Genetic Parameters Of Sperm Subpopulations In A Paternal Rabbit Line

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Introduction

Evidence from several studies suggests that different subpopulations of spermatozoa coexist within any ejaculate. These subpopulations are due to variation in the assembly of individual spermatozoa during spermatogenesis as well as to differential maturational status and age (Bedford 1983; Harrison 1996). Moreover trajectories of individual spermatozoa could reflect their physiological status, and identify motion parameters that best correlate with fertility (Lavara *et al.* 2005; Quintero-Moreno *et al.* 2007). Computer-assisted semen analysis (CASA) systems permit the study of motion characteristics in sperm; these systems provide large sets of data derived from individual sperm measurements. Using multivariate approaches to the CASA derived data, the original observations are classified into multidimensionally defined groups, where the group characteristics distinguish different physiological states. The aim of this study was to estimate the genetic parameters of sperm subpopulations in a paternal rabbit line selected for post-weaning daily weight gain.

Material and methods

Animal and data

A total of 280 males from Line R were used in the experiment. Line R is a paternal line of rabbits selected for 25 generations on the basis of daily weight gain (DG) between 28 and 63 days of age (Estany *et al.* 1992). The selection method is individual selection. The males were reared at two different artificial insemination stations and their data were recorded from 2006 to 2007. At 150–170 days of age, the male rabbits started the training period. The training was performed for 15 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 collections. The semen samples for the experiment were collected during two different periods:

- Period A: 1 week after finishing the training period.
- Period B: more than 3 months after period A.

Only ejaculates that exhibited a white color were used in the experiment; samples containing urine and cell debris were discarded and gel plugs were removed.

Aliquots from each ejaculate (10 µl) were diluted 1:20 in an extender (Tris-citrate acid-glucose) containing bovine serum albumin 0.3% (BSA) to prevent the spermatozoa from sticking to the glassware during motility analysis. Ten microliters of the diluted sample was

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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). Sperm motility was assessed at 37ºC with 10X phase contrast objetive. For each sample four microscopic fields were analyzed. The 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=(VSL/VCL)x100, %), angularity index (AI, %; which is the deviation of linearity calculated as the mean of the cosine of angles, transformed to percentage), angular velocity (AV, µm/s; which is calculated by multiplying angularity index with velocity, divided by 100), amplitude of lateral head displacement (ALHmean, µm; the mean width of the head oscillation as the sperm cells swim), maximun amplitude of lateral head displacement (ALHMáx, µm; the maximun width of the head oscillation as the sperm cells swim), straightness coefficient (STR=(VAP/VCL)x100, %), wobble (WOB = (VAP/VCL) x 100, %; a measure of the oscillation of the actual trajectory about its spatial average path) and beatcross frequency (BCF, Hz) were recorded.

Classification, ordination, and identification of sperm subpopulations

The multivariate analyses were carried out in a number of sequential steps. First a nonhierarchical classification of spermatozoa was undertaken using the VARCLUS procedure (SAS software, Version 9.1). This type of variable clustering will find groups of variables that are as correlated as possible among themselves and as uncorrelated as possible with variables in other clusters. After the VARCLUS procedure only five out of eleven CASA variables were selected. Using these selected variables a disjoint clustering was performed using the FASTCLUS procedure (SAS software, Version 9.1). Spermatozoa with similar motility pattern were assigned to the same cluster. The separation into clusters was performed on 43590 motile spermatozoa from 1226 ejaculates. The final outcome of the cluster analysis was the definition of three subpopulations of spermatozoa, distinguished by their multidimensional motion characteristics. Within different ejaculates, the relative frequencies of spermatozoa belonging to each subpopulation (SS1, SS2, SS3) were computed.

Statistical analyses

Genetic parameters were estimated for SS1, SS2 and SS3 using a bivariate animal model. The model included the station-year-season in which the ejaculate was collected (28 levels), the ejaculate order (2 levels) and the age of the male (3 levels) as systematic effects, and the male additive effect, the male permanent environmental effect, and the residual as random effects. The variance-covariance components for different sperm subpopulation were estimated using a Bayesian approach implemented in the TM program developed by Legarra *et al.* (2008), using flat priors for these components and for the systematic effects. After some exploratory analysis, chains of 600000 samples were used, with a burning period of 30000. Only one sample of each 10 was saved. The convergence was checked on each chain by the Z Geweke criterion (Geweke 1992).

