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Use of computer-assisted sperm analysis and flow cytometry to detect seasonal variations of bovine semen quality

E. Malama [a,*], Y. Zeron [b], F. Janett [a], M. Siuda [a], Z. Roth [c], H. Bollwein [a]

[a] Clinic of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
[b] "Sion" Artificial Insemination Center, Hafetz-Haim, Israel
[c] The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University, Rehovot, Israel

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ABSTRACT

Seasonal fluctuations of climate are considered a major factor affecting spermatogenesis and semen quality in the bovine. Our study aimed to investigate the effect of season on functional parameters of frozen-thawed bovine semen using computer-assisted sperm analysis (CASA) and flow cytometry. For this purpose, 86 ejaculates were collected from five mature Holstein-Friesian bulls kept under subtropical conditions during summer (August to September; n = 43) and winter (December to January; n = 43) months. Semen was diluted with a Tris-egg yolk-based extender and frozen at −196 °C. Computer-assisted sperm analysis was performed immediately after thawing (0h) and after 3 hours of incubation (3h) to evaluate the percentage (%) of total motile, progressively motile, and rapidly motile sperm. In addition, the average path, curvilinear, and straight-line velocities as well as the amplitude of lateral head displacement of sperm were determined. The percentages of sperm with intact plasma membrane and acrosome (PMAI, %), with high mitochondrial membrane potential (HMMP, %), with low intracellular Ca^{+2} levels (LOW-Ca^{+2}, %), and with high DNA fragmentation index (DFI%, %) were flow cytometrically determined at 0 and 3h. The survival rate of sperm under hypotonic conditions (HYPO-SURV, %) and the percentage of sperm with inducible acrosome reaction (IAR, %) were assessed using flow cytometry at 0 and 3h, respectively. The fixed effect of season (winter vs. summer) on the quality parameters of sperm was explored by applying linear mixed-effects models. The results showed an improvement of all CASA parameters, except for the straight-line velocity ($P > 0.05$) in winter compared with summer for both unincubated and incubated sperm ($P < 0.01$ in all cases). Ejaculates collected in summer had lower values of IAR ($P < 0.001$) as well as PMAI, HMMP, and LOW-Ca^{+2} at 0 and 3h ($P < 0.01$ in all cases). On the contrary, HYPO-SURV and DFI% (at 0 and 3h) were not affected by season ($P > 0.05$ in all cases). Concluding, the employment of CASA and flow cytometry revealed season-related alterations in the functional status of cryopreserved bovine sperm, which suggest an adverse effect of summer heat stress on motility, plasma membrane and acrosome integrity, inducibility of acrosome reaction, mitochondrial function and intracellular Ca^{+2} content, but not on the DNA integrity of sperm after freezing–thawing.

* Corresponding author. Tel.: +41 44 635 8204; fax: +41 44 635 8904.
E-mail addresses: lnemalama@gmail.com, emalama@vetclinics.uzh.ch, lnemalama@yahoo.gr (E. Malama).

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1. Introduction

Although bull is not normally considered a seasonal breeder, seasonal variations of bovine semen quality have long troubled researchers and managers of sperm production centers [1]. Variations of semen characteristics between summer and winter months have been partially attributed to related changes of scrotal thermoregulation and heat dissipation mechanisms [2]. Moreover, seasonal changes in the endocrinal profile and responsiveness of bull testes to gonadotropins have also been documented [3–5], further supporting the hypothesis of season-related alterations of semen quality in the bovine.

The majority of so far published studies focus on the seasonal changes of sperm characteristics, which are conventionally used as qualification criteria for cryopreservation of ejaculates in artificial insemination stations; ejaculate volume, total sperm count, sperm motility, and morphology are among the usually studied sperm traits. Indeed, volume and total sperm count appear to be higher in ejaculates collected in summer compared with those collected in winter [6,7]; however, discrepancies associated with the age and the breed of the bull have been reported [8–10]. Opinions are divided regarding the effect of season on sperm motility, viability, and morphology [7–9,11–17]. The genetic background and adaptability of the sire to local microclimatic conditions have been related to the quality of produced semen [10,18] and are considered responsible for the diverse findings of different scientific groups.

