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## The correlation between selected computer assisted sperm analysis parameters and bull fertility

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### ABSTRACT

Computer Assisted Sperm Analysis (CASA) represents an objective, reproducible and reliable method of sperm quality assessment, however, not many reports exist that correlate its accuracy with bull semen fertility. The aim of this study was to evaluate the correlation between selected CASA motility parameters of cryopreserved bull semen and fertility. The total spermatozoa motility (SM %), the progressive spermatozoa motility (PSM %) as well as the percentage of spermatozoa with rapid movement (RAP %) were measured through CASA. All 12 ejaculates were collected from one Holstein Friesian bull. A total of 816 Holstein Friesian cows were used for artificial insemination (AI) and the evaluation of fertility. The fertility success was assessed by pregnancy rates per cycle (PRC %), 90 days after AI of the cows. The sperm variables that were associated with an increase in the PRC were the SM ( $R^2 = 0.6722$ ), the PSM ( $R^2 = 0.6520$ ) and the RAP ( $R^2 = 0.7103$ ). RAP had a greater influence ( $P < 0.001$ ) on fertility (PRC), than SM and PSM ( $P < 0.01$ ). The increase of sperm motility parameters (SM, PSM and RAP) led to increased PRC, i.e. to increased fertility.

**Key words:** bull, computer assisted sperm analysis, fertility, sperm motility

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## Introduction

The sperm cryopreservation process is one of the factors that adversely affect the motility of spermatozoa, and consequently affects their fertility as well (MAZUR, 1970; MORRIS et al., 2012). The reduced fertilizing ability of bull sperm reduces the profitability of livestock production, and contributes to large economic losses.

KASTELIC and THUNDATHIL (2008) reported that sub-fertile bulls delay conception, prolong the calving season, reduce calf weaning weights and increase female culls. This may be attributed to the fact that the cryopreservation process affects the functional integrity of some spermatozoon organelles. Acrosome and mitochondria are responsible for the generation of energy from intracellular stores of ATP, required for sperm motility (FORERO-GONZALEZ et al., 2012).

Bull fertility is a crucial factor influencing animal reproduction, and yield efficiency (PARISI et al., 2014). The link between sperm quality and fertility has been assessed in numerous studies (PETRUNKINA et al., 2007; PEDDINTI et al., 2008; TSAKMAKIDIS et al., 2010). In addition to assessment of sperm morphology and sperm DNA status, sperm motility is an important parameter in quality assessment (KASTELIC and THUNDATHIL, 2008; LOVE, 2011). Sperm quality should be evaluated before (fresh and diluted) and after freezing (diluted). Sperm motility assessment can be done under a phase contrast microscope or, preferably, by a CASA system (Computer Assisted Sperm Analysis). While phase-contrast microscope semen examination represents a subjective method, the CASA system represents an objective and reproducible method (VERSTEGEN et al., 2002; SUNDARARAMAN et al., 2012).

In the CASA system the evaluation of spermatozoa motility and morphology are considered the essential parameters. These systems allow the objective assessment of different cell characteristics (motility, velocity and morphology) (DAVIS and KATZ, 1993; CONTRI et al., 2010; AMANN and WABERSKI, 2014). In addition to an assessment of the sperm quality, the CASA system provides the answer regarding the reaction of the sperm to a variety of factors that act during the process of preparation and storage of sperm, as well as individual variations in bull semen during technological processes (AMANN and WABERSKI, 2014).

The aim of this study was to evaluate the fertility potential of cryopreserved bull semen, assessed by a CASA system, and to compare it with the insemination success. The success of fertility was assessed by pregnancy rates per cycle (PRC, %), after the 90<sup>th</sup> day of artificial insemination of cows (AI).

## Materials and methods

*Animals.* A total of 12 ejaculates were collected from one Holstein Friesian bull, four years old. One bull was used in this study in order to exclude possible individual

variations observed between animals, in terms of the variables related to motility and velocity (VERSTEGEN et al., 2002), and the sperm resistance to the cryopreservation process (WATSON, 2000). The diet met or surpassed the National Research Council (NRC) nutrient recommendations for breeder bulls (NRC, 2001).

