https://doi.org/10.17221/21/2025-CJAS

Impact of microclimatic conditions on sperm production in Czech Holstein bulls: A correlational study

Filipp Georgijevič Savvulidi, Jaromír Ducháček, Martin Ptáček, Matúš Gašparík, Veronika Kovářová, Radim Codl, Luděk Stádník*

Department of Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sceinces (CULS) Prague, Prague – Suchdol, Czech Republic

**Corresponding author: savvulidi@af.czu.cz*

Citation: Savvulidi F.G., Ducháček J., Ptáček M., Gašparík M., Kovářová V., Codl R., Stádník L. (2025): Impact of microclimatic conditions on sperm production in Czech Holstein bulls: A correlational study. Czech J. Anim. Sci., 70: 121–129.

Abstract: In this study, the influence of microclimatic conditions in different seasons of the year (including the hottest months) on the quantitative and qualitative characteristics of ejaculates of Czech Holstein bulls was assessed. Bulls were kept in the facility with no forced ventilation or air conditioning. To determine the influence, the temperature-humidity index (THI) was calculated based on the microclimatic parameters measured directly in the facility with the animals. Bull sperm was obtained using an artificial vagina on five occasions throughout the year, in different seasons of the year including the hottest months. Semen was assessed for its volume, sperm concentration, and motility by CASA, as well as cellular viability by using flow cytometry. In the present study no statistically significant positive correlations between values of THI and sperm degradation were observed. Obtained preliminary results give grounds to believe that Czech Holstein bulls produced high-quality sperm during the summer, the hottest months of the year.

Keywords: bovine; CASA; flow cytometry; fresh sperm; heat stress; temperature-humidity index

In recent years, global warming has been a hotly debated issue with a compelling evidence base (Park 2022; Wang et al. 2023). Particular attention is paid to warming in the European region, where the annual mean temperatures increased with a rate of +0.5 °C per decade, twice as much as the global average (WMO 2023). It must be noted that the intensity of warming observed today in practical

Supported by the Project NAZV (No. QK22010270) and SGS (No. SV24-3-21320).

https://doi.org/10.17221/21/2025-CJAS

measurements exceeds the results of predictive models developed in previous years (Schumacher et al. 2024), which is alarming. Extreme precipitation was confirmed for the European region (Zeder and Fischer 2020) as a warmer atmosphere holds more moisture. All this applies to the Central European region to no lesser extent (Zahradnicek et al. 2022).

Unsurprisingly, many studies in the field of farm animal physiology are devoted to assessing the risks associated with the negative impact of heat stress on livestock (Sabes-Alsina et al. 2019; Morrell 2020; Vanselow et al. 2024; Vinet et al. 2024). Dairy farming is one of the leading areas of livestock production worldwide.

To predict the risks and develop effective methods for combating heat stress in dairy animals, it is necessary to study in detail the effect of increased temperature on production (Bezdicek et al. 2021; Kulaz and Ser 2022; Chen et al. 2023). Here it should not be overlooked that the male population is important in terms of total milk production through reproduction and genetics. Previously, it was established that heat, cold and/or cooling stress might negatively affect at least some qualitative and quantitative parameters of the bull semen of several breeds, which in turn might negatively affect reproduction, and as a consequence – total milk production, leading to an economic loss.

One of the most objective methods for assessing heat stress is calculating the temperature-humidity index (THI) (Garcia-Ispierto et al. 2006; Llamas-Luceno et al. 2020; Gloria et al. 2021). This method considers not so much the temperature itself (for example, in the facility where the animals are kept) but the complex of "temperature and humidity", which more accurately reflects the heat load on animals. According to the available literature, the optimal THI range for semen production in bulls of *Bos taurus* is between 50 and 60, where semen traits are most favourable (Al-Kanaan et al. 2015). Beyond THI 60, detrimental effects on semen volume, output, and quality might be observed in some breeds. Beyond THI 70, an increase in heat stress is supposed; beyond THI 80, the danger of severe heat stress, when declines in semen quality and reproductive performance are likely, is predicted (Al-Kanaan et al. 2015; Llamas-Luceno et al. 2020).

