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Environmental and male variation factors of freezability in rabbit semen

R. Lavara [a,*], I. David [b], E. Mocé [c], M. Baselga [a], J.S. Vicente [a]

[a] Universidad Politécnica de Valencia, Instituto de Ciencia y Tecnología Animal, Valencia, Spain
[b] INRA UR 631 Station d'Amélioration Génétique dês Animaux, Castanet-Tolosan, France
[c] Centro de Investigación y Tecnología Animal (CITA), Instituto Valenciano de Investigaciones Agrarias (IVIA), Segorbe (Castellón), Spain

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ABSTRACT

The aim of this study was to analyze the environmental and male effects that could have an influence on sperm freezability using a recursive model. A total of 853 ejaculates from 217 males belonged to a paternal rabbit line were collected and frozen. Six different traits were evaluated: the sperm concentration (10^6 spermatozoa per mL), the acrosome integrity on fresh (%) and frozen-thawed semen (%), the sperm motility on fresh (%) and frozen-thawed semen (%), and the percentage of viable sperm on frozen-thawed semen (%). In addition, two synthetic traits were computed, the relative reduction of acrosome integrity (%) and relative reduction of motility (%) after the freezing-thawing process. A multiple-trait recursive model was used to analyze the relationships between the semen traits considered. For the fixed effects studied, the season had the highest effect on postthaw semen characteristics. Results of the analysis of recursive coefficients showed that fresh semen concentration and motility influence the future freezability of the semen. All traits studied presented moderate repeatabilities ranging from 0.11 to 0.38. These results provide conclusive evidence that sperm freezability in rabbits could be heritable. Regarding male correlations, there were large positive male correlations between fresh traits ($r_m = 0.77$ to 0.57), and between direct frozen-thawed traits ($r_m = 0.72$ to 1). Male effects on fresh and direct frozen-thawed traits were generally positively correlated. This correlation was moderate to high for fresh semen motility with all frozen-thawed traits ($r_m = 0.41$ to 0.74) and for frozen-thawed semen and all fresh traits ($r_m = 0.5$ to 0.74), these results suggest that these traits could be genetically related. Further studies involving more males and ejaculates should be conducted in the future to estimate the heritabilities and genetic correlations of postthaw semen traits in rabbits.

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1. Introduction

Rabbit artificial insemination is usually performed with cooled semen stored for short periods of time (less than 36 hours) with good results in fertility and prolificacy [1–3]. There is a need to develop this technique to dissociate the days of collection and insemination for biosecurity reasons and because of the interest of commercial rabbit AI stations in establishing sperm cryobanks. To date, in rabbits, there are some freezing-thawing protocols with acceptable results in fertility, such as those reviewed by Mocé and Vicente [4]. These procedures can be used for experimental purposes and in genetic conservation programs in which reproductive performance lower than those achieved with fresh semen are admissible.

Rabbit ejaculates have shown variability in their survival after the freezing-thawing process related to differences between lines [5] and between males within lines [6,7]. These results point out that a genetic component is involved in rabbit sperm freezability that deserves to be studied in association with the currently most acceptable procedure of cryopreserving rabbit semen.

None of the studies found in the literature concerning sperm cryopreservation consider the fact that there is a complex relationship between fresh and frozen-thawed

* Corresponding author. Tel.: +34 963 879 435; fax: +34 963 879 759.
E-mail address: rlavara@dca.upv.es (R. Lavara).

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sperm traits. Obviously, postthawing traits are affected by the fresh semen traits in addition to environmental and male effects. When fresh semen traits are only considered as fixed effects for studying cryoresistance [8], the fact that both fresh and postthawing traits are determined genetically and that they might be genetically correlated is ignored. In contrast, when only the genetic correlation is taken into account [9], the cause-and-effect relationships between both types of trait is ignored. Gianola and Sorensen [10] outlined a solution for this kind of biological system, describing the use of recursive multiple-trait models in a quantitative genetic context. Using this approach allows proper handling of the relationship between fresh and postthaw semen and provides estimates for genetic parameters and estimates of the effect of initial traits on postthaw traits.

