**Original Research Article**

**The influence of seasons on the motility and kinetics parameters of deep-frozen semen of Simmental bulls**

**A B S T R A C T**

The aim of this study was to determine whether there are differences in sperm motility and kinetics parameters in Simmental bulls during different seasons. The study included 43 bulls whose semen was collected and deep-frozen during the summer, autumn, spring, and winter. The semen was analyzed using computer-assisted semen analysis (CASA), whereby 0.25 mL straws were thawed in a water bath at 38 °C for 20 seconds. Our study found that the season had no effect on sperm concentration, total and progressive motility, the percentage of fast and slow sperm, sperm moving in circles or in place, or immotile sperm (p > 0.05). Additionally, no seasonal effect (p > 0.05) was observed on sperm kinetic parameters: velocity curved line (VCL), velocity straight line (VSL), velocity average path (VAP), distance curvilinear line (DCL), distance straight line (DSL), distance average path (DAP), amplitude of lateral head displacement (ALH), head activity (HAC), wobble (WOB), linearity (LIN), and straightness (STR). In our study, only the values for beat cross frequency (BCF) were found to be season-dependent, showing significantly lower values when semen was collected and deep-frozen during the summer compared to spring and winter (p < 0.05).

***Key words****: spermatozoa, deep-frozen semen, Simmental bulls, CASA*

1. INTRODUCTION

The Simmental breed of cattle is one of the oldest multipurpose breeds. In most countries, the Simmental breed is used for meat production through crossbreeding with beef and dairy cattle breeds (Sadikova et al., 2023). Artificial insemination (A.I.), as an important branch of biotechnology, plays a crucial role in improving the genetic quality of animals, especially cattle (Hapsari et al., 2022). Artificial insemination also significantly reduces the risk of spreading infectious diseases (Konenda et al., 2020). The success of A.I. is largely influenced by semen quality. Numerous factors can affect semen quality, with genetic and environmental factors being the most significant (Isnaini et al., 2021). Indicators of good-quality semen include a high concentration of spermatozoa, a high percentage of progressively motile sperm, normal morphology, and an intact acrosome (Dipaz-Berrocal et al., 2024). Spermatogenesis is a complex process of sperm production within the seminiferous tubules of the testes, which can be significantly influenced by environmental factors. Therefore, it is of great importance to ensure optimal conditions for bulls involved in livestock breeding programs (Harrison et al., 2022). It is considered that the optimal ambient temperature for sperm production is between 15 and 18 ºC throughout the entire spermatogenesis period, i.e., 65 to 70 days before semen collection (Parkinson, 1987; Valeanu et al., 2015).

According to Netherton et al. (2022), individual bulls can be classified as either "heat-tolerant," meaning they produce good-quality semen throughout the year regardless of temperature, or "heat-sensitive," meaning they produce good-quality semen only during the summer period.

Regarding the influence of season on the semen quality of bulls, there are differences in the literature. Sabes-Alsina et al. (2017) found that the semen quality of the Holstein breed in Spain was significantly better during the spring. In Simmental bulls, a significantly higher percentage of pathologically altered spermatozoa was observed during the summer compared to the winter period (Nichi et al., 2006). The seasonal influence on semen quality depends on temperature, humidity, day length, and reproductive center management practices (Fuerst-Valtl et al., 2006). Given the contradictory data in the literature regarding the seasonal influence on semen quality—where many authors confirmed a seasonal effect (Vilakazi and Webb, 2004; Nichi et al., 2006; Murphy et al., 2018; Nongbua et al., 2020), while others did not find such an effect (Mathevon et al., 1998; Brito et al., 2002)—the aim of our study was to examine the effect of season on the semen quality of Simmental bulls, whose semen was collected and deep-frozen during different seasons (summer, autumn, spring, and winter).

