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THE INFLUENCE OF BULLS ON THE QUALITY OF SEMEN OF HOLSTEIN-FRIESIAN BULLS

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Abstract: The aim of this research was to investigate the influence of the bull i.e. genotype on the observed traits of semen quality and to determine whether there are significant differences between bulls in terms of semen production. The study included 9 bulls of the Holstein-Friesian breed from the Livestock Center of PKB Corporation. The effect of the bull on certain semen properties (ejaculate volume, sperm concentration, sperm motility in the native ejaculate, sperm dilution, number of doses per ejaculate, sperm motility after thawing) was analyzed and data were collected during 2014. During this period, a total of 326 ejaculates from 9 bulls were collected and average values for the tested properties were determined and the effect of the bull on those properties was analyzed. The results showed that there was a very significant effect of the bull ($p<0.001$) on the degree of sperm dilution and the number of doses per ejaculate. Significant influence ($p<0.01$) was found in ejaculate volume traits, sperm concentration, sperm motility in the native ejaculate, while in sperm motility after thawing no significant effect ($p> 0.05$) of the bull was determined. The LSD test (Least Significant Difference) was used to compare the bulls and the results of this test showed in most cases significant differences between the bulls.

Keywords: bulls, LSD test, sperm, ejaculate, Holstein-Friesian breed

Introduction

Fertility is one of the most important traits in cattle production. The use of artificial insemination has allowed for a much faster genetic progression within the cattle population, since thousands of doses can be made from one bull during the year. Therefore, it is very important to accurately evaluate the potential production of bull semen and, on this basis, to direct selection to bulls with desirable traits.

The quality of the semen significantly affects the fertility of cows because with the poor quality of the semen, more insemination is required for fertilization/conception, thus extending the service period and the calving interval. The production and quality of bull semen is strongly influenced by genetic factors such as breed and individual traits. In addition, sperm production and quality are conditioned by other factors, such as diet, bull housing and care, body size and body weight, age, environment conditions, ejaculation frequency and sperm acquiring, preservation and storage skills. Of all the factors that influence the quality of bull semen in this paper, special emphasis was placed on the influence of the bull i.e. bull genotype on the tested traits.
Mathevon et al. (1998) have established a heritability coefficient for ejaculate volume, sperm concentration, sperm motility, sperm count, sperm count per ejaculate with good motility, of 0.24, 0.52, 0.31, 0.38, and 0.49, respectively, in young bulls and 0.44, 0.36, 0.01, 0.54 and 0.64, respectively, for adult mature bulls. The same authors have also established a coefficient of repeatability for semen quality traits that ranged from 0.41 to 0.64. *Mathevon et al. (1998)* have analyzed, among other things, the effect of the bull on the investigated properties and their results indicate a significant influence ($p<0.001$) of the bull on ejaculate volume, sperm concentration and motility, total sperm count and total number of motile sperm.
Asad et al. (2004) find a significant effect ($p<0.001$) of genotype on ejaculate volume, sperm concentration and motility. The genetic variability of semen quality traits has been confirmed by many authors (*Raju et al. 1982; Rao and Rao 1978; Hafez 1993*).
Estimated heritabilities for ejaculate volume, progressive sperm motility, sperm concentration, sperm count in the ejaculate and number of live sperm were 0.15, 0.12, 0.22, 0.16 and 0.12, respectively, and genetic correlations between these five traits ranged from 0.02 to 0.99 *(Yin et al., 2019). Berry et al. (2019)* have found in their studies that the heritability coefficient for semen quality traits ranges from 0.13 - 0.34 and the repeatability coefficient from 0.22 to 0.45. The results of this work emphasize the great genetic variability of semen quality traits, some of which are highly heritable, so that proper selection should lead to improved semen production. Determination of the coefficient of repeatability for various traits of semen quality allows us to predict the future production potential of young bulls. In this way, it starts with selecting the animal of the preferable traits early on. Assessment of heritability indicates to what extent the traits examined will be passed on from generation to generation. Therefore, the genotype, that is, the hereditary basis of the individual, together with the environmental factors and conditions of care and housing, significantly affect the production of sperm in bulls of the Holstein-Friesian breed.

Material and Methods

Research to produce this work was carried out at the Livestock Center of PKB Corporation in Belgrade. During the data collection period, 12 bulls of the Holstein-Frisian breed were used in the artificial insemination program, and the study included the results of 9 bulls. Data refer to year 2014. The entire process of producing semen doses at the Center was followed, starting with the ejaculation of bulls and ending with the control of semen quality after thawing.

Most bulls ejaculates are take twice a week, usually on Mondays and Fridays. The ejaculate is taken from a bull once a day, while it is rare that bulls are used twice for jumping. Collection of ejaculates is performed by an artificial vagina while another bull is used to cause a sexual reflex or jump.

