ISBN 978-86-82431-73-2

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INTERNATIONAL
SYMPOSIUM

MODERN
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IN LIVESTOCK
PRODUCTION

PROCEEDINGS

11th - 13th October 2017 - Belgrade, Serbia

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BELGRADE - SERBIA

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Publisher
Institute for Animal Husbandry, Belgrade-Zemun, Serbia

Address of the Editor's office
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Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164; e-mail: biotechnology.izs@gmail.com; www.istocar.bg.ac.rs

Circulation 200 copies.

The publication of this Proceedings is sponsored by the Ministry of Education and Science of the Republic of Serbia.
The Proceedings is printed by the Institute for Animal Husbandry, Belgrade, 2017

ISBN 978-86-82431-73-2

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Proceedings of the 11th International Symposium
Modern Trends in Livestock Production
October 11-13, 2017

THE EFFECT OF COLLECTION SEASONS ON THE SEMEN QUALITY OF HOLSTEIN-FRIESIAN BULLS

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Original scientific paper

Abstract: The study, which included 9 Holstein-Friesian bulls from the Livestock Center of the PKB Corporation, analyzed the impact of the ejaculate collection season on certain semen properties (ejaculate volume, sperm concentration, sperm motility in native ejaculate, sperm dilution, number of doses of one ejaculate and mobility of spermatozoa after thawing). For the purposes of this analysis, data for the three-year (2011-2013) and one-year period (2014) were used. In the three-year period, based on 621 ejaculates of three bulls, the average values for examined properties were calculated and the season's impact on these properties analyzed. The results show that the ejaculate collection season had a very significant effect ($p<0.001$) on the sperm motility in native ejaculate, and a significant effect ($p<0.05$) on the level of dilution of ejaculate and the mobility of spermatozoa after thawing. In the single-year period, based on 326 ejaculates from 9 bulls, the average values of the studied properties were calculated, and the influence of the season was also analyzed. The ejaculate collecting season showed a very high impact ($p<0.001$) on the mobility of spermatozoa after thawing, but no significant effect was found on other properties ($p> 0.05$).

Keywords: bulls, season, spermatozoa, ejaculate, Holstein-Friesian breed

Introduction

The production and quality of bull semen are strongly influenced by genetic factors such as breed and individual properties. However, the production and quality of sperm is conditioned by environmental factors such as diet, way of keeping and nursing, temperature and humidity, ejaculation frequency, and seed

collecting skills. One of the most common factors that significantly impairs spermatogenesis is elevated ambient temperature. High daily temperatures (over 28^{o}C) can cause a spermatogenesis disorder, especially if they last for a long time. The spermatozoa in the first stage of spermatogenesis, when they are still located in the semen canals, are most sensitive to the effects of high temperatures. Subsequently, spermatozoids are wrapped in membranes that should enable them to better survive higher temperatures. Therefore, it can be said that the quality of the ejaculate depends on the temperature affecting the spermatozoa 45-60 days ago (*Stančić, 2014*).

The influence of the season on the semen properties was the subject of many studies. *Mathevon et al. (1998)* state that the season significantly affects all the properties of ejaculate (volume, concentration, mobility, total sperm count and total movable sperm count) in young bulls and most of the properties in older bulls (it does not significantly affect the volume of ejaculate and mobility of spermatozoa). Furthermore, the same author states that the concentration and total sperm count were higher during the winter and spring compared to summer and autumn.

Schwab et al. (1987) and *Menendez-Buxadera et al. (1984)* state that the highest values of the volume of ejaculate, concentration, and number of spermatozoa were also achieved during the winter. These results are contrary to the values established by *Fuente et al. (1984)* who have established the lowest results in the winter.

Orgal and Roth (2008) have analyzed the impact of the collecting season on the quality of bull semen. Their results show that there is a progressive decrease in the motility of spermatozoa after thawing during the summer months, although the significant effect of the season on motility has not been confirmed in the native ejaculate. Also, the percentage of spermatozoa in doses after thawing with defective acrosomes is higher in summer months (54.2 ± 3.5%) compared to winter months (51.4 ± 1.9%). The results obtained suggest that the ejaculates collected during the summer are less able to survive the cryoconservation process precisely because of the lower vitality of sperm recorded after thawing.

