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Utilization of frozen–thawed epididymal ram semen to preserve genetic diversity in Scrapie susceptible sheep breeds

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Abstract

The European Union has introduced transmissible spongiform encephalopathy (TSE) resistance breeding programmes for several sheep breeds to cope with the genetic susceptibility to Scrapie infections. Due to the different allele frequencies among breeds, strong selection for ARR alleles is associated with a loss of genetic diversity in small populations and in larger populations with unfavourable ARR allele frequencies. To ensure maintenance of genetic diversity, an adhoc cryopreservation programme was initiated employing epididymal sperm from 109 rams representing 16 different breeds within one breeding season. Epididymal semen was chosen for this adhoc programme because time consuming training of rams for ejaculated semen collection via an artificial vagina was not possible. Prior to freezing, average sperm motility was 79.7% and acrosome integrity was 93.7%. After freezing, these levels were decreased to 60.5 and 72.8%, respectively. An insemination trial using frozen–thawed epididymal semen resulted in a lambing rate of 87.5%. Results show that this semen preservation method is robust and efficient and associated with high fertility. It may also be useful for other animal species.

Keywords: Ram; Epididymal spermatozoa; Cryopreservation; National semen bank; Scrapie eradication

1. Introduction

The European Union has initiated programmes to eradicate Scrapie from its member states and several countries have established breeding programmes to create disease-resistant national flocks with the ram as a major selection target. This allows to introduce Scrapie-resistant genes into sheep populations within a short period. However, especially in small populations and in breeds with unfavourable ARR allele frequencies, such strategy increases the risk that valuable genetic diversity may be lost due to selective breeding for disease-resistant genotypes. The German Expert Panel for Animal Genetic Resources [1] recommended to establish a national semen bank for such sheep breeds with increased risk of losses of genetic variability due to the selection programmes. Here, we sought to establish a model to demonstrate the suitability of an epididymal ram semen freezing protocol for establishing genetic stocks. Epididymal semen was selected because the adhoc nature of the Scrapie elimination programme did not allow the time- and labour consuming training period for the rams to collect ejaculated semen of good enough quality via artificial vagina. Semen was collected from a total of 16 different breeds and up to 11 unrelated rams per breed were used in this study. The viability of frozen/thawed semen samples was tested by intrauterine inseminations. The protocol is robust and allows the freezing of epididymal ram semen after slaughter with high efficiency.

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2. Materials and methods

A total of 109 rams from 16 breeds were used for this study. All animals were healthy and free of any signs of disease. Rams were between 1 and 10 years of age and transported to the institute. One day after their arrival, rams were slaughtered at the institute's slaughterhouse. The entire testes were transported in a thermos (38 °C) to the laboratory within 3 min. The cauda epididymides were separated from surrounding tissue, sliced and suspended in Salamon's one-step freezing medium at a 1 + 4 dilution rate [2]. After two filtrations (75 and 50 μm) through a nylon filter grid at room temperature (20 °C), the sperm suspension was free from any somatic cells. Percent motility was determined by placing a sample aliquot on warm glass slides (38 °C), covered with a coverslip and examined under light microscopy (magnification 200×). Sperm morphology was assessed under a phase contrast microscope at 1000× magnification after fixation in Hancock solution that was produced from a 37% formaldehyde solution mixed with a 2.9% sodium citrate solution. The quality of the sperm was assessed by morphological criteria including acrosome integrity, morphology of head, midpiece and tail. Sperms were always evaluated hierarchically starting with the acrosome, followed by head, midpiece and tail and defects were listed where first noted. At least 200 sperm cells were examined per sample. Cytoplasmic droplets were not considered as abnormal due to the epididymal origin of the sperm. The slicing technology was used to release sperm from the epididymal tissue. The total number frozen samples per ram was limited to a maximum of 400. Thus, we sliced the surface of the epididymal tissue in rams with an obvious high concentration of epididymal semen. A total epididymal sperm count per ram could not be made using this approach. The sperm concentration was determined in a Thoma chamber (Brand, Wertheim, Germany). Semen samples were adjusted to a final concentration of 200×10^6 spermatozoa/ml using the same extender. Diluted sperm samples were transferred into 0.25 ml Ministraws (Fine paillette, 0.25 ml, Tiefenbach, Germany) using a filling machine (IMV, France) and were then sealed on both ends by ultrasound. The straws were cooled from room temperature (20 °C) to 4 °C at a rate of −0.26 °C/min followed by an equilibration period of 1 h. Straws were then kept in nitrogen vapour on a grid, 4 cm above the liquid nitrogen level and were plunged into liquid nitrogen (−196 °C) after 30 min. Thawing was carried out in a water bath at 37 °C for 17 s.

To assess the fertilizing capacity of frozen/thawed epididymal sperm, laparoscopical intrauterine inseminations were performed in eight crossbred ewes in which the estrous cycle had been synchronized according to the schedule described recently [3]. Briefly, the ewes received progestagen-impregnated vaginal sponges (40 mg fluorogestone acetate, Chronogest® Intervet, Boxmeer, The Netherlands) for 13 days. One day prior to removal of the sponges, all animals received a single injection of eCG (500 IU) (Intergonan® Intervet, Unterschleißheim, Germany), followed 1 day after termination of the progestagen treatment by a GnRH injection (0.004 mg buserelin corresponding to 1 ml Receptal® Intervet, Unterschleißheim, Germany). All animals were inseminated once 24 h after GnRH with four doses of 50 million frozen/thawed sperm each, yielding 200 million frozen/thawed spermatozoa per female. The semen donor was a German Blackhead Mutton. All pregnant females were allowed to go to term and the number of lambs was recorded. Data are presented as mean ± S.D.

3. Results

Semen from 102 out of the 109 rams (93.6%) was found suitable for cryopreservation. Semen from seven rams could not be frozen due to degenerated epididymides or poor semen quality. Altogether, more than 33,000 straws could be successfully frozen, with an average of 324 straws per ram. Prior to freezing, the percentage of total motility was 79.7 ± 0.1% and the proportion of normal acrosomes was 93.7 ± 0.1% (Table 1). After freezing/thawing both parameters were decreased by ~20% and amounted to 60.5 and 72.8% in average, respectively. The calculated "cryoresistance" (value after thawing/values before freezing × 100) for motility and acrosomal integrity were 76.2 ± 0.1 and 77.8 ± 0.1%, respectively. Differences between breeds were not calculated because the rams were of a wide range of age and frequently originated from one flock only. The percentage of spermatozoa with looped and bent tails due to adhering plasma droplets in fresh semen was 29.7 ± 0.2%.

Insemination with frozen/thawed epididymal semen resulted in a lambing rate of 87.5%. Fourteen lambs (10 males, 4 females) were born from seven ewes. Average gestation length was 145.6 ± 1.1 days. Birth weights were 4.4 ± 0.4, 3.2 ± 0.7 and 3.6 ± 0.7 kg for single lambs, twins and triplets, respectively.

All male carcasses were marketed. The live weights of the rams varied from 23 and 180 kg and carcass weights after slaughter ranged from 10 to 106.7 kg.