



Effects of season, age, and breed on semen characteristics in different *Bos taurus* breeds in a 31-year retrospective study

T. Snoj^a, S. Kobal^a, G. Majdic^{b,c,*}

^a Institute of Physiology, Pharmacology and Toxicology, Veterinary Faculty, University of Ljubljana, Gerbiceva 60, Ljubljana, Slovenia

^b Center for Animal Genomics, Veterinary Faculty, University of Ljubljana, Gerbiceva 60, Ljubljana, Slovenia

^c Institute of Physiology, Medical Faculty, University of Maribor, Slomskov trg 15, Maribor, Slovenia

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ABSTRACT

Besides genetic factors, the season of semen collection could have an important effect on ejaculate characteristics, although results from previously published studies are somewhat variable. To determine seasonal effects on semen characteristics, we have analyzed 71,983 ejaculates collected from bulls of four different breeds over a 31-year period. Ejaculate volume, semen concentration, and total sperm output were analyzed with the respect to season and age of bull. Results revealed that semen concentration did not vary significantly during seasons, and ejaculate volume and total sperm output are influenced by season in all breeds. The highest ejaculate volume and total number of sperm in ejaculates were observed during the summer, followed by spring, autumn, and winter. Results suggest that the gradual increase in the day length in the spring is most likely responsible for the highest sperm output during the summer months, suggesting that seasonal effects are also present in cattle, which is not normally considered a seasonal species.

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1. Introduction

Artificial insemination is widely used in modern cattle breeding programs. Factors affecting semen production in bull sires must be considered in the insemination industry because they affect the productivity of bulls. Besides genetic factors, environmental factors also have important influences on semen quality in many species. These include nutrition and stress, environmental temperature, and day length. The season in which semen is collected is likely to have an important effect on ejaculate quality, although the results from previously published studies in cattle are somewhat ambiguous. In the current study, the effect of season was examined in a large cohort of bulls with respect to age and breed over a 31-year period.

Seasonal effects on bull semen production have been described, although the results of different studies are not unequivocal. Some studies suggest a stronger seasonal effect

on semen characteristics in breeds originating from *Bos indicus* than in breeds deriving from *Bos taurus* (European) breeds [1–3], although some other studies also have reported seasonal effects in European breeds [4–8]. Reported effects of season are also controversial. Some studies have indicated the highest quality of semen in winter [4,6,9,10], and others in summer and early autumn [3,7,8]. One study did not detect any effect of season on semen characteristics [11] suggesting that seasonal differences are expressed differently in different locations according to latitude, especially because this one study was performed in an area close to the equator where variations in day length between seasons are the smallest. In addition to semen quality, seasonal effects have been studied on other reproductive and/or endocrine parameters such as testosterone blood levels [12–14] and sperm morphology [3,15–17], although these differences also differ between geographic locations [12].

Because there are no conclusive reports about the effects of season on bull semen quality, we have analyzed a large cohort of semen ejaculates (more than 70,000

* Corresponding author. Tel.: +386 1 4779210; fax: +386 1 2832243.
E-mail address: gregor.majdic@vf.uni-lj.si (G. Majdic).

ejaculates from 694 bulls) to determine the possible effects of season, age, and breed on semen quality in the temperate zone. Data on ejaculates were collected over 31 years, and bulls of four different breeds (Holstein [HO], Brown Swiss [BS], Limousin [LIM], and Charolais [CH]) were included in the study.