Ejaculates were collected from the bull by the artificial vagina method twice a week, according to standard AI procedures. All ejaculates were assessed for volume, concentration and gross motility, prior to freezing and the CASA procedure.

Holstein Friesian cows, aged between 3 and 5 years, were used for AI and the assessment of sperm fertility.

A total of 816 cows were inseminated in the study. The estrus of the cows was diagnosed on a basis of the clinical manifestation of general estrus signs, and confirmed by rectal examination and diagnosis of a mature de *Graafian* follicle. AI was performed 12 hours after the manifestation of the first signs of estrus. Pregnancy was diagnosed after 90<sup>th</sup> day of AI, by rectal palpation.

All experimental procedures regarding animal use were in accordance with the guidelines of the European Community (Directive 86/609/EEC) and the guidelines of the Ethics Committee of the Faculty of Veterinary Medicine, University of Belgrade, Serbia.

*Semen collection.* The semen was collected using the artificial vagina method. Immediately after sperm collection, the samples were stored in a water bath at 37° C prior to evaluation. Each sample was then examined, macroscopically (semen volume, consistency and color) and microscopically. The volume was determined on the basis of the volume of sperm in a graduated sperm-collector. Assessment of sperm motility was done using a phase contrast microscope Olympus E51 with a biotherm heating plate (Olympus Corporation, Tokyo, Japan). The mass motility was evaluated at low power (×200 magnification), and the progressive motility was assessed under medium power (×400 magnification) (BELLWOOD and ANDRASIK-CATTON, 2014). The assessment of cell viability was performed by the staining method of BLOM (1950). Since all the samples had progressive motility greater than 70%, and not more than 20% pathologically altered spermatozoa, all of them were used for freezing and CASA evaluation. After determination of the spermatozoa concentration of semen by a SpermaCue™ spectrophotometer (Minitüb GmbH, Tiefenbach, Germany), the semen samples were diluted. AndroMed® semen extender (Minitüb GmbH, Tiefenbach, Germany) was used for dilution, to a final concentration of 80×10<sup>6</sup> spermatozoa per milliliter. Later, semen samples were filled and sealed in 0.25 mL French medium straws, using an MPP Uno automatic filling and sealing machine (Minitüb GmbH, Tiefenbach, Germany). The diluted semen samples were equilibrated for 4 hours at 5 °C. The straws were then horizontally exposed to liquid nitrogen vapors for 15 minutes, and after that immersed in liquid nitrogen for final

freezing and storage at  $-196^{\circ}\text{C}$ . All the equipment used for handling the native semen was tempered at  $37^{\circ}\text{C}$ .

*CASA motility assessment.* The total spermatozoa motility (SM %), the progressive spermatozoa motility (PSM %) as well as the percentage of spermatozoa with rapid movement (RAP %) were examined using Computer Assisted Sperm Analysis (CASA HTM-IVOS version 12, Hamilton Thorne Research, Beverly, MA, USA). The cryopreserved semen samples were thawed in a water bath at  $37^{\circ}\text{C}$  for 30 seconds, and CASA analysis was performed. The analysis set-up for HT-IVOS version 12, used to evaluate bull spermatozoa, was performed as described by SUNDARARAMAN et al. (2012). Only the frozen semen samples with sperm motility over 50% post-thawing were used for AI.

*Statistics.* The software package Prism Pad v. 6.0 (Graph Pad Software Inc., San Diego, CA, USA) was used for statistical calculations. Linear regression was used to determine the correlation between the motility parameters (SM, PSM and RAP) and fertility, expressed by pregnancy rates per cycle (PRC).

## Results

The descriptive statistics of bull spermatozoa motion characteristics after the cryopreservation cycle are presented in Table 1. The obtained results of the percentage of spermatozoa with rapid motility ( $38.68 \pm 7.982$ ) were higher than the percentage of spermatozoa with progressive motility ( $32.11 \pm 4.499$ ). These results were lower than the total sperm motility ( $56.69 \pm 9.457$ ) of the analysed samples of bull sperm. The pregnancy rates per cycle ( $38.15 \pm 3.055$ ) were calculated 90 days after AI of cows using assessed semen.

