GENETIC PARAMETERS FOR SEMEN TRAITS OF RABBIT MALES: I. PRODUCTION, MORPHOLOGY, AND SPERM HEAD MORPHOMETRY

Lavara R.[1]*, García M.L.[2], Torres C.[1], Vicente J.S.[1], Baselga M.[1]

[1]Department of Animal Science, Universidad Politécnica, Camino de Vera s/n ,46022, Valencia, Spain
[2]División de Producción Animal, Dept.Tecnología Agroalimentaria, Universidad Miguel Hernández de Elche, 03312 Orihuela, Spain
*Corresponding author: rlavara@dca.upv.es

ABSTRACT

(Co)variance genetic components of buck semen characteristics and growth traits were estimated using the REML procedure applied to bivariate animal models. The estimations were based on data from 1456 ejaculates from 283 bucks belonging to one of the paternal lines more commonly used in Spanish farms. The ejaculates were collected from 2005 to 2007 at two different insemination stations. The traits studied were: ejaculate volume (V, ml), concentration (Cn, 10^6/ml), total number of spermatozoa per ejaculate (Prod, 10^6), percentage of spermatozoa with normal apical rigde (NAR, %), percentage of sperm morphological abnormalities (ANOR, %), length of head spermatozoa (L, μm), width of sperm head (W, μm), area of sperm head (A, μm^2), perimeter of sperm head (P, μm) and 4 derived head shape parameters: ellipticity FUN1 [L/W], rugosity FUN2 [$4\pi A/P^2$], elongation FUN3 [(L/W)/(L-W)], regularity FUN4 [πLW/4A].
Equal model equations for all semen traits included the artificial insemination station, the period, the year-season and the order of ejaculate as fixed effects; the animal, the permanent environmental and non-additive genetic effect of the male over all its ejaculates, and the residual as random effects. The model of daily weight gain, from 28 days to 63 days of age, included litter size at birth as covariate, parity order and year-season as fixed effects and the animal, the common litter and the residual as random effects. The following heritabilities were estimated: ejaculate volume 0.091, concentration 0.053, total number of spermatozoa per ejaculated 0.097, percentage of spermatozoa with normal apical rigde 0.247, percentage of sperm morphological abnormalities 0.643, length of sperm head 0.309, width of sperm head 0.341, area of sperm head 0.279, perimeter of sperm head 0.105, FUN1 0.508, FUN2 0.518, FUN3 0.220 and FUN4 0.067. The significant genetic correlations with daily gain in the fattening period were: concentration -0.695, total number of spermatozoa per ejaculated -0.360, percentage of spermatozoa with normal apical rigde -0.350, percentage of sperm morphological abnormalities 0.334, width of sperm head -0.617, area of sperm head -0.600, perimeter of sperm head -0.646, FUN1 0.367, FUN2 0.374.

Key words: Genetic parameters, Sperm production, Sperm morphology, Sperm head morphometry, Paternal line.

INTRODUCTION

Automated sperm morphology analysis has been applied in a large number of studies in different species, but only few studies has been reported in rabbit (Gravance and Davis, 1995, Pérez-Sánchez *et al.*, 1998; Marco-Jiménez *et al.*, 2005; Lavara *et al.*, 2006).

There is scarce bibliography about the genetic parameters of seminal traits in rabbits. Nevertheless, Brun *et al.* (2002) and García-Tomás *et al.* (2006) estimated the crossbreeding parameters of seminal traits among different rabbit lines.

The males used in insemination stations have a high percentage of elimination due to their lower output on quality and quantitative semen production and they are submitted to continuous selection for growth rate. So, the aim of this paper is to estimate the genetic parameters for production per ejaculate, morphology of the spermatozoa and sperm head morphometry, and to estimate the genetic correlation between these semen traits and the growth rate. This work is a companion paper to the one by Lavara *et al.* (2008) presented in this Congress, where motility semen traits are studied.

