SURVIVAL EFFECT OF YEAR PERIOD ON BULL SEMEN PROPERTIES

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Abstract. The objective of this study was to examine the effect of year period (month) on bull semen characteristics under the climatic conditions of Lithuania. The semen was collected, evaluated and frozen at 3 to 4 day intervals throughout the year. The semen was collected from Lithuanian Black-and White bulls of 4 years of age. The data were subjected to the analysis of variance using general linear (GLM) procedure. The model included the fixed effect of month. The month of semen collection affected the majority of studied semen parameters. The time of semen collection showed higher effect on the volume of the second than on the first ejaculates. The largest in volume ejaculates were collected in August. The highest values of spermatozoa motility for both ejaculates were obtained in March. The lowest motility in the first ejaculates was found in October and in the second ejaculates in December and August. The highest semen concentration was obtained in October and August in the first and second ejaculates, respectively. The tendency of semen concentration decrease was observed between February and May, and between August and March. However, semen concentration in November showed the increase tendency. The highest least square means for volumes suitable for freezing were in August, May and November. The volume of diluted semen in August was 56.3 ml and 59.6 ml, and in May it was 42.4 ml and 45.6 ml higher compared with February and January, respectively. This led to the result that least square mean number of frozen semen doses in August was by 215.5 higher compared with March. The motility in post-thawed semen from the collection in May was 49.4% vs. 41.7% in November. The time of semen collection did not affect the motility 5 h after semen thawing.

Keywords: semen, quality, collection time, bulls.

SKIRTINGO METŲ LAIKO ĮTAKA BULIŲ SPERMOS KOKYBINIAMS RODIKLIAMS

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Santrauka. Darbo tikslas buvo ištirti skirtingo metų laiko (mėnesių) įtaką bulių spermos kokybės rodikliams Lietuvos klimato sąlygomis. Spermos paėmimo laiko (mėnesio) įtakai, spermos kiekiui ir kokybiniams rodikliams nustatyti bulių sperma imta ir šaldyta ištisus metus kas 3–4 dienos. Kiekvieną mėnesį ištirta ir užšaldyta 1–18 ėmimų sperma iš trijų Lietuvos juodmargių veislės 4 metų bulių. Duomenų analizė atlikta taikant dispersinės analizės apibendrinamąjį linijinį modelį (GLM). Į modelį įtrauktas fiksuotas spermos ėmimo mėnesio faktorius kalendorinių metų laikotarpiu.

Tyrimai parodė, kad bulių spermos ėmimo laikas skirtingais metų mėnesiais didesnę įtaką darė antrųjų negu pirmųjų ejakuliatų tūriui. Didžiausio tūrio ejakuliatai buvo rugpjūčio mėnesį. Didžiausias abiejų ejakuliatų spermatozoidų judrumas nustatytas kovo mėnesį, kai jis ryškiausiai skyrėsi nuo spermatozoidų judrumo pirmuose spalio mėnesio ejakuliatuose ir gruodžio bei rugpjūčio mėnesių antruose. Spermos ėmimo mėnuo turėjo įtakos ir spermos koncentracijai, tačiau didžiausios koncentracijos pirmieji ejakuliatai buvo spalio, o antrieji – rugpjūčio mėnesiais. Spermos koncentracijos mažėjimo tendencija pastebėta tarp vasario ir gegužės bei kovo ir rugpjūčio mėnesių, o didėjimo – tarp kovo ir lapkričio. Didžiausias abiejų ejakuliatų, pripažintų tinkamais kriokonservuoti, vidutinis tūris buvo rugpjūčio, gegužės ir lapkričio mėnesiais. Praskiestos spermos rugpjūčio mėnesį gauta vidutiniškai 56,3 ml ir 59,6 ml daugiau, o gegužės mėnesį – 42,4 ml ir 45,6 ml daugiau, nei atitinkamai vasario ir sausio mėnesiais. Rugpjūčio mėnesį užšaldyta vidutiniškai 215,7 dozių daugiau nei kovo. Didžiausiu judrumu, nustatytu atšildžius kriokonservuotą spermą, išsiskyrė paimta gegužės mėnesį. Jos judrumas buvo 49,4 proc., tuo tarpu lapkričio mėnesį – 41,7 proc. Tačiau spermatozoidų judrumui, nustatytam praėjus 5 val. po kriokonservuotos spermos atšildymo, spermos paėmimo laiko įtaka nenustatyta.

Raktažodžiai: sperma, kokybiniai rodikliai, paėmimo laikas, buliai.