Results and discussion

The characteristics of the three subpopulations are shown in Table 1. Briefly the subpopulation 1 contained the active spermatozoa with the most linear trajectories (the highest LIN and VAP) and 35% of spermatozoa were located in this subpopulation. The subpopulation 2 contained around 22% of total motile sperms which had started the capacitation process (the highest VCL, ALHMáx and BCF and the lowest LIN). The subpopulation 3 was the most represented subpopulation with 43% of the total motile sperms, in which the spermatozoa showed the lowest velocity pattern. Previous results in different species suggest that spermatozoa from subpopulations with high velocity and progressive movement could achieve the greatest fecundity (Cremades *et al.* 2005; Nuñez-Martínez *et al.* 2006).

Table 1: Sperm subpopulations and motility descriptors

	SUBPOPULATION		
	1	**2**	**3**
N (%)	14690 (35)	8950 (22)	17951 (43)
Sperm motility descriptors[1]	**mean±sd**	**mean±sd**	**mean±sd**
LIN (%)	83.30±10.90	29.81±16.61	59.24±21.50
VAP(µm/s)	84.14±19.04	63.24±20.30	41.26±13.56
ALHMax(µm)	5.56±2.54	13.68±7.14	5.48±2.54
BCF(Hz)	15.62±3.60	17.94±6.71	16.22±5.06
VCL(µm/s)	96.42±20.23	123.40±29.06	61.42±16.64

[1] LIN, linearity index; VAP, average path velocity; ALHMax, maximun amplitude of lateral head displacement; BCF, beatcross frequency;VCL, curvilinear velocity

Estimates of genetic parameters are shown in Table 2. Heritability values for SS1, SS2, and SS3 are medium-low but higher than the p^2 estimates. The SS3 showed the highest repeatability value (0.25). Genetic parameters of frequencies of sperm subpopulations have never been estimated, and we could not compare with other authors, but their estimated heritabilities do not widely differ from heritabilities of kinematic variables of rabbit sperm movement (0.10-0.12; Lavara *et al.* 2008). Also, the estimates are comparable with the heritabilities of sperm motility previously obtained in rabbits (Lavara *et al.* 2008) and other farm species like goats (Furstoss *et al.* 2009), cattle (Smith *et al.* 1989; Knights *et al.* 1984) or sheep (David *et al.* 2007) that showed values below 0.2.

Table 2: Descriptive statistics of the posterior marginal distributions of heritability (h^2) and ratio of permanent effects variance to phenotypic variance (p^2) of subpopulations frequencies (SS1, SS2, SS3) within ejaculate (posterior mean ± posterior standard deviation)

	SS1	**SS2**	**SS3**
h^2	0.140±0.047	0.086±0.039	0.166±0.056
p^2	0.038±0.028	0.076±0.035	0.085±0.042

Estimates of genetic, permanent and residual correlations between the frequencies of the sperm subpopulations within ejaculate are shown in Table 3. The SS1 and SS3 showed the highest genetic, permanent and residual correlation. On the other hand correlations in which SS2 was involved showed the lowest value. SS1 and SS3 showed a high and negative genetic correlation (-0.939), permanent correlation (-0.862) and residual correlation (-0.8). In all cases residual correlations were the lowest.

Table 3: Descriptive statistics of the posterior marginal distributions of the genetic (r_g), permanent (r_p) and residual (r_e) correlations between the subpopulations frequencies (SS1, SS2, SS3) within ejaculate (posterior mean ± posterior standard deviation)

	SS1-SS2	SS1-SS3	SS2-SS3
r_g	0.434±0.299	-0.939±0.054	-0.690±0.213
r_p	0.412±0.564	-0.862±0.236	-0.861±0.244
r_e	-0.159±0.031	-0.800±0.011	-0.463±0.026

Conclusion

The results obtained at the present study show that the frequencies of sperm subpopulations within ejaculate have medium-low heritability and repeatability, although SS1 and SS3 are highly and negatively related.

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