MATERIALS AND METHODS

Animals and experimental design

Line R is a paternal line selected during 25 generations for daily gain (DG) between 28 and 63 days of age (Estany *et al.*, 1992). The animals were weighed at weaning (WW, 28 days of age) and at slaughter (WS, 63 days of age). So, the DG was calculated as following:

$$DG = \frac{(WS - WW)}{35}$$

Selection is based on phenotypic values of DG. They were bred at the farm of the Universidad Politécnica of Valencia. A total of 283 males of the line R were used in the experiment. These males were reared in two insemination stations (89 males in Zarzadilla de Totana, Murcia, and 194 males in Aras de Alpuente, Valencia). The environmental conditions were maintained with the aid of a control system for photoperiod, ventilation and temperature. After weaning, the males were housed in individual cages with a photoperiod of 16 hour light/day. Animals were fed "*ad libitum*" a commercial rabbit diet (on dry matter: 17.5% crude protein, 3.5% ether extract, 16.7% crude fibre, 2938 kcal/kg).

Semen sample collection

At 150-170 days of age, males started the training period. The training was performed during fifteen days. For the training and experimental period, each week, two ejaculates per male were collected on a single day using an artificial vagina, with a minimum of 30 min between ejaculate collections. Semen sample for the trial were collected for each male at two different periods:

- Period A: one week after finishing the training period.
- Period B: more than 3 months after Period A.

Only ejaculates exhibiting a white colour were used in the experiment, samples containing urine and cell debris were discarded whereas gel plugs were removed. Then, volume (V, ml) was recorded.

Concentration, morphology and sperm head morphometry

For the morphological analyses, an aliquot from each ejaculate (20 µl) was fixed with 180 µl of a solution of glutaraldehyde 2% in DPBS. A minimum of 100 sperm cells was examined to analyse the status of acrosome from the normal sperm (NAR, %), and the sperm abnormalities (ANOR, %) were evaluated with a differential interference contrast microscope (Nomarski contrast) at a magnification of 500x. The sperm concentration (Cn) was determined using a Thoma-Zeiss counting cell chamber (Marienfield, Germany). To perform the morphometric analyses, a 10 µl drop of fixed sperm with glutaraldehyde was placed onto a slide and covered with a coverslip (20 mm x 20 mm). The slides were placed under an Nikon Elipse-microscope, with a bright field x40 phase optic objective, on which was mounted a Sony CCD AVC-D7CE video camera (Sony Corporation; Tokyo, Japan) with a x3.3 photo-ocular connected to a morphometric module of a Sperm-Class Analyser (SCA) (Microptic; Barcelona, Spain). Sperm cells were displayed on the monitor at equivalent brightness, and all the cells that did not present any overlap with debris or other cells were considered for analysis. If after treatment of the images we observed false correspondence between the original image and its mask, the sperm had to be discarded. At least 100 normal sperm were measured for each ejaculate. Each sperm head was measured for four primary parameters (head area [A, µm2], head perimeter [P, µm],

head length [L, µm], head width [W, µm] and four derived parameters of head shape (ellipticity FUN1 [L/W], rugosity FUN2 [$4\pi A/P^2$], elongation FUN3 [(L/W)/(L-W)], regularity FUN4 [$\pi LW/4A$]).

Statistical Analysis

Variance and covariance components were estimated using the derivate-free multiple trait 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 the semen trait, 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 second ejaculate obtained one day), L_h is the fixed effect of location with 2 levels (Zarzadilla de Totana 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

Summary statistics of the daily gain, production and morphological seminal traits are presented in Table 1. The most significant point to note is the high variability of these seminal traits. Similar means were reported by Vicente *et al.* (2000) and Castellini *et al.* (2003), but in this experiment, the volume was almost half the reported by García-Tomás *et al.* (2006).

Table 1: Summary statistics of daily gain (DG), ejaculate volume (V), concentration (Cn), total number of spermatozoa per ejaculate (Prod), percentage of spermatozoa with normal apical rigde (NAR) and percentage of sperm morphological abnormalities (ANOR)

	DG (g/d)	V (ml)	Cn (10^6/ml)	Prod (10^6)	NAR (%)	ANOR (%)
N	5133	1456	1423	1423	1335	1335
Mean	45.1	0.62	193	110.3	87.7	17.0
Range	11.4-77.4	0.01-2.6	0.5-1470	0.1-1260	1-100	1.1-100.0
S.D.	6.2	0.45	178	119.7	15.2	13.2

N: number of observations, S.D.: standard deviation

The heritability (h^2) and the ratio between the variance of common litter effect and phenotypic variance of DG were 0.122±0.028 and 0.335±0.015, respectively (data not shown in tables). Camacho and Baselga (1989) estimated similar components of variance in this line R.