A general examination of sperm involves an assessment of color, odor and consistency. Ejaculates that are dirty or with blood/pus have to be discarded but noted in records for the current year. If the sperm passes the general examination, the volume of ejaculate, density and motility of the sperm are evaluated. The ejaculate volume is determined volumetrically in a graduated sperm collector. Ejaculate density (sperm count in 1 ml of ejaculate) is measured using a photometer while motility is estimated based on the number of sperm exhibiting progressive motility. Observation is performed using a microscope (magnification 20 - 40x) and ratings are given for motility from 1 to 5.

The ejaculate that meets the basic criteria is then diluted and divided into a number of doses, which are packed in form of paillettes. The most common is the mean degree of dilution (1:10 - 1:15) and the doses obtained have up to 20 million spermatozoids. The most commonly used diluent is AndroMed, and the diluted semen are vacuum packed into 0.22mL straws (paillettes) and then sealed with ultrasound. Subsequently, the usual procedure of deep freezing semen doses 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%.

The semen quality traits analyzed were: 1) ejaculate volume (in mL, 2) sperm concentration (in 10^6/mL,, 3) sperm motility in native ejaculate (grade 1 to 5), 4) degree of sperm dilution (sperm: thinner ratio), 5) number of doses per ejaculate, 6) motility of sperm after thawing (%). For these properties, the basic parameters of the descriptive statistics (average, minimum, maximum, standard deviation) were calculated. The method of analysis of variance (F test) was used to examine the influence of bulls i.e. genotype on semen quality, and the significance of differences of average values of observed traits between individual levels of observation were tested using the Least Significant Difference Test (LSD - test). The number of ejaculates whose quality parameters were analyzed was not the same for all traits tested. The minimum values that the semen must satisfy in order to be used for artificial insemination are: ejaculate volume - 2mL, sperm concentration - 800 x 10^6/mL, motility score - 4- (75-80% progressively motile sperm), motility after thawing - 50 %. This decrease in the total number of ejaculates, which are finally taken for analysis, is about 50% of the total number of ejaculates collected, and there are large differences between the bulls. Statistical processing of the obtained data was performed with the software package "STATISTICA 6.0 StatSoft, 2001".

Results and Discussion

Table 1 lists the trait values of nine bulls in 2014. These data show that the average volume of the ejaculate was 5.1 mL and that the lowest volume was 1 and the highest 14 mL. The average sperm concentration was 1067.8x10^6/mL, the lowest was 30 and the highest was 2600x10^6/mL. Data obtained by *Miljkovic (1995)* show the average sperm concentration of 1200x10^6/mL which is higher than the values obtained by analyzing the examined quality traits of bull semen in 2014. The sperm motility scores in the native ejaculate ranged from 1 to maximal 5, but the mean score was 3.4. The sperm dilution rate averaged 16.3 but the variation in this property was remarkable. The lowest dilution rate was only 9 and the highest was 27. As a result, the number of doses varied from 95 to 920, while the average was 368.8 doses per ejaculate. The average sperm motility after thawing was 51%, the minimum was 10 and the maximum was 75%. It should be noted that ejaculates that are dirty or with admixtures of blood and pus were immediately discarded and not used for analysis, but such ejaculates have been recorded and are attached.

Table 1. Parameters of descriptive statistics for the examined traits of bull semen quality in 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
Mobility, evaluation	326	3.4	1.0	5.0	0.87
Dilution rate (Sperm: Thinner)	158	16.3	9.0	27.0	3.44
Number of doses from single ejaculate	157	368.8	95.0	920.0	145.60
Sperm motility after thawing (%)	153	51.0	10.0	75.0	11.01

Duret et al. (2008) have analyzed the properties of Holstein breed bull semen and found, among other things, an average ejaculate volume of 4.07 mL, a spermatozoid concentration of 1.08^x10^9/mL, and sperm motility of 55.89%. Similar values were obtained by *Ghasemi and Ghorbani (2014)*, they have compared three genotypes for the FSHβ gene and obtained values for ejaculate volume from 3.1 to 4.7 mL; for sperm concentration from $967.8^{x}10^{6}$/ml to $1151.3^{x}10^{6}$/mL and for sperm motility after thawing, values from 58.96% to 60.92%.

By analyzing the collected data on the examined properties of bull ejaculates and comparing them with the results of other authors, it can be concluded that the averages of the traits found in Table 1 satisfy the basic criteria of ejaculate quality.