Introduction. The quality of bovine semen is mostly affected by individual qualities of the sire, animal feeding and housing conditions, bull preparation for semen collection, frequency of collections and the technological process of semen cryopreservation (Chenoweth et al., 1994; Ahmad et al., 203; Alm et al, 2002; Pileckas, 2006). Sire age is also a factor of importance (Brito et al., 2002; Younis et al., 1999). According to T. Haugan et al. (2005), cow conception is influenced by the specific time of the year when the semen is collected. However, Koivisto et al. (2009) and Hansen (2004) indicate that season might influence the quality of semen no more than by 2% and at different periods of the year the quality of semen might be dependent on the breed of the bull. The season might have the effect on the sperm production of both wild and domestic animals (Schopter et al., 1984).

The length of natural photoperiod is also a factor of importance on semen quality (Sancho et al., 2004). The effect of season is highly noticeable on boars. J. Šernienė et al. (1999) found that the highest intensity of boar spermatogenesis is in spring and the lowest in summer. This is in agreement with the findings of M. Mathevon et al. (1998), D.M. Vilikari and E.C. Webb (2004) and V.C. Sekoni and B.K. Gustafson (1987). Although L.F.C. Brito et al. (2002) indicated that ambient temperature and moisture had no significant influence on semen quality as bovine testis temperature is 4 to 6 ^{0}C lower than that of the body (Senger, 2003), yet P. Pakėnas (1993) found that at temperatures higher than 25 ^{0}C semen quality and fecundation power are impaired. There are breeding enterprises that do not collect semen in summer for 4 to 6 weeks, because later, when temperatures become lower, semen quality improves (Fuerst-Waltl et al., 2006).

Animals are bred in different climatic zones with not only temperature differences but also seasonal differences. In various countries, animals that are usually bred are well adapted to the environmental conditions. Literature survey indicated that studies are being carried our regarding seasonal effects on sperm physiological parameters of Brahman (Bos indicus) bulls (Chacon et al., 2002) or buffalos (Koonjaenak et al., 2007), also such wild animals as spotted deer (Axis axis) (Umapathy, 2007) and wild boars (Kozdrovski, Dubil, 2004; Werkerle et al., 1989). Moreover, the majority of studies are related with seasonal effects under tropical climatic conditions (Nichi et al., 2006) and only J. Chacon et al. (2002) investigated semen quality at different month intervals. So far there have been no studies of seasonal variations in semen quality under the climatic conditions of Lithuania.

The purpose of the study was to investigate the influence of different months on bovine semen characteristics under the climatic conditions of Lithuania.

Materials and Methods. The study was conducted at the Department of Animal Reproduction of the LHSU Institute of Animal Science and former joint-stock company "Marijampolės regiono veislininkystė". The semen was collected and frozen at 3 to 4 day intervals throughout the year. Three Lithuanian Black-and White bulls of four years of age were used in the trial for monthly collection, evaluation and freezing of the semen (from 16–18 collections).

Throughout the year, bull feeding was the same in order to avoid the influence of feeds on bull semen. The rations were composed of high quality legume-cereal hay and compound feeds containing oat meal, 58%, wheat meal or oats and crushed wheat grain, 22%, sunflower oil-meal, 18%, premix for breeding bulls, 1% and calcium monophosphate, 1%. The rations were additionally enriched with vitamins A, D, E by pouring on the compound feed individually for each bull twice a week according to the standard requirements. The bulls were fed twice daily (in the morning and evening) from individual troughs and given high quality water from constantly accessible automatic waterers.

On the day of semen collection, the bulls were prepared by compulsory 1 h long motion. Then, the bulls were led one after another in the circle for sexual excitement and mounting on the bull in front. Bull tenders were standing in the middle of the circle and observing that the penis of the mounting bull would not touch the skin of the bull in front. After evident erection, the bull was taken to the manege for semen collection. First ejaculate collection completed, the bull was again led in circles for no less that 15 minutes to activate sexual reflexes and prepare for the second ejaculation. The semen was collected twice a week by the same A.I. worker. There were always two ejaculates per one collection. An artificial vagina (diameter 8 cm, length 50 cm) of 40–45 ^{0}C smeared to half length by sterile non-toxic medical vaseline was used for semen collection. In cool weather, semen collectors were thermoisolated with a cover. Ejaculation completed, the vagina was turned up into vertical position, and the ejaculate sealed by thermal welding and at the same time separating the sperm collector with the ejaculate. The collector was marked with the bull number or name and the ejaculate number and sent to the laboratory. There, the volume of the ejaculate was determined by measuring cylinder and semen quality evaluated (Pakėnas, 1993).