Table 1. The motion characteristics and fertility of bull spermatozoa after cryopreservation (n = 12)

Parameter	$\bar{X} \pm \text{SD}$	Std. Error	CV
SM%	$56.69 \pm 9.457$	2.730	16.68%
PSM%	$32.11 \pm 4.499$	1.299	14.01%
RAP%	$38.68 \pm 7.982$	2.304	20.64%
PRC%	$38.15 \pm 3.055$	0.882	8.01%

SM - total spermatozoa motility, PSM - progressive spermatozoa motility, RAP - spermatozoa with rapid movement, PRC - pregnancy rates per cycle

The calculated correlation between the total spermatozoa motility and pregnancy rates per cycle is shown in Fig. 1. The progressive sperm motility is shown in Fig. 2, and spermatozoa with rapid movement in Fig. 3.

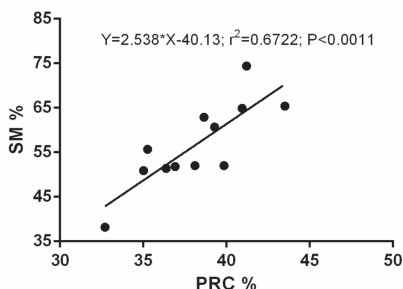


Fig. 1. The relationship between PRC% predicted by SM%. PRC - pregnancy rates per cycle, SM - total spermatozoa motility

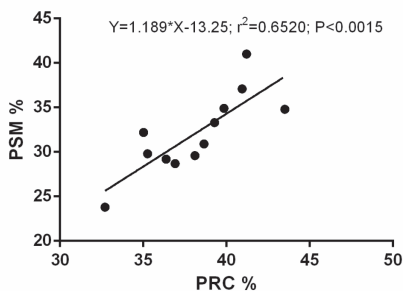


Fig. 2. The relationship between PRC% predicted by PSM%. PRC - pregnancy rates per cycle, PSM - progressive spermatozoa motility

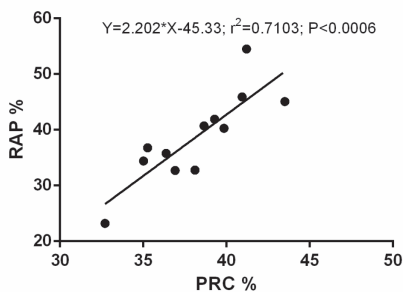


Fig. 3. The relationship between PRC% predicted by RAP%. PRC - pregnancy rates per cycle, RAP - spermatozoa with rapid movement

The sperm variables that were associated with an increase in PRC were the total sperm motility ( $R^2 = 0.6722$ ,  $P < 0.01$ ), the progressive sperm motility ( $R^2 = 0.6520$ ,  $P < 0.01$ ) and the percentage of rapid motility spermatozoa ( $R^2 = 0.7103$ ,  $P < 0.001$ ).

## Discussion

The spermatozoa motility is an important trait for mammalian sperm transport throughout the female reproductive tract, and subsequently for penetration into the oocyte. The processing of semen (equilibration, freezing and thawing) significantly reduces the quality of semen in terms of almost all motion characteristics (SUNDARARAMAN et al., 2012). Also, the dynamic interactions between spermatozoa, luminal fluids and the epithelium of the female reproductive tract, during sperm transit and depositing, can enhance sperm survival and regulate sperm fertilizing ability (SCOTT, 2000).

According to earlier reports (QUINTERO-MORENO et al., 2003; DORADO et al. 2010), ejaculates of mammalian species contain different spermatozoa subpopulations, based on the precise values of sperm motility descriptions obtained using the CASA system.

WATSON (2000) reported lower fertility of frozen compared to fresh semen, and attributed it to both post thaw viability and to the sublethal dysfunction of the surviving subpopulation.

The results from our study indicate that motility parameters, expressed as SM, PSM and RAP, were significantly correlated to fertility ( $R^2 = 0.6722$ ,  $R^2 = 0.6520$ ,  $R^2 = 0.7103$  respectively).

