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EFFECTS OF CLIMATIC CONDITIONS ON BOVINE SEMEN CHARACTERISTICS

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Abstract

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The present study assessed the effects of climatic conditions and year-period (month) on both qualitative and quantitative characteristics of bovine semen in Czech Republic. The semen was collected twice a week using artificial vagina at artificial insemination center Hradištko in period 2011–2013. The effects of collection month, average daily ambient temperature and average daily relative humidity on volume, sperm concentration and progressive motility above head of collected semen and also on number of produced artificial insemination straws of required quality were analyzed using statistical analysis (procedures: UNIVARIATE, STEPWISE, GLM) in software SAS 9.3 (SAS/STAT® 9.3, 2011). Collection month, average daily ambient temperature, average daily relative humidity, and breed had a significant effect ($P < 0.05$) on all evaluated characteristics. The maximum values of motility and sperm concentration were found in March and in April. The highest volume of collected semen was found in July and in September and the lowest in November, when also the lowest number of straws was produced. Motility and sperm concentration were significantly influenced by average daily ambient temperature and average daily relative humidity, where the maximum values were recorded in average daily ambient temperature over 14.85 °C and average daily relative humidity over 72.05 %. The results are important to the management of artificial insemination center responsible for planning of semen collection, processing and artificial insemination straws production.

Keywords: temperature, humidity, motility, sperm concentration, volume

INTRODUCTION

Correlations between characteristics of fresh bovine semen and conception rate of cows inseminated by frozen-thawed semen have been described many decades ago (Linford *et al.,* 1976). These characteristics are known to be affected by both internal and external factors. Breed, genotype, individual traits and age of the bull are mentioned by many authors as important internal factors affecting the semen quality (Hafez and Hafez, 2000; Bryan *et al.,* 2005; Fuertes-Waltl *et al.,* 2006; Hofírek 2009; Beran *et al.,* 2011, Beran *et al.,* 2012; Stádník *et al.,* 2015), while climate, season and especially ambient temperature, relative humidity and daylight period belong to the most important external factors (Hofírek 2009; Bhakat *et al.,* 2014, Stadnik *et al.,* 2014; Alragubi, 2015). Ball and Peters (2004) reported that sperm characteristics are not affected only by the ambient temperature at the time of semen collection, but also during the sperm maturation in epididymis and during spermatogenesis, namely about 70 days prior the collection. The seasonal variations in semen characteristics have been described in many studies (Graffer *et al.,* 1998; Haugan *et al,* 2005; Sarder 2007; Argov-Argaman *et al.,* 2013; Snoj *et al.,* 2013; Bhakat *et al.,* 2014) as well as the effect of the month of collection (Pileckas *et al.*, 2013). However, these studies were conducted mostly, except study from Snoj *et al.* (2013) who collect bull semen in central region Slovenia, under tropical and subtropical climates or under daylight conditions differing from those prevailing in Central Europe. Disorders of thermoregulatory mechanisms and increase of the body internal temperatures above the physiological bounds were found in conditions of high ambient temperatures and relative humidity (Nardone *et al.*, 2010; Alragubi 2015), which prevail in these climates. These disorders, known as heat stress, have a negative impact on quality of bovine semen (Mathevon *et al.*, 1998; Nichi *et al.* 2006), on cow conception rate and embryonal mortality (Hansen *et al.*, 2007). Climate change includes increase of average temperatures and in other parameters of weather. These changes induce biophysical responses of organisms and it is expected that they will affect agriculture production in both, positive and negative ways (Parry *et al.*, 2004).

All above mentioned studies assessing the effect of season and climate conditions were focused on investigation of sperm characteristics in only one cattle breed, except Sarder (2007). However, there are usually kept more than one breed in most artificial insemination (A.I.) centers. Considering climate change, a new research of effects of climatic conditions is desirable. Therefore, objective of this study was to evaluate effect of collection month and climatic conditions represented by average ambient temperature and average relative humidity on parameters of sperm quality.