In the context of this study, it must be clarified that the process of sperm formation and maturation in bulls lasts for about two months. During this entire period, sperm may be exposed to the potentially negative effects of high temperatures, i.e., heat stress. The physiological mechanisms of heat stress in testicular tissues are beyond the scope of this study. However, it is important to note that heat stress can disrupt the normal process of sperm formation and maturation in testicular tissues, or their storage in the epididymis before ejaculation. As a result of heat stress, not only detrimental effects on semen volume and output might be observed, but also several cellular structures of sperm may be damaged: plasma membrane, acrosome, mitochondrial apparatus etc. This damage may be manifested either as cellular pathologies or by subtle changes in the cell functionality. Such damage does not always lead to cell death, but it leads to reduced sperm motility and viability, and consequently to reduced fertilising ability. The fertilising ability of spermatozoa can be studied either *in vivo* [using functional tests, primarily based on artificial insemination (AI), where fertilising ability is assessed directly], or *in vitro*, using various laboratory tests (CASA, flow cytometry) that predict the fertilising ability of spermatozoa. Here it should be noted that the *in vivo* results are heavily influenced by the quality of AI (sampling of the female population, heat synchronisation success, proper timing of AI, practical skills of the AI technician, general zootechnical level of the farm, etc.) (Beran et al. 2013; Boudaoud 2023; Kasna et al. 2023; Bezdicek et al. 2024). On the other hand, the results obtained *in vitro*, although only predictive, are less loaded with error, and therefore are more reproducible in different laboratories (Pytlik et al. 2022; Pytlik et al. 2023; Savvulidi et al. 2023; Grba et al. 2024).

For our study we adopted the following working hypothesis: we assumed that under current climatic conditions, global warming (and especially climate warming in the European region) would negatively affect the sperm production of Czech Holstein bulls kept in buildings without forced ventilation, especially in the hottest months of the year. Therefore, in our study, we asked several scientific questions: namely, would the sperm quality be affected by a high THI value? Or, conversely, would Holstein bulls produce the semen of acceptable quality in the summer months of the year (when the highest negative effect of heat stress on bulls is expected under the Czech climatic conditions) if kept in buildings without forced ventilation or air conditioning?

https://doi.org/10.17221/21/2025-CJAS

MATERIAL AND METHODS

Bulls

In the present study, seventeen adult (2–4 years old) healthy bulls of the Holstein breed were used. Bulls were housed at a commercial breeding station situated in the Central Bohemian Region of the Czech Republic, 285 meters above sea level. Bulls were provided with optimised feeding and drinking regimes. The study design considered the need for minimal intervention in routine activities carried out with bulls at the breeding station. The study was conducted in accordance with the Czech legislation for the Protection of Animals against Cruelty (Act No. 246/1992) and with the Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes.

Ejaculate collection and processing

Ejaculates were collected with an artificial vagina during five collection sessions in different seasons of the year, including the hottest months of the year. Namely, the semen was collected during three sessions in 2023: on July 18 (first session), September 27 (second session) and December 5 (third session), and during two sessions in 2024: on March 6 (fourth session) and June 4 (fifth session). Ejaculates were macro- and microscopically assessed immediately after collection. The obtained semen volume (VOL) and sperm concentration (CONC) were evaluated. For sperm motility analysis, the Axio Scope A1 microscope (Carl Zeiss, München, Germany) and the Androvision computer program (Minitube, Tiefenbach, Germany) were used, evaluating total motility (TMOT), or progressive motility (PMOT). Ejaculates that passed minimal initial qualitative and quantitative thresholds of the insemination centre were extended with OptiXcell extender (IMV Technologies, L'Aigle, France) according to the manufacturer's instructions. Extended semen samples were transported to the laboratory for flow cytometry analysis below 25 °C (transportation time 1 hour). Figure 1 shows descriptive quantitative and qualitative indices of biological samples in native and diluted states. Indices obtained in the present study are quite similar to indices previously reported for semen production in Holstein Friesian bulls kept in a commercial artificial insemination centre (Murphy et al. 2018).

Sperm flow cytometry

For flow cytometry analysis, Hoechst 33342 (H342) and propidium iodide (PI) were purchased from Sigma Aldrich (St. Louis, MO, USA), while lectin FITC-PNA from *Arachis hypogaea* (PNA) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Sperm samples were subjected to the flow cytometry analysis immediately after transportation. The samples were diluted in PBS containing appropriate dyes to achieve a final concentration of 20×10^6 spermatozoa/m. For assessment of basic qualitative parameters, the samples were stained in the dark at 38 °C for 10 min with the following fluorescent dyes, each at its respective final