Lithuanian semen cryopreservation technology was used for semen freezing. The semen was diluted using 10.5 g lactose, 5 cm^3 glycerol, 0.2 g sodium citrate, 20 cm^3 egg yolk and 100 cm^3 bidistilled water extender. The semen was cooled in a thermostat consisting of the frame and electrical part. A stand was placed into the frame and 27±1 ^{0}C temperature maintained. A 500 cm^3 flash with the extender was placed into the thermostat for the initial semen dilution. The semen was diluted twice. Firstly the semen was diluted with a 27±1 ^{0}C extender at a rate of 1:1 and stored at 19±1 ^{0}C for 15 minutes. After that it was diluted for the second time using 19±1 ^{0}C extender to reach the required sperm concentration. The machine M6-APA was used for packaging and sealing the semen in 0.25 cm^3 straws. The straws were placed into racks cooled in the refrigerator at 4±2 ^{0}C. The semen was stored at 4±2 ^{0}C for 240 minutes and then frozen on a freezing device-shield in a storage ChB-0.5. The initial temperature for semen freezing was 150±5 ^{0}C (the temperature depends on the level of liquid nitrogen under the freezing device). The semen was frozen for 6 minutes and then placed into liquid nitrogen. Electronic thermometers TE-200M, ST-200 were used for temperature control in the storage, freezing device and straws.

After 48 hours, the frozen semen was thawed in a water bath at 40±0.5 ^{0}C in 10 seconds and the post-thaw sperm motility was determined after 5 h incubation at 38±0.5 ^{0}C (Pileckas, 2006).

The data were subjected to analysis of variance using general linear (GLM) procedure in Minitab 15. The model included the fixed effect of month. Tukey`s HSD significance test was used to ascertain the existence of significant differences between the traits and their occurrence. The significance was determined at $P<0.05$, but differences of $0.05 \leq P<0.10$ would be considered as trends. The values presented are least squares means with standard errors.

Results and discussion. The highest volumes of the first ejaculates were registered in August. However, the average volume of the first ejaculates collected in August differed significantly from the lowest volume ejaculates collected in January ($P < 0.05$) and March ($0.05 \leq P < 0.10$; Table 1) in 3.43 ml. The time of semen collection in different months had higher influence on the volume of second ejaculates. The second ejaculates were of the highest volume also in August. The difference from the average ejaculate volumes collected in March and January was, respectively, 3.94 and 2.83 ml ($P < 0.05$). There was a tendency for the lower volumes of second ejaculates ($0.05 \leq P < 0.10$) in January and February in comparison with May, August and November. Some researchers indicate that the first and the second ejaculates have the same physiological parameters (Fuerst-Waltl et al., 2006), while others find differences. This could be explained by differences in bull preparation for semen collection (Komisrud, Berg, 1996). In our study, both March ejaculates had the highest sperm motility which was significantly different from the sperm motility in the first ejaculates ($P < 0.05$) collected in October and from the sperm motility in the second ejaculates collected in December ($P < 0.05$) and August ($0.05 \leq P < 0.10$; Table 1). This is in agreement with the findings of M. T. Javed et al. (2000) when the highest buffalo sperm motility was determined in spring. However, this comparison might be only conditional, because the research was carried out in Pakistan, in different climatic zones and with different animal species. Chacon et al. (2002) indicate that *Bos indicus* sperm motility is higher at the period at mating. In our case, the bovine sperm quality of a cultured breed which might have lost seasonal oestrus was investigated. The statistical analysis in our study indicated only the general influence of the month ($P < 0.05$) on the sperm concentration in the first ejaculates whereas the difference between the highest and lowest sperm concentration, respectively, in October and March indicated only a tendency ($0.05 \leq P < 0.10$). Conversely, the general influence on the month on the sperm concentration in the second ejaculates was only a tendency ($0.05 \leq P < 0.10$), but the sperm concentration in the ejaculate collected in August was higher than that collected in March, but lower than that collected in January ($P < 0.05$). Almquist and Cunningham (1967) observed that the sperm motility and concentration of Hereford bulls were lower in June, July and September, but this had no influence on semen volume. In the present study, semen characteristics of the dairy Lithuanian Black-and-White bulls had been analyzed, therefore, the differences might have resulted not only because of semen collection time, but also because of different breeds (Hansen, 2004; Koivisto et al., 2009) and climatic conditions. Moreover, the tendencies in sperm concentration changes ($0.05 \leq P < 0.10$) were observed between February and May and August months, and also between March and November months.

