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Freezability genetics in rabbit semen

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ABSTRACT

The aim of this study was to estimate the heritability of semen freezability and to estimate the genetic correlation between frozen-thawed sperm traits and the growth rate in a paternal rabbit line. Estimated heritabilities showed that frozen-thawed semen traits are heritable (ranged between 0.08 and 0.15). In the case of Live-FT (percentage of viable sperm after freezing), the estimated heritability is the highest one, and suggests the possibility of effective selection. After the study of genetic correlations it seems that daily weight gain (DG) was negatively correlated with sperm freezability, but no further conclusions could be drawn due to the high HPD95%. More data should be included in order to obtain better accuracy for the estimates of these genetic correlations. If the results obtained at present study were confirmed, it would imply that selection for DG could alter sperm cell membranes or seminal plasma composition, both components related to sperm cryoresistance.

1. Introduction

Artificial insemination (AI) is used in rabbit industry, as in other species, to improve breeding management. In rabbit farms AI is performed with fresh or cooled semen rather than frozen because of the poor fertility resulting after thawing [1]. However, frozen-thawed rabbit semen is used for conservation of banking resources (endangered breeds or high-value males); international export (semen from selected lines) and research. The inter-animal, within species, variation in the ability of spermatozoa to survive cryopreservation is evident in many publications [2–5], suggesting that sperm freezability has a genetic component. In fact, selection experiments conducted on avian species showed that sperm freezability has a favourable selection response [6].

Recently in rabbits, Lavara et al. [7] provide estimates of repeatability for some frozen-thawed sperm traits, indicating that sperm freezability in rabbits could be heritable. Previously, Mocé et al. [8], showed differences in fertility and prolificacy after AI with frozen-thawed semen from different selected rabbit lines. The line selected on the basis of growth rate during the fattening period, showed the lowest fertility and prolificacy, despite the fact that fresh semen from this line yielded high fertility and prolificacy rates. In this sense, knowledge of the genetic correlation between frozen-thawed sperm traits and the selection criteria would allow us to predict the future correlated response on semen freezability on this selected rabbit line.

Therefore, the aims of this study were to estimate the heritability of semen freezability traits and to estimate the genetic correlation between frozen-thawed sperm traits and the growth rate in a paternal rabbit line.

2. Materials and methods

2.1. Animals and experimental design

Data were collected from 255 males belonging to a paternal rabbit line (Line R), born between 2006 and 2007. Line R is a synthetic line that has been selected since 1980 for daily weight gain (DG) between 28 and 63 days of age by individual selection [9]. This line was formed by crossing a California line with a synthetic line created by mating commercial crossbred rabbits [10]. After weaning, animals were housed in collective cages (8 rabbits per cage) subjected to a temperature ranging from 15 to 25 °C. At 63 days of age, the weight was recorded and males were moved to two AI stations. Males were placed in individual cages, subjected to a photoperiod of 16 h light/day and fed *ad libitum* with a commercial rabbit diet (on dry matter basis: 17.5% crude protein, 3.5% ether

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extract, 16.7% crude fibre, 2938 kcal/kg). In both stations, environmental conditions were controlled maintaining the temperature between 17 and 24 °C.

Males began the training period at 150–170 days of age. The training was performed for two weeks. After training, the males started the production period. For the training and production period, two ejaculates were collected per male and week on a single day using an artificial vagina, with a minimum of 30 min between collections. Collections from each male during the experiment were performed on the same day of the week. Only ejaculates that exhibited a white colour were used in the experiment. Samples containing urine and cell debris were discarded, whereas gel plugs were removed and the ejaculates processed separately.

2.2. Freezing-thawing protocols

All the chemicals used were purchased from Sigma-Aldrich (Madrid-Spain). Sperm were cryopreserved by diluting the ejaculates 1:1 (v:v) with the freezing extender. The freezing extender was composed of Tris-citric acid-glucose 0.25 M of Tris (hydroxymethyl)aminomethane (Sigma, cat. no. T-1503), 88 mM of anhydrous citric acid (Sigma, cat. no. C-0759), and 47 mM of D (+) glucose (Sigma, cat. no. G-8270)as base media, and 3.5 M of dimethyl sulfoxide (DMSO, Sigma, cat. no. D-5879) and 0.1 M of sucrose (Sigma, cat. no. S-8501), added as cryoprotectants [11]. All sperm manipulations were performed at 22 °C. The sperm were packaged in 0.25 mL plastic straws (IMV® Technologies, L'Aigle, France), sealed with modelling paste (JOVI, S.A. Barcelona, Spain, NRI 8–6650)and then cooled at 5 °C for 30 min. Cooled temperature was provided storing straws in a refrigerator set at 5 °C. To freeze sperm, straws were suspended horizontally in liquid nitrogen vapour 5 cm above the liquid nitrogen level for 10 min before plunging into the liquid nitrogen (LN_2). The straws were kept in an LN_2 bank until use. After storage in LN_2, thawing was performed submerging the straws in a water bath at 44 °C for 12 s.