Our findings are in accordance with the findings of other authors, as far as the SM is concerned (JANUSKAUSKAS et al., 2003; LOVE, 2011; DORADO et al., 2013). However, the total sperm motility after thawing, observed in our study ( $56.69 \pm 9.46\%$ ), was lower than reported by FARRELL et al. (1996), TARDIF et al. (1997) and SUNDARARAMAN et al. (2012).

Interestingly, the value of the PSM recorded in our study was marginally higher ( $32.11 \pm 4.50\%$ ) than the values reported previously (FARRELL et al., 1996; TARDIF et al., 1997; SUNDARARAMAN et al., 2012). The PSM was also in correlation with fertility, as reported by other authors (LOVE, 2011; DORADO et al., 2013). In the study of QUINTERO-MORENO et al. (2003), a subpopulation of high progressive motility stallion spermatozoa conformed with the highest fertilizing capacity. These authors suggested that the study of spermatozoa subpopulations with different motilities can be used in improving the overall semen quality analysis, by introducing a different point of view to the standard semen quality analysis. The ejaculates with the highest subpopulations of rapid and progressive moving sperm were also the most resistant to cryopreservation, and showed the best post-thawing sperm longevity (MUIÑO et al., 2008), which is important for AI, i.e. fertilization. Our findings also supported the above-mentioned facts since the ejaculates with the highest RAP had the best fertilization results, in contrast to SM and PSM. Similarly, the proportions of live spermatozoa after thawing, and their resistance during post-thawing and incubation, were found to be significantly correlated with the incidence of a specific sperm subpopulation in the fresh ejaculates (MUIÑO et al., 2008; MUIÑO et al., 2009).

The percentage of RAP observed in the study was  $38.68 \pm 7.98\%$ . However, we were not able to understand fully the significance of the obtained results due to the fact that we found no records in the literature available to us of RAP measurements in bull semen. It is noteworthy that, in this study, RAP had a higher influence ( $P < 0.001$ ) on fertility (PRC), than SM and PSM ( $P < 0.01$ ). Similarly, the strong effect of the RAP on fertility was described by LOVE (2011) for stallion, and DORADO et al. (2013) for donkey semen.

According to the calculated linear regression formula, we observed that an increase in SM, PSM and RAP led to an increase in PRC, which ultimately led to increased fertility.

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**SAŽETAK**

Kompjutorski potpomognuta analiza sperme (CASA) predstavlja objektivan, ponovljiv i pouzdan način procjene kvalitete spermija. No, malo je izvješća u kojima se provjerava odnos između njezine točnosti i plodnosti sjemena bikova. Cilj ovog istraživanja bio je procijeniti povezanost odabranih pokazatelja kompjutorski potpomognute analize pokretljivosti spermija iz zamrznute sperme bikova s njihovom plodnošću. Ukupna pokretljivost spermija (%), progresivna pokretljivost spermija (%), kao i postotak spermija s brzim kretanjem (%) procijenjeni su kompjutorski potpomognutom analizom sperme. Svih 12 ejakulata prikupljeni su od jednog holštajnsko-frizijskog bika. Ukupno je 816 holštajnsko-frizijskih krava korišteno za umjetno osjemenjivanje (UO) i procjenu plodnosti. Uspjeh plodnosti procijenjen je stopom gravidnosti po ciklusu (%), 90. dana nakon UO krava. Varijable spermija koje su povezane s povećanjem stope gravidnosti po ciklusu su pokretljivost spermija ( $R^2 = 0,6722$ ), progresivna pokretljivost spermija ( $R^2 = 0,6520$ ) i stopa spermija s brzim kretanjem ( $R^2 = 0,7103$ ). Spermiji s brzim kretanjem imali su veći utjecaj ( $P < 0,001$ ) na plodnost (PRC), od ukupne pokretljivosti spermija i progresivne pokretljivosti spermija ( $P < 0,01$ ). Povećanje vrijednosti pokazatelja pokretljivosti spermija (SM, PSM i RAP) dovelo je do povećane stope gravidnosti po ciklusu, tj. povećane plodnosti.

**Ključne riječi:** bik, kompjutorski potpomognuta analiza sperme, plodnost, pokretljivost spermija

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