Table 2 shows the estimates of h^2, the ratio between the variance of the permanent environmental effect and the phenotypic variance (p^2) and the genetic correlation for production and morphology seminal traits (rg). The h^2 of Cn was not different of zero (0.053±0.043). V and Prod showed low heritabilities (0.091±0.048, 0.097±0.034) and the morphological seminal traits showed moderate and high heritabilities (NAR**,** 0.247±0.069; ANOR, 0.643±0.024). Panella *et al.* (1994) reported high heritability for V (0.44), and Cn (0.60). In general, the estimates in pigs were also high and ranged from 0.14 to 0.58 for V and from 0.14 to 0.38 for Cn (Grandjtot *et al.*, 1997; Oh *et al.*, 2003, Smital *et al.*, 2005). These papers differed in the methods and the biological material used, therefore the results are comparable only to a certain degree.

Table 2: Estimates of the heritability (h^2), the ratio between the permanent environmental effect and the phenotypic variance (p^2) and the genetic correlation between daily gain and seminal trait (rg), with their standard error, for ejaculate volume (V), concentration (Cn), total number of spermatozoa per ejaculated (Prod), percentage of spermatozoa with normal apical rigde (NAR) and percentage of sperm morphological abnormalities (ANOR)

	V	Cn	Prod	NAR	ANOR
h^2	0.091±0.048	0.053±0.043	0.097±0.034	0.247±0.069	0.643±0.024
p^2	0.163±0.046	0.120±0.039	0.018±0.027	0.234±0.061	0.000±0.001
rg	0.125±0.233	-0.695±0.333	-0.360±0.191	-0.350±0.181	0.334±0.131

The p^2 was moderate for V, Cn and NAR (0.163, 0.120, 0.234, respectively Table 2) but it was not different of zero for Prod and ANOR. The rg between DG and V was not different of zero (0.125±0.233), but the corresponding values with Prod (-0.360±0.191) and NAR (-0.350±0.181) were moderately negative, moderately positive with ANOR (0.334±0.131), and strongly negative with Cn (-0.695±0.333). Brun *et al.* (2006) obtained higher concentration and lower volume in the H line than in the L lines, being H and L lines, two rabbit lines divergently selected for 63 day body weight during five generations. Nevertheless, the total number of spermatozoa per ejaculate was similar in both lines.

The summary statistics of sperm head traits are shown in Table 3, where it can be noted the low variability of these traits. The estimates of h^2, p^2 and rg are shown in Tables 4 and 5. All the parameters, excepting regularity, had moderate or high heritability and ranged from 0.105 for perimeter to 0.518 for rugosity. The estimates of p^2 were lower than the estimates of h^2 for all traits, excepted regularity. The rg of DG with L, elongation and regularity were not different of zero, moderately positive with ellipticity (0.367) and rugosity (0.374) and highly negative with W (-0.617), A (-0.600) and P (-0.646).

Table 3: Summary statistics of length (L), width (W), area (A), perimeter (P), ellipticity (FUN1), rugosity (FUN2), elongation (FUN3) and regularity (FUN4) of sperm head

	L (μm)	W (μm)	A (μm^2)	P (μm)	FUN1	FUN2	FUN3	FUN4
N	1060	1060	1060	1060	1060	1060	1060	1060
Mean	8.5	4.7	32.7	23.9	1.83	0.29	0.72	0.96
Range	7.7-9.2	4.0-5.4	25.6-39.4	21.1-27.1	1.58-2.09	0.22-0.35	0.62-0.92	0.89-1.05
S.D.	0.2	0.2	1.7	1.0	0.07	0.02	0.04	0.01

N: number of observation, S.D.: standard deviation

Table 4: Estimates of the heritability (h^2), the ratio between the variance of permanent environmental effect of the male and the phenotypic variance (p^2) and the genetic correlation between daily gain and seminal trait (rg), with their standard error, for length (L), width (W), area (A), perimeter (P) of sperm head

	L	W	A	P
h^2	0.309±0.068	0.341±0.077	0.279±0.066	0.105±0.044
p^2	0.147±0.060	0.112±0.065	0.112±0.056	0.158±0.044
rg	-0.205±0.186	-0.617±0.176	-0.600±0.171	-0.646±0.220

Table 5: Estimates of the heritability (h^2 the ratio between the variance of permanent environmental effect of the male and the phenotypic variance (p^2) and the genetic correlation between daily gain and seminal trait (rg), with their standard error, for ellipticity (FUN1), rugosity (FUN2), elongation (FUN3) and regularity (FUN4) of sperm head

	FUN1	FUN2	FUN3	FUN4
h^2	0.508±0.105	0.518±0.111	0.220±0.028	0.067±0.048
p^2	0.034±0.085	0.026±0.089	0.000±0.000	0.193±0.048
rg	0.367±0.151	0.374±0.150	-0.174±0.182	-0.383±0.304

CONCLUSIONS

All semen traits have shown a high variability. Production traits showed a low estimated heritability, but quality and morphology traits showed a medium or high heritability.