Table 1. **The effect of the month of semen collection on the mean fresh semen characteristics**

Month	Characteristics of fresh semen											
	Volume, ml				Sperm motility, %				Concentration, mlr.ml			
	1st ejaculate		2nd ejaculate		1st ejaculate		2nd ejaculate		1st ejaculate		An 2nd ejaculate	
	ESM	SE	ESM	SE	ESM	SE	ESM	SE	ESM	SE	ESM	SE
I	6.90^{a}	0.59	$7.00^{a.\ ct}$	0.61	74.00	1.81	74.00	1.38	1.40	0.03	1.40^{a}	0.03
II	7.17	0.77	6.29^{ct}	0.72	73.33	2.34	72.86	1.65	1.40	0.04	1.40^{ct}	0.03
III	7.00^{ct}	0.63	5.89^{a}	0.64	76.67^{a}	1.91	$76.67^{a,ct}$	1.45	1.29^{ct}	0.29	$1.29^{a,ct}$	0.03
IV	8.43	0.71	7.71	0.72	71.43	2.17	71.43	1.65	1.31	0.03	1.31	0.03
V	9.63	0.66	$9.38^{b,dt}$	0.68	71.88	2.03	71.88	1.54	1.35	0.31	$1.35^{b,\ dt}$	0.03
VI	8.44	0.63	7.78	0.64	73.33	1.91	73.33	1.45	1.38	0.03	1.38	0.03
VII	9.00	0.66	6.75	0.68	68.75	2.03	70.00	1.54	1.31	0.03	1.31	0.03
VIII	$10.33^{b,dt}$	0.77	$9.83^{b,dt}$	0.78	70.83	2.34	70.83^{dt}	1.78	1.38	0.04	$1.38^{b,dt}$	0.04
IX	8.83	0.77	7.50	0.78	70.83	2.34	70.83	1.78	1.40	0.04	1.40	0.04
X	8.86	0.71	7.00	0.72	65.71^{b}	2.17	70.71	1.65	1.43^{dt}	0.03	1.40	0.03
XI	8.83	0.77	9.17^{dt}	0.78	70.00	2.34	70.00	1.78	1.40	0.04	1.40^{dt}	0.04
XII	9.17	0.77	7.83	0.78	67.50	2.34	67.50^{b}	1.78	1.35	0.04	1.35	0.04
P	0.013		0.001		0.029		0.021		0.049		0.088	

LSM–least square means, SE–standard error; Least mean values within the same column having different superscripts indicate significant difference for Tukey HSD test: a-b=$p<0.05$; ct-dt=$0.05 \leq p < 0.10$

The highest average volumes of both ejaculates suitable for cryopreservation were collected in August, May and November (Table 2). The amount of semen suitable for freezing in both ejaculates collected in August was on the average by 3.37, 3.63 and 3.64 ml higher than, respectively, in February, January and March ($P < 0.001$). In May, the amount of suitable semen was on the average 2.79 ml higher than in February ($P < 0.01$). In November, this amount was 2.55 and 2.56 ml higher than, respectively, in January and March ($P < 0.05$). The effect of the month on the level of semen dilution was insignificant ($0.05 \leq P < 0.10$). Extenders are supposed not only to increase the semen volume, but also to protect spermatozoa from the negative influence at the time of semen dilution, cooling and freezing-thawing (Gil et al., 2000). The ingredients of extenders might have a

destabilizing effect on the spermatozoa membranes (Bergeron and Manjunath, 2006). In our study, the semen was diluted with lactose-glycerol-sodium citrate and egg yolk extender. The amount of diluted semen in August was on the average by 56.3 and 59.6 ml higher and in May by 42.4 and 45.6 ml higher than, respectively, in February and January ($P < 0.05$) and 45.4 ml higher than in March ($0.05 \leq P < 0.10$). In August there were on the average by 215.7 frozen doses more than in March ($P < 0.05$).