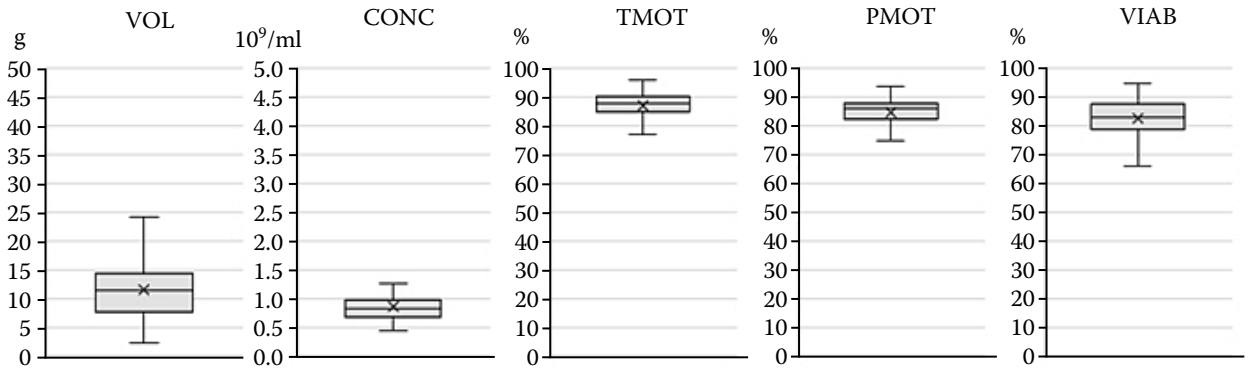

Figure 1. The box and whisker plots represent the descriptive quantitative and qualitative parameters of sperm production measured for all bulls during the entire observation period of the study

The "x" symbol represents the average, and the horizontal line inside the box represents the median

CONC = sperm concentration (10^9/ml); PMOT = progressive sperm motility (%); TMOT = total sperm motility (%); VIAB = sperm viability (%); VOL = semen volume (g)

https://doi.org/10.17221/21/2025-CJAS

concentration: 10 µg/ml H342 for DNA content identification, 8 µg/ml PI to detect damage to the plasma membrane, 0.5 µg/ml PNA for acrosome damage assessment. The evaluation was performed using a NovoCyte 3000 digital flow cytometer (Acea Biosciences/Agilent Technologies, Santa Clara, CA, USA). The flow cytometer was equipped with a set of optimal bandpass filters and solid-state lasers: a violet laser (405 nm, 50 mW) for exciting H342 and a blue laser (488 nm, 60 mW) for exciting PI and PNA. Before the sample analysis, the instrument was calibrated using calibration beads (NovoCyte, QC Particles; Agilent Technologies, Santa Clara, CA, USA). The samples were sat a low speed and a minimum of 10 000 events identified as sperm cells were evaluated from each sample. For automated cytometer setup, performance tracking, and data acquisition, NovoExpress software, v1.3.0 (Acea Biosciences, Agilent, Santa Clara, CA, USA) was used. The data were saved and subsequently analysed using the same software. No compensation was required for the optical filter settings used. The principle gating strategy reported previously (Savvulidi et al. 2021; Vasicek et al. 2022) was followed. The subpopulation of viable spermatozoa ($H342^+/PI^-/PNA^-$; VIAB) was identified.

Recording climatic parameters inside the facility with animals

For permanent recording of the climatic parameters (temperature and relative humidity) inside the facility with animals, the data logger Testo 175 series (Testo, Titisee-Neustadt, Germany) was used. The data logger was installed inside the facility on the wall at a two-meter height above the floor surface. Climatic parameters were acquired every 15 min (observation points). The Testo ComSoft Basic software was used to access climatic data stored in the data logger memory.

Temperature-humidity index calculation

The mean temperature-humidity index (THI) was calculated as previously reported (Garcia-Ispierto et al. 2006):

$$MeanTHI = 0.8 \times MeanT + \left(\frac{MeanRH}{100}\right) \times \left(MeanT - 14.4\right) + 46.4 \quad (1)$$

where:

Mean THI – mean temperature-humidity index;
MeanT – mean temperature (°C);
MeanRH – mean relative humidity.

THI was calculated on the day of semen collection (THI-0), as well as on the day preceding 30 days before (THI-30; time-point for late spermatogenesis), and on the day preceding 60 days before the semen collection (THI-60; time-point for early spermatogenesis). Additionally, the number of days with THI >70 was calculated per one month (d > 70/1 m) and per two months (d > 70/2 m) preceding the semen collection.

Statistics

The statistical analyses were performed with the SAS software v9.4 (SAS/STAT®; SAS Institute, Inc. Cary, NC, USA). The descriptive statistics were calculated using the UNIVARIATE procedure. Pearson correlation coefficients to measure the tightness of the relationship between the analysed variables were determined using the CORR procedure. The correlation coefficients were evaluated as follows: 0.3 to 0.5 (–0.3 to –0.5) fair; 0.5 to 0.8 (–0.5 to –0.8) strong; at least 0.8 (–0.8) very strong. Values of the correlation coefficient between 0 and +0.3/–0.3 were indicated as a weak correlation regardless of the significance level. The significance level for the purpose of this study was set at $P < 0.05$.