Vilakazi and Webb (2004), have examined the effect of the season on the presence of normally developed spermatozoa in the ejaculate. The percentage of normal spermatozoa in the ejaculate was higher during the spring (84.4 ± 2.4%) and winter (82.5 ± 2.4%) compared to the summer (72.8 ± 1.6%) and autumn (79.4 ± 2.2%). Based on these results, it can be again concluded that summer months, or high temperatures, have an adverse effect on the vitality of spermatozoa.

Söderquist et al. (1996) have found significant seasonal variations in the sperm counts with head damage and with total defects. However, the percentage of

spermatozoa with damage was significantly higher during spring and summer compared to colder months of autumn and winter.

Artificial insemination is a process that has significantly improved livestock breeding, and especially cattle breeding. The use of this method has led to more effective use of the bulls, the intensity of selection has increased, the cost of keeping male animals is reduced, the safety of people and animals has increased and the risk of transmission of sexually transmitted diseases is reduced. For this reason, obtaining high quality doses of semen for insemination is an important process in any program of artificial insemination.

Every season is characterized by specific weather conditions that can affect the bull and its production to a lesser or greater extent. The aim of this study is to determine if the ejaculate collection season significantly influences the quality of the doses obtained.

Material and Methods

Research for the purpose of this study was carried out at the Livestock Centre of the PKB Corporation in Belgrade. At the time that data used in the present study was collected, 12 Holstein-Friesian bulls were used in the artificial insemination program in this Centre, and our research included the results of 9 bulls. The entire process of semen production at the Centre was monitored, starting with the collection of bulls' ejaculate, to the final stages of checking of semen quality after thawing.

Taking of the ejaculate in most bulls is done twice a week, most often on Mondays and Fridays. Only one ejaculate is taken from a bull once a day, rarely twice a day. The collection of the ejaculate is performed by artificial vagina, while the second bull is used to induce a full reflex, or jump.

A general overview of sperm involves assessing colour, odour and consistency. Ejaculates that are dirty or with traces of blood/puss are discarded, but are registered in the record for the current year. If the sperm passes the general examination, an estimate of the volume of ejaculate, density and motility of spermatozoa is done. The volume of the ejaculate is determined volumetrically in a graduated sperm collector. The density of the ejaculate (the number of spermatozoa in 1 ml of ejaculate) is measured using a photometer while the motility is estimated based on the number of spermatozoa that exhibit progressive penetration. Observation is performed using a microscope (magnification of 20 - 40x) and score/estimates are given for motility from 1 to 5.

The ejaculate, which satisfies the basic criteria, is then diluted and divided into a number of doses, which are packaged in the form of straws. The most common is the median dilution rate (1:10 - 1:15) and the doses of up to 20 million sperm count. The most commonly used diluent is AndroMed, and diluted semen is packed in vacuums in straws of a volume of 0.22ml, and then closed with ultrasound. Subsequently, a conventional method of deep freezing of the semen dose is applied. The first control of deep-frozen semen is carried out 24 hours after freezing when the percentage of progressively moving spermatozoa is determined, which should be at least 50% in the dose.

The analysis is based on the examination of the impact of the season of semen collection, and semen quality of bulls in the three-year period (2011 - 2013) and the one-year period (2014).

For the three-year period (2011 - 2013), data was collected from 3 bulls who produced between them 621 ejaculates, and for the purpose of the present analysis the year was divided into four seasons:

1) Winter (January, February, March)
2) Spring (April, May, June)
3) Summer (July, August, September)
4) Autumn (October, November, December)

For the one-year period (2014), data was collected from 9 bulls, which produced between them 326 ejaculates, and due to limited data, the year was divided into three seasons:

1) Winter (January, February)
2) Transitional period (March, April, May)
3) Summer (June, July)