2.3. Semen evaluation and traits

Three traits were measured directly in frozen-thawed semen: the percentage of viable sperm, the acrosome integrity and the sperm motility.

The percentage of viable (plasma membrane intact) sperm (Live-FT, %) in each frozen-thawed sample was determined using flow cytometry, as described by Mocé et al. [12]. Briefly, a sample from each thawed straw was diluted with Tris-BSA to 30 × 10^6 sperm/mL. Then, each sample was stained for flow cytometric analysis by transferring a 0.1 mL aliquot into a tube containing 0.45 mL Tris-BSA diluent, 2.5 μL SYBR-14 (stock solution: 10 μM in DMSO) and 2.5 μL propidium iodide (PI) (stock solution: 1.5 mM in distilled water). The samples were incubated for 10 min at room temperature and filtered through a 40 μm nylon mesh before being analysed using an Epics XL-MCL flow cytometer (Beckman Coulter, IZASA, Barcelona, Spain) equipped with an argon laser tuned to 488 nm at 15 mW power. Fluorescence from 10,000 cells was measured using a 550 long pass filter (LP) combined with a 525 nm band pass filter (BP) to detect SYBR-14 and a 645 nm LP combined with a 620Nm BP filter to detect PI. Using this protocol, all cells stain with SYBR-14, but only non-viable cells stain with PI.

For the acrosome status evaluation, an aliquot from each frozen-thawed straw (20 μL) was fixed with 180 μL of a 0.2% solution of glutaraldehyde (Electron Microscopy Science, Washington) in Dulbecco's Phosphate Buffered Saline (DPBS). A minimum of 100 spermatozoa were evaluated at ×400 by phase positive contrast microscopy. Acrosome status of normal sperm was classified as intact (AI) or reacted (AD). The percentage of sperm with normal acrosome status (Nar-FT, %) was calculated as the ratio: [AI/(AI + AD)] x 100. For motility analyses, an aliquot from each frozen-thawed straw (10 μL) was diluted 1:20 in an extender (Tris-citric acid-glucose) containing bovine serum albumin 0.3% (BSA) to prevent the spermatozoa from sticking to the glassware during the image capture analysis. Then, 10 μL of the diluted 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). Sperm motility was assessed at 37 °C with ×10 negative phase contrast objective. Four microscopic fields were captured for each sample, and then revised and corrected manually in order to avoid the possible problems due to sperm granules present in the rabbit semen plasma [13]. The percentage of total motile sperm cells (Mot-FT, %) was recorded.

In addition, two synthetic traits were computed, the relative reduction of acrosome integrity (Rnar, %) and relative reduction of motility (Rmot, %) after the freezing-thawing process. The two variables were defined as the reduction of the trait between fresh and frozen-thawed semen divided by the value of the trait in fresh semen.

A total of 12,908 records for DG were used in the experiment. DG data used belonged to animals from twelve generations before. In addition to DG, the sperm traits described above were recorded involving 1292 ejaculates from 255 males. The pedigree file included 14,700 animals.

2.4. Statistical analyses

To reduce bias in the estimation of the genetic parameters of sperm traits resulting from the selection for DG, the sperm traits were analysed jointly with DG [14]. A set of two-trait analyses were thus performed to estimate the correlations among traits.

The mixed model used for the semen traits was:

$$y_{sijopkl} = \mu_s + S_{si} + O_{sj} + T_{so} + P_{sp} + a_{sk} + p_{sk} + c_{sl} + e_{sijopkl}$$

where $y_{sijopkl}$ is the frozen-thawed semen trait recorded, μ_s is the overall mean, S_{si} is the systematic effect station–year–season in which the ejaculate was collected, with 47 levels (two AI station with 28 and 19 weeks of collection for each one, where each week of collection on each station represents one different level), O_{sj} is the systematic effect of ejaculate order with two levels (first and second ejaculate on the same day), T_{so} is the systematic effect of thawing session with 19 levels, P_{sp} is the systematic effect of age of the male with 3 levels (≤6 months, 6–8 months, more than 8 months), a_{sk} is the animal additive genetic effect, p_{sk} is the permanent environmental effect over all the ejaculates of the male *k*, c_{sl} is the random effect of the litter in which the male *k* was born, and $e_{sijopkl}$ is the residual. It was assumed that the different random effects (additive, permanent, litter of birth and residual) followed normal distributions and were independent among and within the effects, excepting the additive values of the animals, which were correlated though the numerator relationship matrix.

The mixed model used for DG was:

$$y_{dijkl} = \mu_d + b \times LS_{dl} + YS_{di} + OP_{dj} + a_{dk} + p_{dk} + c_{dl} + e_{dijkl}$$

where y_{dijkl} is the daily gain of animal *k*, μ_d is the overall mean, LS_{dl} is the covariate litter size at birth and b the corresponding regression coefficient, YS_{di} is the systematic effect of year–season in which the animal was weaned, with 30 levels, OP_{dj} is the systematic effect of parity order in which the animal was born, with three levels (first, second, and higher), a_{dk} is the animal additive genetic effect, c_{dl} is the random effect of the litter in which the animal *k* was