1. MATERIALS AND METHODS

2.1. OVERVIEW OF THE QUALITY OF DEEP-FROZEN BULL SEMEN

The deep-frozen bull semen was delivered to the Laboratory of the Department of Obstetrics and Sterility for analysis, transported in a container with liquid nitrogen at –196 ºC, where semen motility and kinetics parameters were assessed using the computer-assisted sperm analysis (CASA) system (Minitube, AndroVision, Germany). A total of semen samples from 43 Simmental bulls were analyzed, with semen collected and frozen during the summer, autumn, spring, and winter. Before analysis, 0.25 mL straws were thawed in a water bath at 38 ºC for 20 seconds. After thawing, the semen was transferred from the straws into Eppendorf tubes on a heating plate (Minitube, Tiefenbach, Germany). After careful mixing, a 2.7 µL sample of semen was examined using the CASA system in a Leja chamber (Leja, GN Nieuw Vennep, Netherlands). Semen was analyzed under ten fields of view using a phase-contrast microscope (Motic BA310, Barcelona, Spain) equipped with a heating plate. Motility and kinetics parameters were automatically analyzed using the AndroVision software (Minitube Manual 12500/0000, AndroVision, Germany). The CASA system measured the following parameters: concentration, total and progressive motility, the percentage of fast and slow sperm, sperm moving in circles or in place, and immotile sperm. Additionally, kinetic parameters were measured: velocity curved line (VCL), velocity straight line (VSL), velocity average path (VAP), distance curvilinear line (DCL), distance straight line (DSL), distance average path (DAP), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), head activity (HAC), wobble (WOB), linearity (LIN), and straightness (STR).

**2.2. STATISTICAL DATA ANALYSIS**

The normality of the data distribution was tested using the Shapiro-Wilk test. Since the data were normally distributed (Shapiro-Wilk test), analysis of variance (ANOVA) was applied, and subsequent comparisons were made using the Tukey test. The data are presented in graphical form. Statistical analysis was performed using GraphPad Prism version 8 software (GraphPad, San Diego, CA, USA).

1. RESULTS AND DISCUSSION

The quality of bull semen can be significantly influenced by the characteristics of the breed itself (Sabés‐Alsina et al., 2017). It is well-known that semen quality varies between different cattle breeds, with genetics playing a significant role (de Lucio et al., 2014). However, there is no consensus in the literature regarding the effect of season on bull semen quality. Some studies have confirmed the seasonal influence (Vilakazi and Webb, 2004; Nichi et al., 2006; Murphy et al., 2018; Nongbua et al., 2020), while others have not found any seasonal effect on semen quality (Mathevon et al., 1998; Brito et al., 2002).

Figure 1 shows the summarized results of total sperm motility, progressive sperm motility, the percentage of fast, slow, and immotile sperm, as well as their concentration.

Slika na kojoj se nalazi tekst, snimak ekrana, dijagram, Četvorougao

Sadržaj koji generiše veštačka inteligencija može biti netačan.

**Fig 1.** Assessment of total and progressive sperm motility, percentage of fast, slow, immotile sperm, and sperm concentration (mean ± SE) in Simmental cattle depending on the seasons (summer, autumn, spring, and winter).

*Statistically significant difference p > 0.05*

 The computer-assisted sperm analysis (CASA) system is crucial for the objective determination of sperm motility, kinetics, and concentration (Bastan, 2024). In our study, the highest percentage of total motile sperm was found in the winter period, with 6.08% compared to summer (4.32%), autumn (1.49%), and spring (p > 0.05, Fig. 1). Also, as seen in Fig. 1, the highest percentage of progressively motile sperm was found in the winter period, with 7.79% compared to summer (4.02%), autumn (1.03%), and spring, but without statistical significance (p > 0.05). Similar to our findings, Nongbua et al. (2020) observed no significant differences in volume, concentration, or sperm motility between winter and summer in bulls in Thailand. Although no statistical significance (p > 0.05) was found in our study, the highest percentage of fast sperm was noted in spring, with 10.68% compared to summer (9.30%), autumn (3.12%), and winter (Fig. 1). The highest percentage of slow sperm was found in autumn (Fig. 1), with 7.92% compared to summer, 9.16% compared to spring, and only 0.86% compared to winter, but without statistical significance (p > 0.05). Although no statistically significant differences (p > 0.05) were observed, the highest percentage of immotile sperm was found in the summer period, with 2.81% vs. autumn, 9.47% vs. spring, and 9.64% vs. winter (Fig. 1). High temperatures and humidity are believed to have negative effects on sperm production (Suriyasomboon et al., 2004). Similar to our research, Sabés‐Alsina et al. (2017) found higher values of total and progressive sperm motility in Holstein cattle samples collected in spring compared to those collected in winter.