MATERIALS AND METHODS

Collection and Semen Samples

The study was conducted at artificial insemination (A.I.) center Hradištko, NATURAL, Czech Republic in the period 2011–2013. For this purpose, total number of 3064 semen samples from 29 bulls (8 Holstein, 5 Dairy Simmental, 4 Aberdeen Angus, 4 Limousine, 4 Beef Simmental and 4 Belgian Blue) was collected. The donor bulls were maintained in identical and optimal conditions of feeding, watering and management during the entire period. The bulls were healthy, free from diseases, sexually mature, with good libido and clinically normal.

Ejaculates from bulls were collected two times a week by the same A.I. technician using sterilized bovine artificial vagina warmed to 38 °C. Ejaculate was collected from each bull two times in a day of collection. The same artificial vagina set was used for both collections. Each semen bag with two ejaculates was placed in the laboratory (room temperature) immediately after the semen collection. The semen quality analysis was accomplished up to 10 minutes after the collection. All measurements were performed by professional lab technician in accordance to the Semen evaluation protocol of A.I. center Natural.

Sperm quality characteristics

Volume of the ejaculate was measured using calibrated laboratory scales, considering 1 g = 1 ml. Spermatozoa concentration (million/ml) in fresh semen was measured on regularly calibrated photometer (Carl-Zeiss Jena, Oberköchen, Germany). To sperm forward motility (forward progressive motility in direction of head) (motility) evaluation, a small drop of fresh semen was diluted in 0.5 ml saline solution (0.9 % NaCl solution) and incubated in water bath 39 °C for 3 minutes. Thereafter, a small droplet was placed on clean, pre-warmed slide and covered with a pre-warmed cover slip and examined at a magnification of 100 = under light microscope. Sperm motility was scored as a percentage of spermatozoa with normal progressive movement and it was estimated by one trained laboratory person. Sperms showing circle or oscillating movement were considered as immotile.

Semen processing and A.I. straws production

Only ejaculates showing minimum concentration $0.7 = 10^9$ sperms per mm^3 and minimum forward motility (motility) 70 % were processed. These ejaculates were diluted with Andromed extender (Minitübe, Tiefenbach, Germany) pre-warmed to 38 °C in wather bath to final concentration $33 = 10^9$ sperms per straw. Semen was filled in ministraws (0.25 ml) using IMV (L'Aigle, France) filling and sealing machine, placed on racks, cooled and equilibrated for 2 hours in 5 °C and frozen in DigitCool freezer (IMV, L'Aigle, France) to −140 °C and then placed into bath with Liquid Nitrogen (−196 °C). Two samples from each batch (n = 2556) were thawed in water bath (40 °C, 40 seconds) and evaluated exactly the same way as the fresh semen. Only samples with minimum motility 40 % were accepted (n = 2256). Batches with lower motility were disposed.

Area and climatic conditions

The A.I. center Hradištko is situated in altitude 285 meters above the mean sea. Records about climatic conditions were obtained from the Czech Hydrometeorogical Institute (available on: www.chmi.cz). The records show average daily climatic characteristics as average daily ambient temperature (TEM) and average daily relative humidity (HUM) in particular months during the period of this study.

For a purpose of TEM effect evaluation, limits of temperature < 7.24 °C; 7.24–14.85 °C and < 14.85 °C were determined based on average temperature during the whole period of this study (11.04 ± 7.61 °C). The same model was used in effect of HUM evaluation. Average HUM in the whole period was 68.15 ± 7.80 %. Based on average HUM, groups < 64.25 %; 64.25–72.05 %; > 72.05 % were determined with the aim of effect of HUM evaluation.

Statistical analyses

The recorded data were subjected to statistical analyses using software SAS 9.3 (SAS/STAT® 9.3, 2011). Procedures MEANS and UNIVARIATE were used to the basic characteristics calculation. Descriptive characteristics for semen traits, temperature and relative humidity used in this study are shown in Tab. I. Procedure REG, method STEPWISE was used to select an appropriate model for evaluation. The differences between animals and groups were evaluated by GLM, followed by a detailed evaluation using the Tukey-Kramer test. Total numbers of 29 bulls were included into the evaluation (8 Holstein, 5 Dairy Simmental, 16 beef breeds: 4 Limousine, 4 Belgian Blue, 4 Aberdeen Angus, 4 Beef Simmental). Model equation was following:

$$Y_{ijklmno} = \mu + BREED_i + AGE_j + MONTH_k + YEAR_l + TEM_m + HUM_n + e_{ijklmno}$$

where:

