VAP and VCL than Higher line, but the lines didn't show significative differences in MOT or ALH (Brun *et al.*, 2006).

Table 3: Estimates of the heritability (h^2), the ratio between the variance of the permanent environmental effect of the male and the phenotypic variance (p^2) and genetic correlation between daily gain and seminal traits (rg), with their standard error for the percentage of total motile sperm (MOT) cells, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP) and linearity index (LIN)

	MOT	VCL	VSL	VAP	LIN
h^2	0.156±0.041	0.077±0.039	0.175±0.056	0.171±0.062	0.189±0.062
p^2	0.111±0.038	0.092±0.036	0.062±0.045	0.049±0.046	0.044±0.052
rg	-0.927±0.160	-0.156±0.230	-0.354±0.165	-0.292±0.170	-0.203±0.170

In general, the ratio between the variance of the permanent environmental effect of the male and the phenotypic variance (p^2) was lower than the heritability for all the motility traits, except for VCL,

ALH and DNC The repetability was close to 0.2 (0.169 VCL; 0.267 MOT) for all motility traits, excepting DNC (0.092).

Table 4: Estimates of the heritability (h^2), the ratio between the variance of the permanent environmental effect of the male and the phenotypic variance (p^2) and the genetic correlation between daily gain and seminal traits (rg), with their standard error, for straightness (STR), wobble (WOB), amplitude of lateral head displacement (ALH) and dance(DNC)

	STR	WOB	ALH	DNC
h^2	0.119±0.046	0.176±0.064	0.074±0.041	0.021±0.029
p^2	0.078±0.041	0.050±0.051	0.124±0.042	0.071±0.032
rg	-0.229±0.208	-0.128±0.188	0.177±0.285	-0.113±0.386

The daily gain in the fattening period and the percentage of total motile sperm cells were strongly and negative correlated (-0.927±0.160, Table 3). The daily gain had low and negative genetic correlation with VSL (-0.354±0.165). The other motility traits could be considered independent of growth rate, because the genetic correlation was not different of zero.

CONCLUSIONS

The parameters of sperm motility studied with CASA methodology had low estimated heritabilities, although they were higher than the estimated permanent effects. The majority traits had a negative and moderate genetic correlation with daily gain, but this correlation was -0.927 for percentage of total motile sperm.

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