The following properties of semen quality were analysed: 1) the volume of the ejaculate (in ml), 2) the concentration of spermatozoa (in 10^6/ml), 3) the motility of spermatozoa in the native ejaculate (grade 1 to 5), 4) the level of sperm dilution (sperm ratio: thinner), 5) number of doses of single ejaculate, 6) sperm motility after thawing (%). For these properties, the basic parameters of descriptive statistics (average, minimum, maximum, standard deviation) were calculated. By the method of variance analysis (F test), the influence of the ejaculate collection season on the semen quality was examined, and the significance of differences in the average values of the observed properties between the individual levels of observation was tested, by the least significant difference test (LSD - test). The number of ejaculates, whose quality parameters were analysed, was not the same for all investigated traits. The minimum values that the semen had to meet in order to be used for artificial insemination are: volume of ejaculate - 2 ml, concentration of spermatozoa - 800 x 10^6/ml, motility score – 4 - (75-80% of progressively

moving spermatozoa), motility after thawing - 50 %. This decrease in the total number of ejaculates, which are finally included in the analysis, is about 50% of the total number of ejaculate collected, and there are huge differences between the bulls. Statistical processing of the received data was performed with the program "STATISTICA 6.0 StatSoft, 2001".

Results and Discussion

Table 1 shows the values of the examined properties for three bulls over a three-year period. During this period, the average volume of the ejaculate for these three bulls was 4.9 ml, the minimum ejaculate was 0.5 and the maximum 11 ml. The lowest concentration of spermatozoa was 100 $x10^6$/ml, and maximum $2780x10^6$/ml, but the average was $1118.7x10^6$/ml. Scores for the motility of spermatozoa in native ejaculate were from 1 to 5, while the average score was 3.5. The average dilution rate was 14, the minimum was 5 and the maximum level of dilution 27. The number of doses per ejaculate was on average 298.5; the lowest number of doses were 68, and the maximum of 730. The average motility of spermatozoa after thawing was 52.8%, minimum 10 and maximum motility 75%.

Table 1. Parameters of descriptive statistics for studied quality traits of bull semen in a three-year period

Trait	N	Average	Minimum	Maximum	Std.Dev.
Ejaculate volume, ml	621	4.9	0.5	11.0	1.58
Concentration (10^6/ml)	620	1118.7	100.0	2780.0	423.64
Motility, score	620	3.5	1.0	5.0	1.06
Dilution (semen : thinner)	386	14.0	5.0	27.0	3.41
Single ejaculate doses, number	386	298.5	68.0	730.0	122.77
Post-thawing sperm motility	374	52.8	10.0	75.0	11.92

Tables 2 and 3 show the average values and errors of the average for the tested traits depending on the season of the year. It is determined that the season significantly influences the following traits: the motility of spermatozoa in native ejaculate, the level of dilution and the motility of spermatozoa after thawing. The remaining three properties (volume of ejaculate, sperm concentration and number of doses) were not under the significant impact of the season.

Table 2. The impact of the season on the quality of bull semen in the three-year period (2011-2013)

Season	Ejaculate volume, average	Ejaculate volume, std. error	Concentration (10^6/ml), average	Concentration (10^6/ml), Std. error	Motility, average	Motility, std. error	N
1	4.7	0.117	1105.7	31.41	3.5	0.078	181
2	5.0	0.108	1170.6	29.23	3.7	0.073	209
3	4.9	0.179	1124.5	48.15	3.4	0.120	77
4	4.9	0.127	1060.4	34.16	3.3	0.085	153
F exp.	0.857 n.s.		2.085 n.s.		5.931***		620

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

Table 3. The impact of the season on the quality of bull semen in the three-year period (2011-2013)

Season	Level of dilution, average	Level of dilution std. error	Number of doses, average	Number of doses, std. error	N	Post-thawing motility, average (%)	Post-thawing motility, std. error (%)	N
1	14.4	0.316	310.0	11.384	115	54.4	1.120	111
2	13.3	0.275	283.5	9.902	152	53.7	0.970	148
3	14.2	0.529	277.4	19.066	41	48.3	1.865	40
4	14.4	0.384	321.7	13.823	78	50.8	1.362	75
F exp.	2.949*		2.449 n.s		386	3.692*		374

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

The motility of spermatozoids in native ejaculate is the only studied trait on which the season had a very significant effect ($p<0.001$) as determined by the application of F test. The best average score of motility was achieved during the 2nd season (3.7), while the lowest score was recorded in the 4th season (3.3). After a significant seasonal effect on the mobility of spermatozoa was identified, LSD test was also conducted to determine the significance of the differences between the seasons. The results of the conducted test indicate that the values during the 2nd season were significantly different compared to the other seasons, while between the 1st and the 4th season there was no significant difference.