$Y_{ijklmno}$ – dependent variable (motility, density, volume, number of straws),

μ – mean value of dependent variable,

$BREED_i$ – fixed effect of breeding purpose (i = dairy – Holstein, n = 569; i = double purpose – Dairy Simmental, n = 243; i = beef breeds – Aberdeen Angus, Belgian Blue, Limousine, Beef Simmental, n = 2253),

AGE_j – fixed effect of bull age at months (j < 25, n = 64; j = 25 – 36, n = 667; j = 37–48, n = 310; j = 49 – 60, n = 357; j = 61 – 72, n = 357; j = 73 – 84, n = 297; j = 85 – 96, n = 145; j = 97 – 108, n = 71; j = 121 – 132, n = 32; j = 133 – 144, n = 101; j > 144, n = 64),

$MONTH_k$ – fixed effect of month of collection (k = 1 – January, n = 258; k = 2 – February, n = 291; k = 3 – March, n = 322; k = 4 – April, n = 310; k = 5 – May, n = 224; k = 6 – June, n = 304; k = 7 – July, n = 273; k = 8 – August, n = 202; k = 9 – September, n = 197; k = 10 – October, n = 249; k = 11 – November, n = 252; k = 12 – December, n = 183),

$YEAR_l$ – fixed effect of year of collection (l = 2011, n = 1268; l = 2012, n = 1192; l = 2013, n = 605),

TEM_m – fixed effect of groups of daily average ambient temperature (m = 1 < 7.24 °C, n = 1150; m = 7.24 – 14.85 °C, n = 798; m = > 14.85 °C, n = 1116),

HUM_n is fixed effect of groups of daily average relative humidity (n = 1 < 64.25 %, n = 1295; n = 64.25 – 72.05 %, n = 687; n = > 72.05 %, n = 1082),

$e_{ijklmno}$ – random error.

Significance levels P < 0.05, P < 0.01 and P < 0.001 were used to evaluate differences between groups.

RESULTS

In this study, an average motility 78.11 ± 16.91 %, average sperm concentration 1.03 ± 0.41 = 10^9 sperms per mm³, average volume 8.88 ± 3.32 ml and average number of produced straws per bull 424.33 were detected. The effects of CM, TEM and HUM on qualitative and quantitative characteristics of bull semen are presented in Tabs. II–IV.

Tab. II shows that collection month (CM) had significant effect (P < 0.05) on all characteristics of bull semen in this study. The maximum motility and sperm concentration were found in March and April (89.2 ± 2.17 % and 89.8 ± 2.21 %; respectively 1.68 ± 0.06 and 1.68 ± 0.06 × 10^9 sperms per mm³) and, in contrary, the lowest ones in August and September (69.9 ± 1.83 % and 70.3 ± 1.50 %; respectively 0.97 ± 0.05 and 0.97 ± 0.05 × 10^9 sperms per mm³). These differences have been statistically significant (P < 0.01) and characteristics were also different in other months (P < 0.05).

We observed the highest volumes of ejaculates collected in July and September which were significantly different from those collected in November, December, February and March (P < 0.05).

Number of produced straws is important factor to A.I. centers economic outcome. In spite of the fact that month of the semen collection had a significant effect on all qualitative and quantitative characteristics of fresh bull semen, we have found that there were only few significant differences between months in straws production, with peaks in January (599.1 ± 34.4) and May (604.8 ± 30.2), which significantly differs from November, December and June (478.8 ± 34.7; 522.2 ± 35.6 and 533.3 ± 31.9 respectively).

Motility and sperm concentration values were significantly highest in average ambient temperatures (TEM) > 14.85 °C (P < 0.01). But volume of ejaculates was insignificantly lowest in these temperatures, which was probably the reason of lowest production of straws in this temperature level. There was found none significant effect of TEM on volume and number of straws in this study (Tab. III).