RESULTS

Fluctuation of THI during the observation period

Based on the objectives of this study, we first assessed the fluctuation of climatic parameters (temperature-humidity index) inside the facility with animals in different seasons of the year. The results are represented graphically in Figure 2.

As can be seen from the graph of fluctuations, the index value was below 70 for most of the observation period. The average value for the entire obser-

https://doi.org/10.17221/21/2025-CJAS

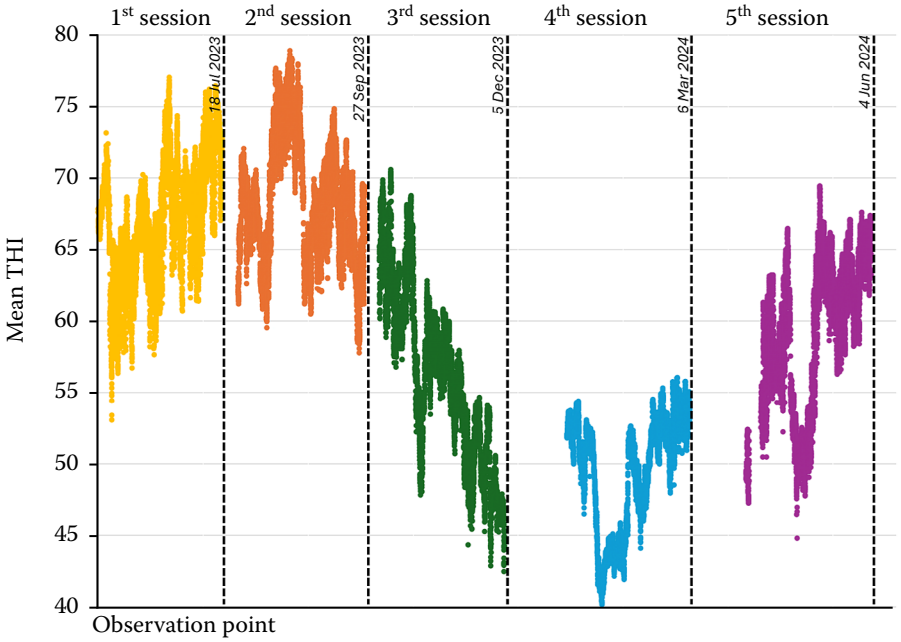

Figure 2. Fluctuations of the temperature-humidity index (THI) inside the bull-rearing facility during the observation period

Five ejaculate collection sessions during the year and THI fluctuations 60 days before each session are shown. The black dotted lines represent the semen collection time-points

vation period was 59.00; the min. THI was 40.15; the max. THI was 78.93; the median was 59.90. Additionally, the fluctuations of mean temperature (°C), and mean relative humidity (%) inside the facility with animals in different seasons of the year during five semen collection sessions are represented graphically in Figure 3.

Correlation between climatic conditions and sperm quantitative and qualitative parameters

In our study, we conducted a correlation analysis to identify correlations between temperature, relative humidity, and ejaculate characteristics. We did

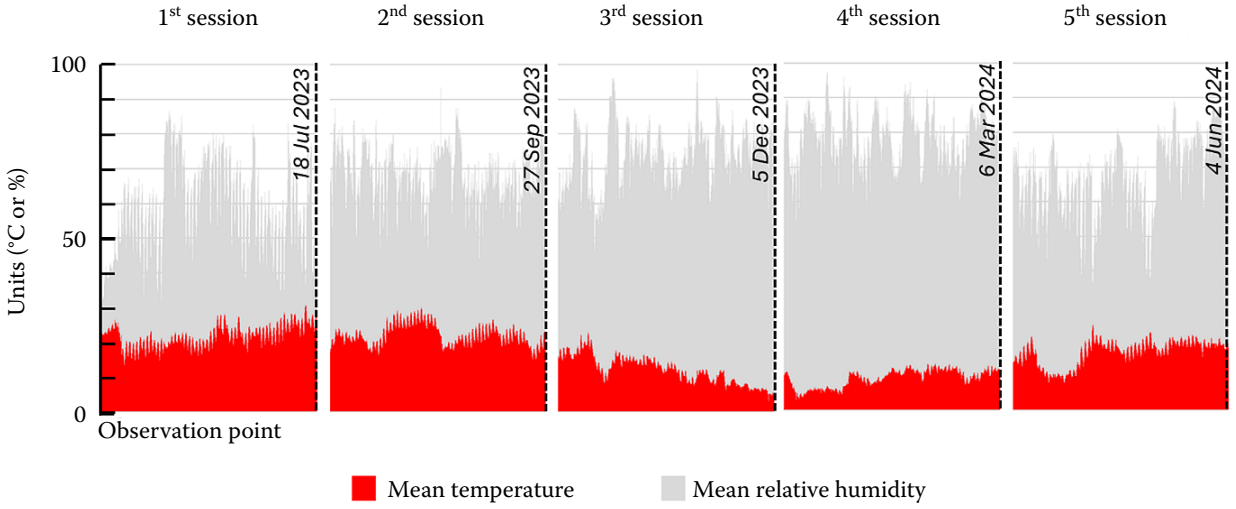

Figure 3. Fluctuations of mean temperature and mean relative humidity inside the bull-rearing facility during the observation period