In Fig. 2, the summarized values of kinetic parameters of deep-frozen semen from Simmental bulls (VCL, VSL, VAP, DCL, DSL, and DAP) are presented during the summer, autumn, spring, and winter periods.

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**Fig 2.** Assessment of sperm kinetics parameters: velocity curved line (VCL), velocity straight line (VSL), velocity average path (VAP), distance curvilinear line (DCL), distance straight line (DSL), distance average path (DAP) (mean ± SE) in Simmental cattle depending on the seasons (summer, autumn, spring, and winter).

*Statistically significant difference p > 0.05*

Although no statistically significant difference was found (p > 0.05), the highest values for VCL were observed in spring, with 6.27% compared to summer, 6.91% compared to autumn, and 1.29% compared to winter (Fig. 2). It is considered that VCL values are associated with low fertilizing capacity of sperm (Oliveira et al., 2012). Also, in our study, the highest values for VSL were observed in spring (Fig. 2), with 7.19% compared to summer, 5.61% compared to autumn, and 2.07% compared to winter, but without statistical significance (p > 0.05). Sabés‐Alsina et al. (2017) found higher average values of VAP and VSL in spring compared to summer in Holstein cattle, which is in line with our findings. In spring, the highest values for DCL were also observed, although without statistical significance (p > 0.05, Fig. 2), with 5.38% compared to summer, 5.92% compared to autumn, and 0.91% compared to winter. Additionally, in our study, the highest values for DSL were observed in spring, with 7.64% compared to summer, 4.02% compared to autumn, and 0.99% compared to winter, but without statistical significance (p > 0.05, Fig. 2). In the study by Sinha et al. (2021), the highest values for VSL, VCL, DSL, and LIN were found during the winter period, with values significantly decreasing during the summer period.

In Fig. 3, the summarized values of the kinetic parameters of the deep-frozen semen of Simmental bulls (ALH, BCF, HAC, WOB, LIN, and STR) are shown for the summer, autumn, spring, and winter periods.

Slika na kojoj se nalazi tekst, dijagram, snimak ekrana, Četvorougao

Sadržaj koji generiše veštačka inteligencija može biti netačan.

**Fig 3.** The evaluation of spermatozoa kinetic parameters: amplitude of lateral head displacement (ALH), beat cross frequency (BCF), head activity (HAC), wobble (WOB), linearity (LIN), and straightness (STR) (mean ± SE) in Simmental cattle, depending on the seasons (summer, autumn, spring, and winter).

*Statistically significant difference (\*) p < 0.05 BCF summer vs spring and winter*

Although no statistically significant difference was found (p > 0.05, Fig. 3), the highest values for ALH in our study were observed in spring, being 5.80% higher compared to summer, 5.96% higher compared to autumn, and 1.69% higher compared to winter. The highest values for BCF (Fig. 3) were found in the winter period, with a 11.18% increase compared to summer (P < 0.05), a 3.14% increase compared to autumn, and a 1.20% increase compared to spring. Statistically significant lower values for BCF were found in the summer period compared to spring, with a 10.10% decrease (P < 0.05). According to Oliveira et al. (2012), total motility, progressive motility, or BCF can be predictors of high fertilization potential of spermatozoa.

In our study, the highest values for HAC were found in the winter period, with an increase of 6.51% compared to summer, 6.77% compared to autumn, and 1.41% compared to spring. However, this increase was not statistically significant (p > 0.05, Fig. 3). Nearly uniform values (p > 0.05, Fig. 3) for WOB, LIN, and STR were found across all seasons. These results are consistent with the findings of Sabés‐Alsina et al. (2017), who reported that spring semen samples from Holstein cattle in Spain exhibited better quality compared to those collected during other seasons. Similarly, Sinha et al. (2021) found that values for VAP, DAP, and STR were significantly higher during the winter and autumn periods compared to the spring period. These authors also noted that there were no statistically significant differences in ALH and BCF values across the investigated seasons.

1. CONCLUSIONS

In our study, it was found that the seasons had no impact on the quality of deep-frozen semen in Simmental cattle, except for the beat cross frequency (BCF) of spermatozoa, whose values were significantly lower when semen was collected and deep-frozen during the summer period compared to spring and winter.

DECLARATION OF COMPETING INTERESTS

The authors declared no potential confl icts of interest with respect to the research, authorship, and/or publication of this article.

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