The trend for the effect of average relative humidity (HUM) was similar to the trend of effect of temperature in this study (Tab. IV). Significantly highest values of motility (83.97 ± 1.01 %) and sperm concentration (1.52 ± 0.041 × 10^9 sperms per mm³) were recorded under conditions of higher relative humidity (> 72.05 %). Sperm motility significantly (P < 0.05) differed among HUM groups.

Volume and number of straws were insignificantly lower in group of HUM > 72.05 %. Therefore, we can expect that even slight changes of HUM have impact on sperm concentration.

The effect of breed on qualitative and quantitative parameters of ejaculates was confirmed in this study. We have found the highest motility (80.39 ± 0.90 %) in Holstein (dairy breed) compared to Dairy

Simmental (double purpose breed) and beef breeds. These differences have been statistically significant ($P < 0.05–0.01$). On the other hand, the highest sperm concentration and average volume of ejaculate ($1.27 \pm 0.03 \times 10^9$ sperms per mm^3, resp. 10.36 ± 0.023 ml; $P < 0.05 – 0.01$) as well as the highest number of straws produced per bull (606.57 ± 19.47; $P < 0.01$) were observed in Dairy Simmental. Results are shown in Tab. V.

DISCUSSION

These results for factor CM are consistent with findings from Germany, where the climatic conditions are similar to the Czech Republic (Al-Kanaan *et al.,* 2015). We can also partially agree with Pileckas *et al.* (2013) who reported in their study conducted in Lithuania (similar climate) significantly highest motility of bull sperms in March, while recorded significantly lowest sperm concentration in the same month. This difference may be explained by different breed of donor bulls (Lithuanian Black and White). Fiaz *et al.* (2009) reported that bulls of different breeds produce semen of different qualitative and quantitative parameters in different seasons. They explain it by different tolerance ability against environmental stress. Valeanu *et al.* (2015) found no significant differences in motility of bull sperms between seasons, while other authors report significantly better characteristics of bull semen in different seasons. Winter season was stated as the best by Argov-Argaman *et al.* (2013); Bhakat *et al.* (2014) and Alragubi (2015), summer season by Sarder (2007) and Snoj *et al.* (2013). Differences can be explained by different climatic conditions in followed studies. The negative effect of summer season on sperm quality and quantity mentioned in some studies could be explained by negative effect of heat stress. High ambient temperatures and relative humidity disrupt thermoregulatory mechanisms and the body temperature increases over physiological limits consequently (Nardone 2010). Bulls have an ability to compensate changes of temperatures from 2.1 to 21.6 °C effectively and without distinct changes in sperm production. Long-term temperatures over 22 °C cause degenerative changes of sperm epithelium and metabolism are decreasing

I: *Descriptive characteristics for semen traits, temperature and relative humidity used in this study*

characteristic	units	n	$\bar{x}$	s	min.	max.	s.e.	V (%)
MOT	%	3003	78.11	16.91	10	95	0.31	21.65
CON	mil/mm^3	3059	1.03	0.41	0.1	2.4	0.01	40.05
VOL	ml	3062	8.88	3.32	1	23.5	0.06	37.38
NS	pcs	2256	424.33	258.60	57	1751	5.44	60.94
TEM	°C	3064	11.04	7.61	–2.3	27	0.14	68.92
HUM	%	3064	68.15	7.80	56.7	81.2	0.14	11.44

MOT – motility; CON – sperm concentration; VOL – volume; NS – number of straws; TEM – average ambient temperature; HUM – average relative humidity; n – number; – arithmetic means; s – standard deviation; min. – minimal value; max. – maximal value; s.e. – standard error of the arithmetic means; V – variance coefficient.