Five ejaculate collection sessions during the year and fluctuations 60 days before each session are shown. The black dotted lines represent the semen collection time-points

https://doi.org/10.17221/21/2025-CJAS

not find any statistically significant correlations. Furthermore, bearing in mind the fact that climatic factors (temperature and humidity) do not affect the animals separately, but in combination, in our study we focused on a correlation between the climatic conditions (in the sense of THI measurements) on the one hand and the characteristics of the ejaculates on the other. The assessment was carried out at two levels. At the first level, we assessed the correlation between the climatic conditions measured on the day of obtaining the ejaculate, 30 days and 60 days before obtaining the ejaculate, and sperm quality. We assessed the influence of the climatic conditions on the origin, development, and maturation of spermatozoa and on mature spermatozoa. Weak, statistically insignificant correlations were found between microclimatic conditions and the characteristics of the obtained semen (Figure 4).

Of note is the trend that the THI value measured on the day of semen collection, 30 or 60 days prior to the day of semen collection correlated (albeit weakly) positively with semen volume and negatively with sperm concentration in the ejaculate. Further, a trend was found that THI values on the day of sperm collection and on the day preceding 30 days before sperm collection were negatively correlated with sperm motility, especially with total motility, and positively correlated with sperm viability. However, these particular correlations were at an extremely weak level ($r < 0.1$).

The second level of the analysis was devoted to the evaluation of correlations between the number of days with THI values above 70 during the entire period of spermatogenesis and sperm quality. In general, at the second level of the analysis for the parameters VOL, CONC, TMOT, PMOT, and VIAB, we observed trends similar to those de-

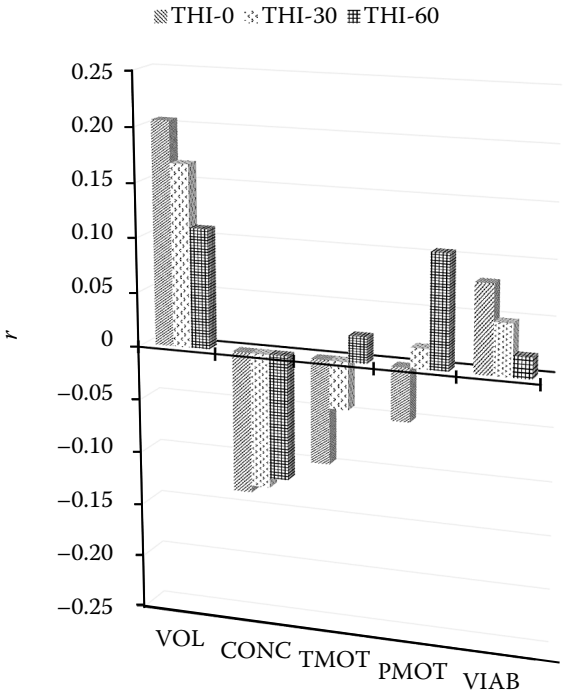

Figure 4. Correlations (r) between climatic conditions and quantitative and qualitative parameters of sperm
All observed correlations were above the significance level ($P > 0.05$) and therefore they were statistically insignificant
CONC = sperm concentration (10^9/ml); PMOT = progressive sperm motility (%); THI-0 = index calculated on the day of semen collection; THI-30 = index calculated on the day preceding 30 days before the semen collection; THI-60 = index calculated on the day preceding 60 days before the semen collection; TMOT = total sperm motility (%); VIAB = sperm viability (%); VOL = semen volume (g)