ACKNOWLEDGEMENTS

The authors thank all the farmers involved in the experiment for housing facilities and the care with the animals. This work has been supported by the Spanish Research Project (CICYT AGL2004-02710/GAN).

REFERENCES

Brun J.M., Theau-Clement M., Bolet G. 2002. Evidence for heterosis and maternal effects on rabbit semen charateristics. *Anim. Res., 51, 433-442.*

Camacho J., Baselga M. 1990. Genetic correlation between reproductive and growth taris in rabbits. *In: Proc. 4th World Rabbit Congress, Vol. 1, 366-369.*

Castellini C., Lattaioli P., Dal Bosco A., Minelli A., Mugnai C. 2003. Oxidative status and semen characteristics of rabbit buck as affected by dietary vitamin E, C and n-3 fatty acids. *Reprod. Nutr. Dev., 43, 91-103.*

Estany J., Camacho J., Baselga M., Blasco A. 1992. Selection and genetic response of growth rate in rabbits for meat production. *Genet. Sel. Evol., 24, 527-537.*

García-Tomás M., Sánchez J., Rabel O., Ramon J., Piles M. 2006. Heterosis, direct and maternal genetic effects on semen quality traits of rabbits. *Livest. Sci., 100, 111-120.*

Grandjot G., Brandt H., Glodek P. 1997. Genetische und phänotypsiche Untersuchungen zu Eigenleistungs, sperma und fruchtbarkeismerkmalen von besamungsebern. 1. Mitteilung: Systematische einlussfaktoren auf genetische parameter. *Arch. Tierz., 40, 421-432.*

Gravance C.G., Davis R.O. 1995. Automated sperm morphometry analysis (ASMA) in the rabbit. *J. Androl., 16, 88-93.*

Marco-Jiménez F., Balasch S., Vicente J.S, Lavara R., Viudes-De-Castro M.P., Moce E. 2005. Detection of subfertile rabbits by means of sperm head morphometric analysis. Poster 124. 30th Annual Meeting of American Society of Andrology, Seattle, Washington. *J. Androl., supplement March/April, pp. 80.*

Neumaier A., Groeneveld E. 1998. Restricted maximum likelihood estimation of covariances in sparse linear models. *Genet. Sel. Evol., 30 (1), 3-26.*

Lavara R., García M.L., Torres C., Vicente J.S., Baselga M. 2008. Genetic parameters for semen traits of rabbit males: II. Motility. *In: Proc. 9th World Rabbit Congress, 2008 June, Verona, Italy, 159-162.*

Lavara R., Marco-Jiménez F., Cortell C., Safaa H.M., Vicente J.S. 2006. Sperm head morphometry parameters in two selected Rabbit lines. *Reprod. Dom. Anim. 277.*

Oh S.H., See M.T., Long T.E., Glavin J.M. 2003. Genetic correlations between boar semen traits. *J. Anim. Sci., 81 (Suppl.1), 317.*

Panella F., Castellini C., Facchin E. 1994. Heritability of some male reproduction traits in rabbit. *Cahiers Options Méditerranéennes, 8, 279-283.*

Pérez-Sánchez F., Tablado L., Soler C. 1998. Quantitative changes in sperm head morphology during passage through the male excurrent duct system of the Rabbit. *Mol. Reprod. Dev., 51, 203-209.*

Smital J., Wolf J., De Sousa L.L. 2005. Estimation of genetic parameters of semen characteristics and reproductive trait in AI boars. *Anim. Rep. Sci., 86, 119-130.*

Vicente J.S., Viudes de Castro M.P., Lavara R., Lavara F. 2000. Effect of male line on prolificacy from does inseminated with low sperm doses. *In: Proc. 7th World Rabbit Congress, 2000 July, Valencia, Spain, Vol. A, 1273-1277.*