2. Materials and methods

2.1. Animals and location

Raw data on bull semen, collected at the Semen Collection Center Preska, in Slovenia, were obtained from the Department of Agriculture and Forestry, Ljubljana, Slovenia. The center is located in the central region of Slovenia (46.1337° N, 14.4096° E) in a temperate zone. Climate conditions are continental with hot summers and cold winters. Average annual temperatures range from 10 °C to 12 °C with average temperatures of 11.0 °C in spring, 20.3 °C in summer, 10.8 °C in autumn, and 1.1 °C in winter [18]. Ejaculates collected from 233 HO, 369 BS, 52 LIM, and 40 CH bulls were analyzed in the study. Bulls were fed 9 kg of hay and 1.5 kg of complementary feed twice per day throughout the study period and had drinking water *ad libitum*. Bulls were kept in a standardized environment throughout the observation period, without any significant changes during the entire 31-year period. For semen collection, bulls mounted a teaser animal and semen was collected with an artificial vagina. Semen collection was performed every 4 to 7 days with one or two collections per day, depending on individual bull semen quality and libido. When performed, second collections were made 15 minutes after the first one. Only data for semen characteristics obtained during the first collections were included in the present study.

2.2. Evaluation of semen characteristics

Ejaculate data from the 31-year collection period (1977–2007) were included in the study. Altogether, 71,983 ejaculates were analyzed. Ejaculate volume was measured directly in the graded collection tube. Throughout the 31-year period, sperm concentration was measured using a spectrophotometer (IMV Technologies; L'Aigle; France) according to the manufacturer instructions. Raw data on parameters of fresh semen were transferred to a computer from original protocols for the purpose of this study. Total sperm output was calculated by multiplying ejaculate volume and semen concentration.

Data related to fresh ejaculate volume, semen concentration, and total sperm output were grouped by season. Seasons were determined as spring (March 1 to May 31), summer (June 1 to August 31), autumn (September 1 to November 30), and winter (December 1 to February 28 or 29). Because age has an important influence on semen characteristics, ejaculates were further grouped with respect to the age of the bull at the time of semen collection. Bulls were divided into 7 age groups: 12 to 24 months, 25 to 36 months, 37 to 48 months, 49 to 60 months, 61 to 72 months, 73 to 84 months, and older than 84 months of age.

2.3. Statistical analysis

All statistical analyses were performed with the NCSS software package (NCSS 2007, Kaysville, UT, USA). First, descriptive statistical parameters (mean, standard deviation, and standard error of mean) were calculated separately for breeds and seasons. To compare differences between seasons, data were analyzed using ANOVA, followed by Bonferroni post hoc test, to determine differences between individual groups. Differences were considered statistically different when $P < 0.05$. Data are expressed as mean \pm SEM.

3. Results

3.1. Ejaculate volume

At the ages of 25 to 84 months, all breeds included in the study exhibited their lowest ejaculate volumes in the winter period. Interestingly, in bulls younger than 24 months from HO, BS, and CH breeds, the lowest ejaculate volume was detected in spring. At the age of older than 84 months, the lowest ejaculate volumes in HO and CH bulls were observed in autumn and summer, respectively, and in BS and LIM bulls, the lowest values were noted in the winter period (Tables 1–4). As shown in Figure 1, the highest ejaculate volumes at all ages combined were observed in all breeds in summer, followed by spring, autumn, and winter, with significant differences between seasons ($P < 0.001$).

There was a marked increase in the mean ejaculate volume with age (Tables 1–4), and a moderate decrease in BS bulls after 84 months of age and in LIM and CH bulls at the ages from 73 to 84 months.

3.2. Semen concentration

As presented in Tables 1–3, semen concentrations in HO, BS, and LIM bulls varied little between seasons. No reproducible pattern of seasonal semen concentration fluctuations at different ages was observed. In CH bulls, the lowest semen concentrations were found in autumn at almost all ages (Table 4). However, the highest values were dispersed and seasonality varied with age.

3.3. Total sperm output

The lowest mean total sperm output was observed in most groups during the winter period. Higher values were observed in autumn and spring, and the highest observed during the summer period (Tables 1–4). Total sperm output increased with age in all four breeds. In the LIM breed a decrease of total sperm output was observed at ages from 73 to 84 months, and in BS and CH breeds a lower total sperm output was observed in bulls older than 84 months. In HO bulls, no age-related decrease in total sperm output was observed. The data for all bulls regardless of age revealed the highest number of sperm in all breeds in summer and lowest in winter (Fig. 2) with significant differences between seasons ($P < 0.001$).

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