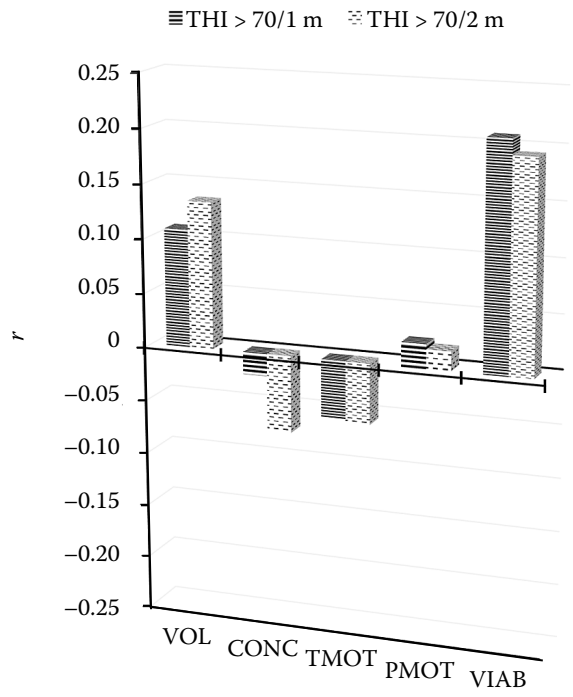

Figure 5. Correlations (r) between climatic conditions and qualitative and quantitative parameters of sperm
All observed correlations were above the significance level ($P > 0.05$) and therefore they were statistically insignificant
CONC = sperm concentration (10^9/ml); THI > 70/1 m = number of days with THI >70 per one month preceding the semen collection; THI > 70/2 m = number of days with THI >70 per two months preceding the semen collection; PMOT = progressive sperm motility (%); TMOT = total sperm motility (%); VIAB = sperm viability (%); VOL = semen volume (g)

https://doi.org/10.17221/21/2025-CJAS

scribed for the first level. Interestingly, a positive, albeit again weak (r = 0.194–0.208) correlation was observed between the number of days with THI >70 during spermatogenesis and the percentage of viable sperm (Figure 5). All observed correlations were above the significance level (insignificant).

DISCUSSION

In the last decade, the problem of an alarming increase of air temperature in the European region (WMO 2023) has attracted close attention from the wide audience, including livestock breeders. Previous studies have shown that heat stress can lead to a decrease in both the productive and reproductive performance of animals, including cows and bulls (Garcia-Ispierto et al. 2006; Llamas-Luceno et al. 2020). It is known that heat stress occurs in bulls at high values of the temperature-humidity index (THI). There is no consensus on the exact threshold for heat stress in bulls. According to most literature sources, the threshold value of THI >80 is supposed to be the danger of severe heat stress, when declines in semen quality and reproductive performance are very likely (Al-Kanaan et al. 2015; Llamas-Luceno et al. 2020). However, more recent studies suggest that heat stress can already occur at lower THI values (Pinto et al. 2020; Capela et al. 2022). These discrepancies in the literature prompted us to conduct the study in which we investigated the issue of seasonal fluctuations in sperm quality of Czech Holstein bulls kept indoors without forced air conditioning in the current climate. We assessed seasonal fluctuations in sperm quality depending on microclimatic factors. The median THI took a relatively low value of 59.9, which is obviously due to no more than 10% of the total number of days included in this study with THI >70. From this observation, it can be concluded that the animals in our study were not exposed to severe heat stress. In the present study, a positive (albeit weak) correlation was found between the THI value on the day of sperm collection, 30 and 60 days prior to sperm collection and semen volume, whereas interestingly, the correlation between THI values and sperm concentration was negative. It is known that more voluminous ejaculates diluted by accessory gland secretions often show lower sperm concentrations (Murphy 1967). As for the qualitative characteristics of sperm, we observed that the THI values on the day of ejaculate collections were negatively correlated with sperm motility. We assume that mature sperm stored in the epididymis are most susceptible to the negative effects of extreme high temperature-humidity environmental conditions. It should be noted here that despite the significant number (seventeen) of bulls used in our study, which allows us to state that the results of our study are based indeed on a large sample, it turned out that all the described correlation relationships between THI on the day of sperm collection, 30 or 60 days prior to sperm collection, on the one hand, and the quantitative and qualitative parameters of sperm, on the other, were statistically insignificant at the level of $P < 0.05$.

A similar situation was observed when assessing the correlation between the number of days with THI over 70 during the early and late stages of spermatogenesis and the quantitative and qualitative parameters of sperm. Here we were able to determine a positive correlation (albeit again only numerical; with no statistical significance at the level of $P < 0.05$) between the number of days with THI over 70 on the one hand and the number of viable sperm on the other. This observation, coupled with the absence of any negative effect of the high THI value on the day of ejaculate collection, 30 days, or 60 days preceding the day of ejaculate collection on VIAB parameter, is counterintuitive only at a first glance.