Given the complexity of sperm's nature, a multiparametric approach of semen quality is recommended to increase the precision of quality control schemes and fertility prognostics in semen production centers [19–21]. In a previous study in the bovine, Argov et al. [13] suggested that conventionally assessed sperm traits, including sperm concentration and motility, offer a limited insight into season-related variations of semen quality. The authors provided evidence on the differential expression of the very-low-density lipoprotein receptor between summer and winter sperm and supported that seasonal alterations of lipid utilization by sperm could be partially responsible for the deterioration of semen quality in summer [13]. Nowadays, the implementation of computer-assisted sperm analysis (CASA) and flow cytometry can provide useful information on a wide array of functional and structural attributes of sperm associated to its fertilizing potential [19,21]. Recently, Valeanu et al. used CASA and flow cytometry to describe the seasonal changes of a set of functional parameters of frozen-thawed bovine sperm. Although only one ejaculate per bull per season was included in the study, the authors were able to reveal season-related alterations in the integrity of sperm plasma membrane, acrosome, and chromatin structure even in bulls kept under temperate climate in Northern Europe [16].

Because artificial insemination in cattle is mainly implemented with the use of cryopreserved semen [22], the ability of sperm to maintain its functional status post-thaw is of major importance. There is strong indication that the freezability of bovine sperm is affected by the season of semen collection [12,23]. Orgal et al. (2012) showed that season-related changes in sperm motility were detectable in cryopreserved but not in fresh semen of Holstein-Friesian bulls kept under subtropical climatic conditions [12]. Their findings raised further questions regarding the effect of season on the functional status of sperm following cryopreservation. The present study was an attempt to further characterize the seasonal changes in the quality of cryopreserved bovine semen using advanced laboratory techniques (CASA and flow cytometry). For the purposes of our research, sperm motility and kinematics were determined using CASA and were compared between frozen-thawed semen collected in winter and summer. In parallel, season-related changes were explored for a set of flow cytometrically assessed functional parameters, including sperm plasma membrane, acrosome and DNA integrity, mitochondrial membrane potential, intracellular Ca^{+2} levels, and inducibility of acrosome reaction.

2. Materials and methods

2.1. Animals and semen collection

Semen samples were collected from five mature Holstein-Friesian bulls (7.3 ± 0.6 years of age) housed in the Israeli Artificial Insemination Center ("Sion", Hafetz-Haim, Israel) during winter (December to January) and summer (August to September). Bulls did not receive any hormonal or other treatment, were exposed to natural photoperiod and climatic conditions of the area, and were fed the same total mixed ration [68.4% (wt/wt) dry matter, 7.2% (wt/wt) protein, 36.2% (wt/wt) neutral detergent fiber, 20.0% (wt/wt) acid detergent fiber, 1.45 net energy Mcal/kg, and 3.5 g minerals/kg (NaCl, Ca, and P) on a dry matter basis] throughout the experimental period.

Bulls were ejaculating twice per week in a disposable tube attached to a prewarmed (38 °C) sterile artificial vagina, after mounting on a teaser bull. For each bull, only the first ejaculate collected each week was used for experimental purposes. In total, 86 ejaculates were collected during winter (n = 43) and summer (n = 43). In a preceding phase of our research project, the collected samples were used for the investigation of seasonal changes in the concentration of chemical elements in seminal plasma and the quality of sperm after cryopreservation using fluorescence microscopy [12].

The climate of the region where the study was conducted is considered "subtropical dry" or Mediterrenean. It is characterized by moderately cool, rainy winters (November to March) and hot, humid summers (June to October) with no rainfall. Day temperatures in summer exceed 30 °C and relative humidity ranges from 50% to 90% [24]. Microclimatic data (ambient air temperature and relative humidity) during the experimental period of our study were made available by the central meteorological station of Bet-Dagan, Israel. Daily maximum ambient temperature (mean ± standard error of the mean) observed in summer and winter was 31.8 ± 1.4 and 16.8 ± 2.3 °C, whereas maximum values of relative humidity were 84.3 ± 3.8% and 46.0 ± 15.0%, respectively [12]. The values of microclimatic parameters in summer and winter were in the range expected for the region. No heat wave was reported during the experimental period.