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Short communication

The quality and fertilizing ability of the ram semen cryopreserved around 40 years ago

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Abstract

The aim of the study was to verify the quality of ram semen, frozen in 1982-1983, from the historical collection of the Bank of Biological Material of the National Research Institute of Animal Production. A total of 18 ejaculates from 3 Świniarka type rams were analyzed to assess sperm motility (subjectively), total motility, progressive motility, sperm concentration (CASA), membrane integrity (SYBR-14/PI) and chromatin structure (SCSA). In order to determine sperm fertilizing ability 49 ewes were intracervically inseminated (200×106 sperm per AI) with frozen-thawed semen 12 and 24 hours after detection of estrus. Sperm motility parameters, membrane intact spermatozoa and DFI did not differ among the analyzed rams. Spermatozoa concentration was significantly higher for ram no. 2 than for rams no. 1 and 3. The lambing rates (27.3 to 36.0%) did not differ significantly for individual rams. The ram semen, which had been stored for around 40 years, showed satisfactory quality and fertilizing capacity, allowing for its use in artificial insemination.

Key words: ram semen, ex situ storage, gene banks, cryopreservation, sperm quality, fertility

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Table 1. Quality parameters and fertility of ram semen (Mean±SD).

Ram (no. of ejaculates)	PM (subjective) (%)	CASA		Sperm concentration (mln/ml)	Membrane intact spermatozoa (%)	DFI (%)	Lambing rate (%)	Lambs born
		TM (%)	PM (%)					
1 (n=4)	45.0±10.0	76.7±8.3	61.5±10.1	1359.0±468 a	37.2±4.6	0.5±0.2	27.3 (3/11)	3
2 (n=8)	50.0±15.1	72.1±7.1	55.9±8.5	1916.0±327 b	44.7±9.3	0.5±0.2	36.0 (9/25)	12
3 (n=6)	54.2±9.7	80.0±3.2	65.1±5.4	1152.0±234 a	38.5±8.9	0.7±0.3	30.8 (4/13)	5

a,b – values in columns with different letters differ significantly ($p \leq 0.05$)
PM – progressive motility, TM – total motility
DFI – DNA fragmentation index

Introduction

Crucial to the operation of the banks of biological material is not only to regularly increase the genetic reserve pool, but also to assess the quality of collected material. In the case of semen collection, it is recommended to systematically monitor basic quality parameters at least once in every ten years of its liquid nitrogen storage (FAO 2012). Periodical analysis of valuable genetic material should therefore be a generally accepted practice that allows verifying whether it is legitimate to continue its storage and use. In the years 1982-1983 the Department of Reproductive Biotechnology and Cryoconservation of the National Research Institute of Animal Production collected biological material in the form of frozen semen pellets of Świniarka type rams. Although this semen collection has been stored at the National Research Institute of Animal Production since freezing in 1982-1983, it has never been assessed for quality since then.

The aim of the study was to verify the quality of stored ram semen using advanced andrological diagnostic tests combined with the use of semen for insemination to assess its fertilizing capacity.

Materials and Methods

A total of 18 ejaculates from 3 Świniarka type rams, frozen in 1982-1983, were used in the study. The semen was diluted with a milk-egg yolk diluent and frozen in pellets on dry ice (detailed methodology is not known). After thawing in a special thawing device (dry thawing at 60°C) semen was assessed at a laboratory specializing in andrological diagnosis at the Department of Reproductive Biotechnology and Cryoconservation, National Research Institute of Animal Production in Balice, Poland. The assessment included estimation of the percentage of progressive motile sperm (subjectively), total motility, progressive motility, sperm concentration (CASA) and cytometric assessment of sperm cell membrane integrity (SYBR-14/PI) and DNA fragmentation (SCSA).

Semen progressive motility was evaluated subjectively by two individuals with long-standing expertise under a Nikon optical phase-contrast microscope with heating table (38°C) at 100× magnification. Total and progressive motility and sperm concentration was assessed using a computer-assisted semen analyzer (Sperm Class Analyzer, S.C.A V5.1, Microptic, Barcelona, Spain) after 30× semen dilution in 0.9% NaCl in 20 micron Leja 8-chamber slides (Leja Products B.V., The Netherlands) (Gogol et al. 2019). The sperm membrane integrity was cytometrically assessed using a LIVE/DEAD® Sperm Viability Kit (SYBR-14 and propidium iodide) (Molecular Probes, Inc., Eugene, Oregon, USA) (Szczęśniak-Fabiańczyk et al. 2021). The sperm chromatin structure assay (SCSA) was performed using flow cytometry (Evenson and Jost 2000). Fluorescence measurements were carried out using a CytoFlex cytometer (Beckman Coulter, Brea, CA, USA).

Forty-nine Świniarka ewes 2-3 years old were used to determine the fertilizing capacity of frozen-thawed semen. Ewes were intracervically inseminated (200×10^6 sperm per AI) in a standing position with frozen-thawed semen 12 and 24 hours after detection of estrus. Insemination was performed during natural estrus in October by experienced technician, who remained the same throughout the study. After parturition the lambing rate (ewes lambed/ewes inseminated × 100) was calculated.

Means were compared using the t-test and differences were considered significant at $p<0.05$. A chi-square test was carried out to analyze the effect of the ram on the lambing rate.

Results and Discussion

Sperm motility parameters, membrane intact spermatozoa and DFI did not differ significantly among

the analyzed rams (Table 1). In various studies total ram sperm motility after thawing has been ranged from 14 to 85% (Alvarez et al. 2012, Gogol et al. 2019, Fang et al. 2020, Vozaf et al. 2021). The values of this parameter obtained in our study were high and ranged from 72 to 80%. Percentage of membrane intact sperm cells post-thawing obtained in our study, was comparable with those of Gil et al. (2003) and Alvarez et al. (2012) and DFI was lower than reported by Garcia-Alvarez et al. (2010) (1.95). Spermatozoa concentration was significantly higher for ram no. 2 than for rams no. 1 and 3. The lambing rate (27.3 to 36.0%) did not differ significantly for individual rams and were comparable with those obtained after intracervical insemination with frozen semen in studies by O'Meara et al. (2008) and Gogol et al. (2019). Salamon and Maxwell (2000) showed that a period of 27 years of ram semen storage had no effect on fertility. The present study demonstrated that fertility is retained even after 40 years of storage, which confirms that long-term frozen storage of ram semen is feasible and makes possible the banking of genetic resources in sheep breeding.

In conclusion, the ram semen, which had been frozen in 1982-1983, showed satisfactory quality and fertilizing capacity, allowing for its use in artificial insemination.

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