To address the question of whether genetic selection for freezability in rabbit could be possible, we need to know which part of the observed variation is because of a genetic additive component (heritability) or at least which part is because of variation within males (upper limit of heritability). In addition, better knowledge about the relationship between fresh and frozen-thawed traits could be interesting to facilitate an eventual selection program. Because of the absence of such estimates in the literature, we aim to analyze the environmental and male effects that could have an influence on sperm freezability using a recursive model and linked to the currently acceptable procedure for freezing-thawing rabbit semen.

2. Materials and methods

2.1. Animals and experimental design

Data were collected from 217 males belonging to a paternal rabbit line (Line R). Line R was selected for daily weight gain between 28 and 63 days of age by individual selection [11]. After birth, number of total born was recorded. At weaning (28 days of age), number of weaned and individual weight were recorded. After weaning, animals were housed in collective cages (8 rabbits per cage) subjected to a temperature ranging from 15 °C 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 hours light per day and fed *ad libitum* with a commercial rabbit diet (on dry matter basis: 17.5% crude protein, 3.5% ether extract, 16.7% crude fiber, 2938 kcal/kg). In both stations, environmental conditions were controlled maintaining the temperature between 17 °C and 24 °C. Males began the training period at 150 to 170 days of age. The training was performed for 2 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 minutes between collections. Collections from each male during the experiment were performed on the same day of the week. The mean number of collections per male was five. Only ejaculates that exhibited a white color were used in the experiment (N = 853). Samples containing urine and cell debris were discarded, and 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 [12]. All sperm manipulations were performed at 22 °C. The sperm were packaged in 0.25 mL plastic straws (IMV Technologies, L'Aigle, France) and sealed with modeling paste (JOVI, S.A., Barcelona, Spain; NRI 8-6650). Sperm were cooled at 5 °C for 30 minutes. To freeze sperm, straws were suspended horizontally in liquid nitrogen vapor 5 cm above the liquid nitrogen level for 10 minutes 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 seconds.

2.3. Semen evaluation and traits

2.3.1. Fresh semen traits

Three different variables were assessed in fresh semen: the sperm concentration, the acrosome status, and the motility.

Sperm concentration (10^6 spermatozoa per mL) was determined using a Thoma-Zeiss counting cell chamber (Marienfield, Germany).

For the acrosome status evaluation, an aliquot from each ejaculate (20 μL) was fixed with 180 μL of a 0.2% solution of glutaraldehyde (Electron Microscopy Science) in Dulbecco's phosphate buffered saline. A minimum of 100 spermatozoa were evaluated at a magnification ×400 by phase positive contrast microscopy. Acrosome status of normal sperm was classified as intact (IA) or reacted, for details see Figure 1. The percentage of sperm with normal acrosome status (%) was calculated as the ratio: [IA/(IA + reacted acrosome)] × 100. For motility analyses, an aliquot from each ejaculate (10 μL) was diluted 1:20 in an extender (Tris-citric acid-glucose) containing bovine serum albumin (BSA) 0.3% to prevent the spermatozoa from sticking to the glassware during the image capture analysis. Then, 10 μL of the diluted sample was placed into a 10 μm deep Makler counting chamber (Sefi Medical Instruments, Haifa, Israel) for motility analysis using a computer-assisted sperm analysis system (Sperm Class Analyzer, S.C.A.; Microptic, Barcelona, Spain). Sperm motility was assessed at 37 °C with magnification ×10 negative phase contrast objective. Four microscopic fields were captured for each sample. Individual sperm tracks were visually assessed to eliminate possible debris and misdiagnosed tracks. The percentage of total motile sperm cells was recorded.

2.3.2. Frozen-thawed semen traits

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

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