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Seasonal variation in sperm quality parameters in Swedish red dairy bulls used for artificial insemination

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ABSTRACT

The aim of the present study was to investigate the extent to which seasonal factors such as temperature, atmospheric pressure or visible light length affect dairy bull sperm quality. Straws from 10 dairy bulls, from semen collections in three different seasons, were available for this study. The following quality parameters were assessed: motility, measured by computer assisted sperm motility analysis (SpermVision motility analyzer), membrane integrity (staining with SYBR14/PI (Propidium Iodide)with flow cytometric measurement of fluorescence), content of reactive oxygen species (ROS) measured using HE(Hydroethidine) and DCFDA (2′, 7′-dichlorodihydrofluorescein diacetate), mitochondrial membrane potential (using JC-1 fluorescence stain), acrosome reaction (measured with fluorescein isothiocyanate-PNA (FITC-PNA) labeling combined with calcium ionophore A23187 and PI), DNA fragmentation index (%DFI, Sperm Chromatin Structure Assay) as well as morphology, using the William's staining protocol. Data was analyzed by analysis of variance (PROC MIXED), using SAS software. The proportion (%) (mean ± SD) of living, dying and dead sperm cells varied between seasons, with the proportion of living spermatozoa being lowest in summer, although a significant difference ($P<0.05$) was only observed between spring and summer. The %DFI assessed by SCSA was lowest in spring and differed significantly ($p < 0.04$) from summer. A trend towards significance was observed between spring and summer for the motility parameters linearity (LIN) ($P=0.068$) and straightness (STR) ($P=0.062$).

1. Introduction

The identification of the environmental factors which may affect sperm production and the quality of the semen has a major importance in improvement of bull fertility. Sperm quality is itself influenced by environmental factors such as temperature, humidity, atmospheric pressure, and day length. Elevated testicular temperatures, either by exposure to high ambient temperatures or high body temperature, have been identified as causing a disruption in spermatogenesis, producing sperm abnormalities with a consequent decrease in semen quality, which eventually may result in a decrease in embryo quality or in the failure of the embryo to signal maternal recognition of pregnancy (Bhuiyan and Shamsuddin, 1998; Mathevon et al., 1998). Maintaining testicular temperature below body temperature is essential for production of fertile spermatozoa in species with extra-abdominal testes (Waites, 1970). The optimal environmental temperature for sperm production is estimated to range between 15 and 18 °C for the entire

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spermatogenesis period, i.e. for 65–70 days before collection (Parkinson, 1987).

According to Tilbrook et al. (1992), increasing levels of plasma corticosteroids due to heat stress may inhibit luteinizing hormone (LH), which is responsible for spermatogenesis. Reports on the effects of season on semen production are contradictory. While in many studies, season significantly influenced semen production (Fiaz et al., 2010; Koonjanek et al., 2007; Mathevon et al., 1998; Nichi et al., 2006; Stålhammar et al.,1989), other investigations (Brito et al., 2002), failed to detect an effect of the seasons on sperm production. Although Stålhammar et al. (1989) observed the highest concentration and total number of spermatozoa during the summer season, in a study done in Sweden, Mathevon et al. (1998), reported the highest values of these parameters during winter and spring, in a study that was conducted in Canada. According to Parkinson (1987), the optimal environmental temperature for sperm production is between 15 and 20 °C, but this applies to the whole period of sperm production, not just to the day of collection.

Despite its northern latitude, most of Sweden has a temperate climate due to the Gulf Stream, with the result that it is warmer and drier than other places at a similar latitude, and even somewhat farther south (Swedish Meteorological and Hydrological Institute). Temperatures vary greatly from north to south, with southern and central parts of the country experiencing average high temperatures of 20–25 °C and lows of 12–15 °C in the summer, −4–3 °C in the winter and 8 °C in spring.The aim of this retrospective study was to investigate the influence of climatic factors such as temperature, atmospheric pressure or amount of daylight on semen quality of Swedish Red and White (SRB) dairy bulls on a bull station in southern Sweden, using CASA and flow cytometry.

2. Material and methods

2.1. Semen collection

Semen was collected from ten Swedish Red and White (SRB) dairy bulls, aged between 4 and 7 years, housed at a bull stud in Sweden. Semen was collected using an artificial vagina and those ejaculates having more than 500×10^6 spermatozoa/mL and 70% progressive motility were extended in AndroMed® (Minitüb, Tiefenbach, Germany) and cryopreserved. Only straws with an acceptable post-thaw quality (conforming to the company's internal standards) were included in this study. One straw of frozen semen was selected for each bull from collections made in spring (April–May), summer (July–August) and winter (December–January).

2.2. Climate factors

The daily climatic parameters registered in the period November 2010–January 2012, were obtained from Sweden's Meteorological and Hydrological Institute website (SMHI www.smhi.se). The daily and monthly average of the following parameters were calculated: temperature (°C), Humidity (%), atmospheric pressure (hPa), Visible light length (minutes per day). Based on the monthly information, for the bull stud region, available on the Swedish Meteorological and Hydrological Institute website, the data was processed, analyzed and then correlated with the spermatic parameters. The bulls were housed indoors in a barn with natural light, with supplementary lighting during normal working hours (7 h during the day).

2.3. Sperm thawing and determination of concentration

The straws were thawed in a water bath at 37 °C for 12 s. Sperm concentration was measured using a Nucleocounter-SP 100 (Chemometec, Allerød, Denmark) according to the manufacturer's instructions. An aliquot (50 μL) from each sample was mixed with 5 mL reagent S100 in a sample cup. A cassette containing propidium iodide (PI) was filled with the mixture before being placed in the Nucleocounter-SP100 to measure fluorescence. The Total Cell Count was displayed after approximately 30 s.

2.4. Computer-assisted sperm motility analysis (CASA)

Sperm motility was assessed by computer-assisted semen analysis (CASA) using the SpermVision™ (MiniTűb, Tiefenbach, Germany) with an Olympus BX 51 microscope (Olympus, Japan). A 5 μL aliquot was pipetted on to a pre-warmed (38 °C) microscope slide, covered with a coverslip (18×18 mm^2) and the motility patterns were analyzed. The following parameters were measured: total motility (%), progressive motility (%), DAP (distance average path, μm), DCL (distance curved line, μm), DSL (distance straight line, μm), VAP (velocity average trajectory, μm/s), VCL (velocity curved line, μm/s), VSL (velocity straight line, μm/s), STR (straightness, VSL/VAP,%), LIN (linearity, VSL/VCL, %), WOB (wobble, VAP/VCL, %), ALH (amplitude of lateral head displacement, μm), and BCF (beat cross frequency, Hz) (Boyers et al., 1989), (Farrell et al., 1995).

2.5. Morphology

Wet smears were prepared using the “feathering” technique (Anon, 2010), air dried and stained with carbol-fuchsin–eosin, (Kavak et al., 2004). Using × 1000 magnification with oil immersion, 500 sperm cells were evaluated on each slide to establish the percentage of sperm cells with normal morphology (NM) and morphological abnormalities such as proximal cytoplasmic droplets, detached heads, acrosome defects, nuclear pouches and tail defects. In addition, further aliquots of semen fixed in formol-saline were used to make smears for evaluation of 200 spermatozoa at × 1000 magnification. Morphology evaluation was carried out by skilled personnel according to the standard protocol in the Swedish Sperm Reference Laboratory at SLU.