The average level of dilution was the best during the 1st and 4th season (14.4) while the worst was during the 2nd season (13.3). The analysis of the data showed a significant influence ($p<0.05$) of the season on the level of dilution. The LSD test was carried out which examined the significance of the differences between the seasons. The results of this test show that the 2nd season significantly

differed ($p<0.05$) from the 1st and 4th season, while in other cases of mutual comparisons of the seasons, no significant difference was found.

By analysing the collected data, a significant influence ($p<0.05$) on the motility of sperm count after thawing was established, similar to results presented by *Orgal and Roth (2008)* and *Vilakazi and Webb (2004).* The average values for this trait were the highest in the 1st season (54.4%), and at lowest in the 3rd season (48.3%). After having determined the significant impact of the season, the LSD test was performed in order to check the significance of the differences between the seasons. The results of this test show that the 3rd season was very different ($p<0.01$) from the 1st and 2nd seasons, and that the 4th did not differ significantly. Season 4 was significantly different from the 1st season ($p<0.05$), while in all the remaining cases of mutual comparison there was no significant difference. The influence of the season on the operation of the PKB Corporation's Livestock Centre is reflected in the number of collected ejaculates per season, which is significantly higher during the spring compared to the summer (Table 2). Reduction in the number of collected ejaculates during the summer is carried out with the aim of minimizing the impact of heat stress on animals.

Table 4 shows the values of the examined properties for nine bulls, which they achieved in 2014. These data show that the average volume of the ejaculate was 5.1 ml, with the lowest volume being 1ml, and a maximum of 14 ml. *Rasbech (1983)* states that the bull's ejaculate volume varies from 2 to 10 ml. The average concentration of spermatozoa was 1067.8×10^6/ml of ejaculate, the lowest was 30, and the highest as high as 2600×10^6/ml. The data reported by *Miljković (1995),* which show an average concentration of spermatozoa of 1200×10^6/ml, are slightly higher than the results obtained in this analysis of the examined quality traits of bull semen in 2014Motility scores of spermatozoa in native ejaculate ranged from 1 to maximal 5, but the average score was 3.4. The level of sperm dilution was on average 16.3, but the variation of this property was remarkable. The lowest level of dilution was only 9, and the highest was 27. The number of doses ranged from 95 to 920, with an average of 368.8 doses per ejaculate. As stated by *Stančić (2014),* the modern dose of semen contains 12 - 15 $\times 10^6$ progressively motile spermatozoa corresponding to the results in this paper. The average motility of spermatozoa after thawing was 51%, the minimum was 10% and the maximum 75%. It can be concluded that sperm tolerated well the deep freezing, which justifies the use of artificial insemination in cattle breeding.

Table 4. Parameters of descriptive statistics for studied quality traits of bull semen in year 2014

Trait	N	Average	Minimum	Maximum	Std.Dev.
Ejaculate volume, ml	326	5.1	1.0	14.0	1.95
Concentration (10^6/ml)	326	1067.8	30.0	2600.0	490.73
Motility, score	326	3.4	1.0	5.0	0.87
Dilution (semen : thinner)	158	16.3	9.0	27.0	3.44
Single ejaculate doses, number	157	368.8	95.0	920.0	145.60
Post-thawing sperm motility, %	153	51.0	10.0	75.0	11.01

Table 5. The impact of the collection season on the quality of bull semen (2014)

Season	Ejaculate volume, average	Ejaculate volume, std. error	Concentration (10^6/ml), average	Concentration (10^6/ml), Std. error	Motility, average	Motility, std. error	N
1	5.4	0.238	1089.9	60.113	3.3	0.106	67
2	5.2	0.144	1068.3	36.473	3.4	0.064	182
3	4.8	0.222	1047.5	56.074	3.4	0.099	77
F exp.	1.438 n.s.		0.133 n.s.		0.016 n.s.		326

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

Table 6. The impact of the collection season on the quality of bull semen (2014)

Season	Level of dilution, average	Level of dilution std. error	N	Number of doses, average	Number of doses, std. error	N	Post-thawing motility, average (%)	Post-thawing motility, std. error (%)	N
1	15.9	0.635	29	394.6	26.833	29	48.3	1.894	29
2	16.8	0.369	86	379.1	15.582	86	48.6	1.107	85
3	15.7	0.521	43	330.0	22.297	42	58.3	1.634	39
F exp.	1.869 n.s.		158	2.1967 n.s.		157	13.485***		153