Table 2. **The effects of the month of semen collection on the suitability of both ejaculates for cryopreservation, level of dilution and amount of diluted semen**

Month	Average amount of semen suitable for freezing per ejaculate, ml		Level of dilution		The amount of diluted semen from two ejaculates, ml	
	LSM	SE	LSM	SE	LSM	SE
I	6.45^{a,g}	0.43	7.60	0.17	109.5^{a}	9.00
II	6.71$^{e, g}$	0.51	7.50	0.22	112.7^{a}	11.62
III	6.44^{a,h,g}	0.45	6.83	0.18	109.7^{ct}	9.48
IV	8.07	0.51	6.93	0.21	125.4	10.75
V	9.50^{f,h}	0.48	7.19	0.19	155.1$^{b, dt}$	10.06
VI	8.11	0.45	7.39	0.18	134.1	9.48
VII	7.88	0.48	7.00	0.19	123.9	10.75
VIII	10.08^{h}	0.55	7.42	0.22	169.0^{b}	11.62
IX	8.17	0.55	7.42	0.22	138.8	11.62
X	7.93	0.51	7.58	0.22	131.7	11.62
XI	9.00^{b}	0.55	7.42	0.22	152.7	11.62
XII	8.50	0.55	7.30	0.24	131.0	12.74
P	<0.0001		0.084		0.002	

LSM–least square means, SE–standard error; Least mean values within the same column having different superscripts indicate significant difference for Tukey HSD test: a-b=p<0.05; e-f=p<0.01; g-h=p<0.001; ct-dt=0.05≤p<0.10.

Table 3. **The effects of the month of semen collection on cryopreserved semen characteristics**

Month	Number of frozen doses		Post-thaw motility, %		Post-thaw motility after 5 h storage, %	
	LSM	SE	LSM	SE	LSM	SE
I	398.00	35.70	47.0	1.27	15.00	1.24
II	422.50	46.09	46.67	1.63	13.33	1.60
III	402.70^{a}	37.63	47.78	1.33	15.56	1.30
IV	464.60	42.67	47.14	1.52	10.71	1.48
V	536.20	46.09	49.38^{a}	1.42	12.14	1.48
VI	501.00	37.63	47.22	1.33	12.22	1.30
VII	448.40	50.49	45.71	1.51	10.00	1.96
VIII	618.40^{b}	50.49	44.17	1.63	11.25	1.96
IX	502.50	46.09	44.17	1.63	11.67	1.60
X	475.00	46.09	46.67	1.63	11.67	1.60
XI	561.50	56.45	41.67^{b}	1.63	11.25	1.96
XII	445.30	56.45	44.00	1.79	10.00	1.96
P	0.032		0.060		0.170	

LSM–least square means, SE–standard error; Least mean values within the same column having different superscripts indicate significant difference for Tukey HSD test: a-b=p<0.05.

The semen collected in May was distinguished by the highest post-thaw motility. The motility accounted for 49.4% whereas in November it was 41.7% ($P < 0.05$). The month of semen collection had no effect on the sperm post-thaw motility after 5 h storage. However, L. Soderquist et al. (1996) indicate that in hot weather, in spring and summer the number of spermatozoa with defects of head, proximal drops and general pathological abnormalities is higher than in autumn or winter, but this fact had no effect on the semen culling rate. Culling resulted in 718 doses in May, 825 in July, 525 in August, 1118 in November and 590 doses in December. The bulls were fed complete rations, because if the ration composition varies throughout the year, this might

influence the semen quality as the length of spermatogenesis is 62–63 days (Pakėnas, 1993) or 65 days (Dorst, 1991).

Conclusions. The month of bull semen collection had influenced many parameters studied. The volume of the second ejaculates was more affected by the semen collection time in different months than the volume of the first ejaculates. The highest volumes were registered in August.

The highest sperm motility in both ejaculates was determined in March. Then the difference in sperm motility was most significant in comparison with that in the first ejaculates in October ($P < 0.05$) and in the second ejaculates in December ($P < 0.05$) and August ($0.05 \leq P < 0.10$).

The month of semen collection had influenced sperm concentration too. The highest sperm concentration was found in the first ejaculates in October and in the second ejaculates in August. The tendencies to sperm concentration changes were also observer between February, May and August months, also between March and November.

The highest average volume of both ejaculates suitable for cryopreservation was in August, May and November. The amount of diluted semen was on the average by 56.3 and 59.6 ml higher in August and by 42.4 and 45.6 ml higher in May in comparison with, respectively, February and January ($P < 0.05$). In August the average number of frozen semen doses was by 215.7 doses higher than that in March ($P < 0.05$).

The semen collected in May had the highest post-thaw motility that was 49.4%, whereas the post-thaw motility of the semen collected in November was 41.7% ($P < 0.05$).

The effect of semen collection time was not determined for the post-thaw sperm motility after 5 h storage.

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