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Short communication

Selected qualitative and biochemical parameters of cryopreserved semen of Holstein-Friesian (HF) AI bulls

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Abstract

Selected qualitative and biochemical parameters were determined in cryopreserved semen used for artificial insemination, sampled from 120 bulls reared at the Animal Breeding and Insemination Center in Bydgoszcz. The total average motility of the analyzed sperm samples was determined at 62.51%. The percentage of motile spermatozoa displaying progressive forward motility was 21.65%. Analyzed samples were characterized by a high percentage of sperm cells with a intact plasma membrane (71.21%) and active mitochondria (71.32%). High efficiency of the enzymatic antioxidant system of the evaluated sperm cells was demonstrated by high activity of CAT, GPx and SOD (494.37, 2847.83 and 5.31U/1x10^9 spermatozoa, respectively) values and low values of the DNA Fragmentation Index (9.32). The results of the study, obtained with the involvement of advanced analytical methods, indicate a high fertilizing capability of the analyzed sperm samples.

Key words: bull, spermatozoa, cryopreservation, sperm characteristics

Introduction

Artificial insemination (AI) with cryopreserved semen is the predominant method used in cattle reproduction around the world. The success of AI programs depends on many different factors. The most important is high quality of semen, but genetics, physiology, nutrition and management of cows are also significant (Walsh et al. 2011). The technology of cryopreservation causes various structural changes in cells. Spermatozoa, after the freeze-thaw process are characterized by decreased motility and altered kinematics. These changes may be caused by mechanical damage to the plasma membrane, peroxidation of membrane phospholipids and DNA fragmentation (Tasdemir et al. 2013). In Polish animal insemination centers motility is propably one of the most important parameters used for sperm quality evaluation in cryopreserved semen samples. Analyses that rely on advanced laboratory methods can significantly improve the reliability of tests evaluating the suitability of semen samples for insemination. The aim of this study was to analyze selected qualitative and biochemical parameters of cryopreserved bull semen.

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Table 1. Selected qualitative and biochemical parameters of cryopreserved semen of HF bulls, used for insemination.

Parameter	Unit	Mean ± SD
Total motile sperm	%	62.51 ± 6.94
Progressively motile sperm (PMOT)	%	21.46 ± 6.51
Sperm cells with intact plasma membrane	%	72.21 ± 2.72
Sperm cells with active mitochondria	%	71.32 ± 1.85
ATP concentrations	nmol/10^8 cells	15.59 ± 2.16
CAT	U/1x10^9 cells	2847.83 ± 420.57
GPx	U/1x10^9 cells	494.37 ± 138.52
SOD	U/1x10^9 cells	5.31 ± 1.04
DNA fragmentation	DFI	9.32 ± 1.93

Materials and Methods

The experimental material comprised cryopreserved semen from 120 HF bulls reared at the Animal Breeding and Insemination Center in Bydgoszcz. Sperm motility parameters were determined using the CASA system (VideoTesT Sperm 2.1, St. Petersburg, Russia). Plasma membrane integrity was assessed by staining with a combination of SYBR-14/PI fluorochromes (Fraser et al. 2002). Mitochondrial activity was analyzed using JC-1/PI fluorochromes (Thomas et al. 1998). ATP content was determined with the use of a CLSII Bioluminescence Assay Kit (Roche Molecular Biochemical) in accordance with the manufacturer's instructions. The activity of antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) – was determined following the method proposed by Koziorowska-Gilun et al. (2013). DNA fragmentation analysis was performed following the method developed by Fraser and Strzeżek (2005), and the results were expressed in terms of DFI (DNA Fragmentation Index) values (ratio of damaged to intact cells). The results were processed using the Statistica 10 application (StatSoft).

Results and Discussion

In accordance with EU standards, cryopreserved bull semen should be characterized by a minimum of 50% total motile sperm. The data presented in Table 1 indicate that semen samples analyzed in this study had 62.51 ± 6.94% total motile sperm. Similar results were reported by Kasimanickam et al. (2007). The evaluated semen samples were also characterized by a high percentage of progressively motile spermatozoa (PMOT, 21.46 ± 6.51%), which indicates that the analyzed semen is highly suitable for insemination. These results are validated by the values of other sperm kinematic parameters: VCL – 55.47 ± 6.77, VSL – 27.92 ± 4.99 μm/s, STR – 88.64 ± 3.56 and LIN – 49.52 ± 2.89%. These parameters are highly correlated with male fertility (Hirai et al., 2001). Low ALH and BCF values at 1.20 ± 0.15 and 7.62 ± 0.24Hz, respectively, indicate that spermatozoa are not hyperactivated and are characterized by high fertilizing capacity. Plasma membrane integrity (72.21 ± 2.72%) and the percentage of sperm cells with active mitochondria (71.32 ± 1.85%) confirm the high viability of cryopreserved semen. ATP concentration (15.59 ± 2.16 nmol/10^8 sperm cells) suggests the high metabolic efficiency of the analyzed semen samples. The activity levels of antioxidant enzymes – CAT, GPx and SOD (Table 1) – were similar to those reported by Kasimanickam et al. (2007). Mentioned results indicates that the analyzed sperm samples are characterized with efficient enzymatic antioxidant system, which can be confirmed by the low value of the DNA Fragmentation Index.

Our findings indicate that additional laboratory tests are needed to evaluate cryopreserved bull semen. The results of our study point to the high fertilizing capability of the analyzed semen samples, and they can be used in evaluations of cryopreserved spermatozoa characterized by loss of viability.

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