One should not lose sight of the fact that in our study the THI with a value >80 was not observed during the entire period of observation (all THI values recorded in the present study were below the severe heat stress threshold reported for bulls, and therefore our animals were not definitely under severe heat stress during the study), and also that it was already shown previously that bulls of some breeds produce the highest quality semen in the summer (hottest) season under European conditions (Filipcik et al. 2023). This may be explained in some part by the grazing nature of taurine cattle (i.e., the mating season should optimally fall in the summer so that the offspring will be born in the spring when plenty of plant material is available), notwithstanding the fact, that cattle are cycling, and bulls are producing sperm all the year round. Here it is essential to add that the studies of seasonal variation of sperm quality in Holstein bulls are yet scarce. Our study, in our opinion, brings new knowledge to this issue.

https://doi.org/10.17221/21/2025-CJAS

CONCLUSION

The most important conclusion that can be drawn from the results we have obtained in our study is that Holstein bulls can be kept indoors without any forced ventilation or air conditioning in the conditions of the current climate of the Czech Republic, without detectable adverse effect on their sperm quality. We were not able to detect THI >80 during the entire period of observation (including the hottest months of the year). Moreover, in the present study no statistically significant positive correlations between high values of THI and sperm degradation were observed. We concluded that the obtained preliminary results give grounds to believe that Czech Holstein bulls produced high quality sperm during the summer, the hottest months of the year. To clarify and generalise the obtained results, further parallel studies should be conducted in several breeding centres of the Czech Republic, or better in different Central European countries. In addition, in our opinion, further studies assessing the influence of microclimatic conditions on the quality of frozen doses made from the ejaculates of Holstein bulls kept in the Central European region will be of considerable future interest.

Acknowledgement

The authors thank Professor Jane Morrell (Swedish University of Agricultural Sciences, Uppsala, Sweden) and Professor Marek Petřivalský (Palacký University Olomouc, Olomouc, Czech Republic) for reading the manuscript and for valuable comments on the text and research. The authors express gratitude to management authorities and to the personnel of the commercial breeding station for troubles-free assistance in carrying out this study.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Al-Kanaan A, Konig S, Brugemann K. Effects of heat stress on semen characteristics of Holstein bulls estimated on a continuous phenotypic and genetic scale. Livestock Sci. 2015 Apr 2;177:15-24.

Beran J, Stadnik L, Duchacek J, Okrouhla M, Dolezalova M, Kadlecova V, Ptacek M. Relationships among the cervical mucus urea and acetone, accuracy of insemination timing, and sperm survival in Holstein cows. Anim Reprod Sci. 2013 Nov 1;142(1-2):28-34.

Bezdicek J, Nesvadbova A, Makarevich A, Kubovicova E. Negative impact of heat stress on reproduction in cows: Animal husbandry and biotechnological viewpoints: A review. Czech J Anim Sci. 2021 Jul 28;66(8):293-301.

Bezdicek J, Nesvadbova A, Duchacek J, Sekaninova J, Stadnik L, Janku M. Changes in the oxidative–biochemical status in dairy cows during the transition period affecting reproductive and health parameters. Czech J Anim Sci. 2024 Sep 30;69(9):345-55.

Boudaoud A. Timed artificial insemination protocols in dairy cattle: Functioning, shortcomings, and improvements. Czech J Anim Sci. 2023 Jun 14;68:231-44.

Capela L, Leites I, Romao R, Lopes-da-Costa L, Pereira RMLN. Impact of heat stress on bovine sperm quality and competence. Animals (Basel). 2022 Apr 9;12(8):975.

Chen X, Li Y, Xiao J, Dong J, Zhao W, Han Z, Xin L, Qin G, Wang T, Zhen Y, Sun Z, Zhang X. Heat stress affects the milk yield, milk composition, serum oxidative status, and metabolites of Holstein cows during mid-lactation. Czech J Anim Sci. 2023 Aug 21;68(8):333-45.

Filipcik R, Reckova Z, Pesan V, Konoval O, Kopec T. Evaluation of semen parameters from Fleckvieh-Simmental bulls and the influence of age and season of collection. Arch Anim Breed. 2023 Mar 2;66(1):113-20.

Garcia-Ispierto I, Lopez-Gatius F, Santolaria P, Yaniz JL, Nogareda C, Lopez-Bejar M, De Rensis F. Relationship between heat stress during the peri-implantation period and early fetal loss in dairy cattle. Theriogenology. 2006 Mar 1;65(4):799-807.

Gloria A, Candeloro L, Wegher L, Robbe D, Carluccio A, Contri A. Environmental temperature and relative humidity differently affect the sperm characteristics in Brown Swiss and Belgian Blue bulls. Int J Biometeorol. 2021 Dec;65(12):2189-99.

Grba J, Kuzelova L, Makarevich A, Balazi A, Dragin S, Tekic D, Chrenek P. The effect of ellagic acid on rabbit sperm in vitro parameters after cryopreservation. Czech J Anim Sci. 2024 Mar 7;69:110-7.

Kasna E, Zavadilova L, Krupova Z, Slosarkova S, Fleischer P. The most common reproductive disorders of cows in Holstein cattle breeding. Czech J Anim Sci. 2023 Dec 1;68(11): 433-42.