***(p<0.001) **(p<0.01) *(p<0.05) n.s.(p>0.05)

Tables 5 and 6 show the average values and standard errors for the examined traits depending on the season. Of all the examined traits, the only significant influence of the season was registered on the mobility of spermatozoa after thawing. In the other examined traits, the average values of the ejaculate properties were somewhat better in the first two seasons compared to the third season, but F test showed that these differences were not statistically significant ($p>0.05$). A very significant influence ($p<0.001$) of the season on the motility of spermatozoa after thawing was determined, which enabled the LSD test to determine the significance of the differences between the seasons. The results of the test showed that the 3rd

season was very significantly different ($p<0.001$) from the 1st and 2nd seasons, while between the 1st and 2nd season there was no significant difference. The results of this analysis were probably affected by the shortening of the season in 2014 due to the reduced number of data, however, it is certainly interesting that the motility of spermatozoa after thawing during January and February (season 1) was below the average and amounted to 48.3% and during June and July (season 3) was as high as 58.3% (Table 6). Climatic conditions that define a season can be unpredictable as well as their effect on bulls, therefore testing the impact of the season on semen quality should include many years to make the available data relevant and comparable to other surveys.

Conclusion

Based on the obtained results of the research carried out at the Livestock Centre of the PKB Corporation on the influence of different factors on production and the quality of bull sperm, the following conclusions can be drawn:

- by analysing the data obtained during the three-year and one-year period, it was found that ejaculates on average met basic criteria with a higher or lower variation of properties,

- ejaculate collecting season exhibited a very high impact ($p<0.001$) on sperm motility in native ejaculate and a significant influence ($p<0.05$) on the motility of spermatozoa after thawing in a three year period; especially the summer season is highlighted by its negative impact on the motility of spermatozoa.

- in a one-year period, a very significant effect ($p<0.001$) of the season on the motility of spermatozoa after thawing was recorded; the summer season has shown a positive effect on this property; the justification for such a result probably lies in the specific effect of the climate in the year of testing,

- future studies could analyse the impact of the semen doses from different seasons on the success of the insemination of cows.

Uticaj sezone kolekcionisanja na kvalitet semena bikova holštajn-frizijske rase

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Rezime

Ovim istraživanjem je obuhvaćeno 9 bikova holštajn-frizijske rase iz Centra za stočarstvo PKB Korporacije i analiziran je uticaj sezone kolekcionisanja ejakulata na određene osobine semena (zapremina ejakulata, koncentracija spermatozoida, pokretljivost spermatozoida u nativnom ejakulatu, razređenje sperme, broj doza od jednog ejakulata, pokretljivost spermatozoida posle odmrzavanja). Za potrebe analize korišćeni su podaci iz trogodišnjeg (2011-2013.god) i jednogodišnjeg perioda (2014. god). U trogodišnjem periodu su na osnovu 621 ejakulata od tri bika izračunate prosečne vrednosti za ispitivane osobine i analiziran je uticaj sezone na ta svojstva. Rezultati pokazuju da je sezona kolekcionisanja ejakulata imala vrlo visoko značajan uticaj ($p<0,001$) na pokretljivost spermatozoida u nativnom ejakulatu, i značajan uticaj ($p<0,05$) na stepen razređenja ejakulata i pokretljivost spermatozioda posle odmrzavanja. U jednogodišenjem periodu su na osnovu 326 ejakulata od 9 bikova izračunate prosečne vrednosti ispitivanih svojstva i takođe analiziran je uticaj sezone. Sezona kolekcionisanja ejakulata ispoljila je vrlo visoko značajan uticaj ($p<0,001$) na pokretljivost spermatozoida posle odmrzavanja ali na ostala svojstva nije utvrđen značajan uticaj ($p>0,05$).

Ključne reči*:* bikovi, sezona, spermatozoidi, ejakulat, holštajn-frizijska rasa

Acknowledgment

The authors would like to thank the Livestock Centre of the PKB Corporation (Industrijsko naselje bb, 11213 Padinska Skela - Belgrade) for the cooperation with hope that it will continue in the future. Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 31053.

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