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Short communication

Sperm concentration and viability of bull semen frozen in 2004–2010, from the collection of the National Bank of Biological Material

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Abstract

The aim of this study was to carry out a follow-up evaluation of cryopreserved semen of native breed cattle collected in the National Bank of Biological Material (NBBM). The oldest material, from 2004–2010, was included in the study. A total of 70 ejaculates from 62 bulls (5 Polish Red-and-White, 7 Polish Black-and-White, 11 Whitebacked, 39 Polish Red) were used in the study. After thawing sperm concentration and viability (plasma membrane intactness) were determined using a fluorescence-based instrument – NucleoCounter SP-100. Sperm concentration was higher ($p \leq 0.05$) for the semen of Polish Red-and-White and Polish Red bulls compared to the semen of Polish Black-and-White and Whitebacked bulls. For the viability, no significant differences were observed between breeds. The number of viable spermatozoa per straw in the examined semen ranged from 4.09 to 18.29 × 10^6 . This study has shown large differences in the quality of bull semen frozen between 2004 and 2010, from the collection of the National Bank of Biological Material while its quality parameters allow it to be used for insemination.

Keywords: bull semen, *ex situ* storage, gene banks, cryopreservation, sperm quality

Introduction

During the 10 years of the National Bank of Biological Material (NBBM)'s operation, its resources have been successively expanded with semen from native breed bulls. This has created a unique collection of biological material in Poland, original in terms of both genotype and quantity of material collected. This valuable collection of biological material can be used as an element of *in situ* and *ex situ* conservation activities for cattle genetic resources in Poland carried out by the National Research Institute of Animal Production. However, the effectiveness of using the collection of biological material depends on its quality. Therefore, an accurate and objective assessment of the quality of semen collected in the resources of the NBBM is essential for the evaluation of its biological value. Cyclic evaluation of cryogenic bank collections is also recommended by the Food and Agriculture Organization of the United Nations (FAO). In 2012, FAO published a guide: "Cryoconservation of animal genetic resources. FAO Animal Production and Health Guidelines" which provides guidelines for collecting *ex situ* material in a way that ensures the reproducibility of breeds, as well as systematically checking the basic parameters for the evaluation of semen at least once every ten years of its storage in liquid nitrogen. The material collected in the NBBM was only subject to basic in-process evaluation at bull semen production centers. Such assessment generally included macroscopic evaluation (volume, color, consistency, pH of the ejaculate) and subjective microscopic evaluation (mass movement, progressive motility of spermatozoa). The aim of the study was to carry out a follow-up evaluation of cryopreserved semen of native breed cattle collected in the NBBM. The oldest material, from 2004-2010, was included in the study. Sperm concentration and sperm viability (percentage of sperm with intact cell membranes) were evaluated.

Materials and Methods

A total of 70 ejaculates from 62 bulls (5 Polish Red-and-White, 7 Polish Black-and-White, 11 Whitebacked, 39 Polish Red) were used in the study. The semen was frozen in 0.25 ml French straws (detailed methodology is not known) in Polish commercial AI centers in 2004--2010. After thawing in a water bath at 37°C for 40 seconds sperm concentration and viability (plasma membrane intactness) were determined using a fluorescence-based instrument – NucleoCounter SP-100 (ChemoMetec A/S, Allerod, Denmark) according to the manufacturer's instruction. Briefly, to determine sperm concentration (Total count), 25 µl of thawed semen was diluted in 500 µl of S100 Reagent. Exposure of the sperm to this solution results in the permeabilization of the sperm membranes. After thorough mixing, 60 µl of the sample was drawn into a disposable microfluidic SP-1 cassette (ChemoMetec A/S) pre-loaded with propidium iodide (PI) and placed into the instrument. Placing the cassette inside the instrument resulted in the automatic transfer of the contents into a measurement chamber within the cassette. This chamber is exposed to a green fluorescent light that excites PI, and the red fluorescent signal emitted by sperm is registered in a compact fluorescence microscope integrated with a camera inside the instrument. After the sperm concentration in the sample was determined, a second sample of 25 µl of thawed semen was diluted in 500 µl PBS. Exposure of sperm to PBS will result only in PI uptake by intrinsically membrane-damaged sperm (Non-viable count). After mixing, 60 µl of the sample was drawn into an SP-1 cassette, which was then inserted into the instrument. The percentage of viable sperm was calculated using the following formula:

Viability (%) = 100% * (Total – Non-viable) / Total.

All values were expressed as means ± standard deviation (SD). Data were processed by ANOVA and means were compared with the t-test using the Statistica software package (StatSoft Inc., USA). Differences between means were regarded as significant at $p \leq 0.05$.

Results and Discussion

Sperm concentration was higher ($p \leq 0.05$) for the semen of Polish Red-and-White and Polish Red bulls ($74.74 \pm 12.24 \times 10^6$ and $84.64 \pm 20.92 \times 10^6$ respectively) compared to the semen of Polish Black-and-White and Whitebacked bulls ($55.77 \pm 9.83 \times 10^6$ and $55.02 \pm 13.06 \times 10^6$ respectively) (Table 1). For the viability, no significant differences were observed between breeds. The number of viable spermatozoa per straw in the examined semen ranged from 4.09 to 18.29×10^6. Until recently, the issue of the quality of bull semen in the production centers was regulated by relevant provisions in legal acts, which specified in detail its minimum quality parameters. Currently, these regulations are no longer in force and therefore producers of insemination doses set their own criteria, monitoring the effectiveness of individual ejaculates through information obtained from the field (Christensen et al. 2005, Karoui et al. 2011, Holden et al. 2017). One Polish AI station has recently introduced a lower limit for qualifying semen for insemination after the freezing process at 45% of sperm with intact cell membranes. Of the 70 samples tested in this study, 14 were below this threshold. Studies indicate that by using $2 \times \mathbf{10^6}$ bull sperm in

Table 1. Quality parameters of bull semen for individual cattle breeds.

Breed (no. of ejaculates)	Sperm concentration (10^6/ml)		Viability (%)	
	Mean ± SD	Range	Mean ± SD	Range
Polish Red and White (6)	74.74 ± 12.24[a]	61.30 - 89.86	49.66 ± 14.95	26.62 - 63.90
Polish Black and White (8)	55.77 ± 9.83[b]	39.42 - 70.93	60.76 ± 9.38	43.95 - 72.08
Whitebacks (11)	55.02 ± 13.06[b]	37.86 - 78.09	53.30 ± 8.11	36.61 - 66.42
Polish Red (45)	84.64 ± 20.92[a]	48.04 - 149.50	52.99 ± 12.64	15.53 - 74.40

Values with different letters (a, b) are statistically significant at $p \leq 0.05$
Viability – membrane intact spermatozoa

the insemination dose, it is possible to achieve satisfactory fertility with good animal management (Bodmer et al. 2005, DeJarnette et al. 2011).

This study has shown large differences in the quality of bull semen, frozen between 2004 and 2010, from the collection of the National Bank of Biological Material while its quality parameters allow it to be used for insemination.

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