II: *Effect of collection month (CM) on motility, concentration of sperms, volume of collected ejaculate and number of produced straws of required quality*

CM	motility (%)	sperm concentration ($\times 10^9$ sperms per mm^3)	volume (ml)	number of straws
	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
January	$78,83 \pm 2,067^{A,a}$	$1,48 \pm 0,052^{a}$	$10,12 \pm 0,397^{A}$	$599,09 \pm 34,366^{A,a}$
February	$82,27 \pm 2,045^{C,c}$	$1,37 \pm 0,052^{c}$	$9,97 \pm 0,397^{a}$	549,94 ± 33,643
March	$89,17 \pm 2,172^{D,E,b}$	$1,68 \pm 0,055^{A,d,e}$	$9,19 \pm 0,418^{c}$	548,23 ± 35,358
April	$89,82 \pm 2,210^{G}$	$1,68 \pm 0,057^{C,g}$	10,06 ± 0,432	577,82 ± 36,370
May	$75,18 \pm 1,380^{F,H,e}$	$1,13 \pm 0,044^{E,i}$	$11,33 \pm 0,336^{C,b,d,e}$	$604,79 \pm 30,186^{c}$
June	$71,29 \pm 1,250^{F,H}$	$1,05 \pm 0,047^{D,f}$	$10,01 \pm 0,356^{g}$	$533,26 \pm 31,896^{d}$
July	$71,18 \pm 1,910^{F,H}$	$1,02 \pm 0,048^{B,D}$	$11,54 \pm 0,362^{E,i}$	568,63 ± 32,369
August	$69,89 \pm 1,830^{F,H,d,f}$	$0,97 \pm 0,050^{B,D,F}$	$11,05 \pm 0,376^{G}$	572,05 ± 33,806
September	$70,33 \pm 1,500^{F,H,d}$	$0,97 \pm 0,047^{B,D,b,j}$	$11,55 \pm 0,353^{I,b,d,k}$	580,77 ± 32,508
October	$74,42 \pm 1,780^{F,H}$	$1,12 \pm 0,043^{h}$	$9,49 \pm 0,323^{l}$	558,24 ± 28,651
November	$74,52 \pm 2,040^{F,H,d}$	1,17 ± 0,052	$8,76 \pm 0,393^{B,D,F,H,J,h}$	$478,76 \pm 34,671^{B}$
December	$71,72 \pm 2,125^{B,D,H,f}$	1,17 ± 0,054	$9,06 \pm 0,409^{J,f,j}$	$522,19 \pm 35,554^{b}$

CM – collection month; LSM – least square means; SELSM – standard error of last square means.
$^{A, B, C, D, E, F, G, H}$ – values in columns with different letters differ significantly ($P < 0.01$).
$^{a, b, c, d, e, f, g, h, i, j, k, l}$ – values in columns with different letters differ significantly ($P < 0.05$).

III: *Effect of average ambient temperature (TEM) on motility, concentration of sperms, volume of collected ejaculate and number of produced straws of required quality*

TEM (°C)	motility (%)	sperm concentration (× 10^9 sperms per mm^3)	volume (ml)	number of straws
	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
< 7.24	68.68 ± 1.470^{A}	1.08 ± 0.044	10.89 ± 0.337	582.69 ± 28.874
7.24–14.85	72.20 ± 1.500^{A}	1.07 ± 0.039^{a}	10.00 ± 0.292	557.91 ± 26.302
> 14.85	88.77 ± 2.076^{B}	1.64 ± 0.053^{b}	9.56 ± 0.399	532.85 ± 34.971

TEM – average ambient temperature; LSM – least square means; SELSM – standard error of last square means.
$^{A, B}$ – values in columns with different letters differ significantly ($P < 0.01$).
$^{a, b}$ – values in columns with different letters differ significantly ($P < 0.05$).

IV: *Effect average relative humidity (HUM) on motility, concentration of sperms, volume of collected ejaculate and number of produced straws of required quality*

HUM (%)	motility (%)	sperm concentration (× 10^9 sperms per mm^3)	volume (ml)	number of straws
	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
< 64.25	71.05 ± 1.300^{A}	1.05 ± 0.035^{A,a}	10.36 ± 0.266	560.80 ± 24.036
64.25–72.05	74.64 ± 1.110^{B}	1.14 ± 0.032^{B}	10.58 ± 0.243	561.65 ± 21.473
> 72.05	83.97 ± 1.010^{C}	1.52 ± 0.041^{b}	10.04 ± 0.307	550.99 ± 26.096

HUM – average relative humidity; LSM – least square means; SEM – standard error of last square means.
$^{A, B, C}$ – values in columns with different letters differ significantly ($P < 0.01$).
$^{a, b}$ – values in columns with different letters differ significantly ($P < 0.05$).