Kulaz E, Ser G. A meta-analysis of heat stress in dairy cattle: The increase in temperature humidity index affects

https://doi.org/10.17221/21/2025-CJAS

both milk yield and some physiological parameters. Czech J Anim Sci. 2022 Jun 29;67(6):209-17.

Llamas-Luceno N, Hostens M, Mullaart E, Broekhuijse M, Lonergan P, Van Soom A. High temperature-humidity index compromises sperm quality and fertility of Holstein bulls in temperate climates. J Dairy Sci. 2020 Oct;103(10): 9502-14.

Morrell JM. Heat stress and bull fertility. Theriogenology. 2020 Sep 1;153:62-7.

Murphy DP. The volume of the semen. A study of 3 544 childless men. Fertil Steril. 1967 Jan-Feb;18(1):124-6.

Murphy EM, Kelly AK, O'Meara C, Eivers B, Lonergan P, Fair S. Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre. J Anim Sci. 2018 Jun 4; 96(6):2408-18.

Park S. Application strategy for sustainable livestock production with farm animal algorithms in response to climate change up to 2050: A review. Czech J Anim Sci. 2022 Nov 30;67(11):425-41.

Pinto S, Hoffmann G, Ammon C, Amon T. Critical THI thresholds based on the physiological parameters of lactating dairy cows. J Therm Biol. 2020 Jan 22;88:102523.

Pytlik J, Codl R, Duchacek J, Savvulidi FG, Vrhel M, Stadnik L. Low-density lipoprotein supplementation improves the quality of Holstein bulls' insemination doses. Czech J Anim Sci. 2023 Feb 23;68(2):64-71.

Pytlik J, Savvulidi FG, Duchacek J, Codl R, Vrhel M, Nagy S, Stadnik L. Effect of extender on the quality and incubation resilience of cryopreserved Holstein bull semen. Czech J Anim Sci. 2022 Mar 28;67(3):75-86.

Sabes-Alsina M, Lundeheim N, Johannisson A, Lopez-Bejar M, Morrell JM. Relationships between climate and sperm quality in dairy bull semen: A retrospective analysis. J Dairy Sci. 2019 Jun;102(6):5623-33.

Savvulidi FG, Ptacek M, Malkova A, Beranek J, Stadnik L. Optimizing the conventional method of sperm freezing in liquid nitrogen vapour for Wallachian sheep conservation program. Czech J Anim Sci. 2021 Feb 15;66(2):55-64.

Savvulidi FG, Ptacek M, Nagy S, Stadnik L. Meta-analysis of fluorochrome concentrations to assess bull sperm quality by flow cytometry in 2021–2023. Czech J Anim Sci. 2023 Dec 20;68(12):469-85.

Schumacher DL, Singh J, Hauser M, Fischer EM, Wild M, Seneviratne SI. Exacerbated summer European warming not captured by climate models neglecting long-term aerosol changes. Commun Earth Environ. 2024 Apr 6; 5(1):59.

Vanselow J, Wesenauer C, Eggert A, Sharma A, Becker F. Summer heat during spermatogenesis reduces in vitro blastocyst rates and affects sperm quality of next generation bulls. Andrology. 2024 Nov;12(8):1897-906.

Vasicek J, Balazi A, Svoradova A, Vozaf J, Dujickova L, Makarevich AV, Bauer M, Chrenek P. Comprehensive flow-cytometric quality assessment of ram sperm intended for gene banking using standard and novel fertility biomarkers. Int J Mol Sci. 2022 May 25;23(11):5920.

Vinet A, Mattalia S, Vallee R, Bertrand C, Barbat A, Promp J, Cuyabano BCD, Boichard D. Effect of temperature-humidity index on the evolution of trade-offs between fertility and production in dairy cattle. Genet Sel Evol. 2024 Mar 29;56(1):23.

Wang L, Wang L, Li Y, Wang J. A century-long analysis of global warming and earth temperature using a random walk with drift approach. Decis Anal J. 2023 Apr 28;7: 100237.

WMO – World Meteorological Organization. State of the Climate in Europe 2022. No. 1320. Geneva, Switzerland: WMO Press; 2023. 40 p.

Zahradnicek P, Brazdil R, Rehor J, Lhotka O, Dobrovolny P, Stepanek P, Trnka M. Temperature extremes and circulation types in the Czech Republic, 1961–2020. Int J Climatol. 2022 Jan 31;42(9):4808-29.

Zeder J, Fischer EM. Observed extreme precipitation trends and scaling in Central Europe. Weather Clim Extrem. 2020 Jun 16;29(11-12):100266.

Received: February 13, 2025
Accepted: March 4, 2025
Published online: April 9, 2025