V: *Effect of breed on motility, concentration of sperms, volume of collected ejaculate and number of produced straws of required quality*

purpose of breeding	motility (%)	concentration (sperms mm^{-3})	volume (ml)	number of straws
	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
Dairy (Holstein)	80.39 ± 0.904^{A,a}	1.11 ± 0.023^{a}	10.14 ± 0.173^{A}	544.15 ± 15.464^{A}
Double purpose (Dairy Simental)	77.12 ± 1.188^{C,b}	1.27 ± 0.030^{b,c}	10.36 ± 0.226^{B,C}	606.57 ± 19.468^{B,C}
Beef breeds (Aberdeen Angus, Beef Simmental, Limousine and Belgian Blue)	72.15 ± 0.552^{B,D}	1.12 ± 0.014^{d}	9.60 ± 0.105^{B,D}	522.72 ± 11.820^{D}

$^{A, B, C}$ – values in columns with different letters differ significantly ($P < 0.01$).
$^{a, b}$ – values in columns with different letters differ significantly ($P < 0.05$).

at the same time. This stress results in decrease of gonadotropic hormones (Hofírek *et al.*, 2009). Oppositely to our study in Romania, the lowest volume of semen was observed in August (Igna *et al.*, 2010) in contrary to Pileckas (2013), who reported August as a month when the highest volume of semen had been gained. Also Snoj *et al.* (2013) confirmed the best results in volume and sperm concentration by milk and beef breeds in Slovenia rather in summer months. Sarder (2007), Bhakat (2014) and Alragubi (2015) found no significant difference in volume of ejaculates collected in different seasons. The minimum of differences in number of produced straws can be explained by lower sperm concentration and motility in ejaculates of higher volumes (comparing months June–September with other months). Therefore, continual production of similar numbers of straws per month is possible. Pileckas *et al.* (2013) also reported minimum significant differences in straws production in particular month, however their peak of the production was in August. It is obvious, that results of CM are important for management responsible for planning of any disruptions of semen processing, which would bring lower losses in summer months and the lowest in November. On the other hand, disruptions in winter and spring months are undesirable.

No effect of TEM on volume is consistent to results of Sarder (2007), who, on the other hand, found a significant difference in number of produced straws. Nevertheless, his study took place in Bangladesh where TEM groups were determined as follows: < 21 °C; 21–25 °C; 25–29 °C and > 29 °C. He also described significantly highest values of motility in the group of TEM 25–29 °C. Decrease of motility towards low TEM (< 7.24 °C) recorded in our study may be explained using knowledge of sperm cold shock mechanism. Cold shock, defined as permanent changes in sperms membrane and motility loss, occurs in quick cooling from 30 to 0 °C (Buhr *et al.*, 1989; Ghetler *et al.*, 2005).

The effect of HUM also influences sperms parameters. Higher motility of sperms in the conditions of higher relative humidity was also found in research of Stadnik (2014), who, on the other hand, reported that higher concentration of sperms was detected in the lowest relative humidity. Sarder (2007), who investigated effect of HUM using limits < 65 to> 85 %, detected similar results. However, he determined that significantly lowest sperm concentration occurred when HUM was from 65 to 75 %. Regarding the motility of sperms, our findings are different from Sarder (2007), who showed significantly highest values of motility in group < 65 %.

CONCLUSION

We can conclude that, results of this study clearly demonstrated that year-phase (collection month), daily average ambient temperatures and daily average ambient humidity have significant effect on semen characteristics of bulls used in A.I. programme. The month of bull semen collection had influenced many parameters studied. March and April were most favorable months considering the semen characteristics, while January and May considering the number of produced straws. Eventually, any interruption of semen collection plans in A.I center Hradištko shall be planned considering the climatic conditions of particular months. Finally, it is almost impossible to provide a uniform recommendation concerning semen processing interruptions valid worldwide due to different climate conditions of world regions.

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