Table 2. Average values and average errors for bull semen quality traits examined in 2014

Bull	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	4.4	0.238	795.8	61.701	2.92	0.112	40
2	6.5	0.289	1297.4	75.100	3.85	0.136	27
3	5.9	0.250	1187.9	65.039	3.50	0.118	36
4	4.5	0.229	720.0	59.510	2.84	0.108	43
5	3.1	0.229	1520.0	59.510	3.86	0.108	43
7	6.3	0.284	682.9	73.747	2.42	0.134	28
8	5.2	0.232	1126.2	60.214	3.67	0.109	42
9	4.4	0.229	897.2	59.510	3.16	0.108	43
10	7.9	0.307	1548.7	79.656	4.21	0.145	24
F exp.	28.889**		24.619**		21.219**		326

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

Table 3. Average values and average errors for bull semen quality traits examined in 2014

Bull	Dilution rate, Average	Dilution rate Std. error	N	Number of doses Average	Number of doses Std. error	N	Sperm motility after thawing Average (%)	Sperm motility after thawing Std. error (%)	N
1	13.7	0.816	12	312.7	35.467	11	47.8	3.590	9
2	16.2	0.632	20	444.2	26.303	20	53.5	2.408	20
3	17.3	0.666	18	415.3	27.726	18	44.7	2.539	18
4	15.8	1.413	4	398.8	58.815	4	42.5	5.385	4
5	19.6	0.508	31	273.6	21.127	31	51.3	1.934	31
7	12.0	1.264	5	334.0	52.606	5	54.0	4.817	5
8	14.7	0.534	28	303.8	22.230	28	50.0	2.112	26
9	14.8	0.686	17	326.8	28.529	17	54.1	2.612	17
10	16.9	0.589	23	534.8	24.528	23	54.3	2.246	23
F exp.	10.404***		158	11.3774***		157	$1.846^{n.z.}$		153

Tables 2 and 3 show the average values and standard errors of the average for the tested semen quality traits for each bull separately. Table 4 shows the results of the LSD test, out of 36 cases of bull comparisons, those with significant differences were identified ($p<0.001$). Only the most stringent level of significance was used ($p <0.001$).

The average for ejaculate volume was the highest for bull number 10 and it was 7.9 mL, while the average was the lowest in bull number 5 and it was 3.1 mL (Table 2). The analysis showed that the bull had a significant effect on ejaculate volume. As the significant influence of the bulls was determined, in the following analysis, using the LSD test, a comparison was made between the bulls to determine the significance of the difference between them (Table 4). The results of that test found that in 11 of the 36 cases of comparison, there was no significant difference between the bulls, but in the remaining 25 cases of comparison, a very significant difference ($p<0.001$) between the bulls was established. These results particularly highlight bulls 10 and 5, which showed a very high difference compared to all other bulls.

The average values for sperm concentration showed the highest concentration of 1548 $x10^6$/mL in bull 10, while the lowest average concentration of $683x10^6$/mL was established in bull 7 (Table 2). It is interesting that in case of the bull 5, which had the lowest average ejaculate volume, almost the best average concentration ($1520x10^6$/mL) was established. Using the F test, it was found that the bull significantly affected the concentration of spermatozoids in the ejaculate. The bulls were then compared with each other using the LSD test to determine the significance of the difference between them (Table 4). The results of this test showed that in 14 out of 36 cases of comparison, there was no significant difference between the bulls, but in the remaining 22 cases of comparison, a very significant difference ($p <0.001$) was found between the bulls.

Average scores for sperm motility in the native ejaculate showed that the bull 10 had the best average score of 4.21 and the bull 7 had the worst average score of 2.42 (Table 2). This trait has been found to be under a significant effect of the bull. Therefore, an LSD test was also performed to determine the significance of the difference between the bulls (Table 4). Applying this test, it was found that in 16 out of 36 cases of comparison, there was no significant difference between the bulls but in the remaining 20 cases of comparison, there was a very high difference between them ($p<0.001$).

The next three studied properties (dilution rate, number of doses, and motility after thawing) resulted in a decrease in the total number of ejaculates used to calculate their average values, which are presented in this paper (Table 3). This is because ejaculates that lack the proper sperm concentration and motility score are already rejected. This decrease was about 50% of the total number of ejaculates, with differences between the bulls. The worst in this regard were the bulls 4 (4/43) and 7 (5/28) and the best was the bull 10 (23/24) which had only one ejaculate that was not prepared for freezing.

The degree of dilution is a property that is highly dependent on sperm concentration and motility score. The results in Table 3 show the best average dilution rate for bull 5 of 19.6. Such a high average dilution rate is explained by the fact that this bull's ejaculates were characterized by a very good sperm concentration and motility score, but at the same time by a low average ejaculate volume, which required such dilution. The lowest average degree of dilution was recorded for bull number 7 - 12. Analysis of this property, using the F test, revealed a significant influence of the bull on its expression. Further analysis involves conducting an LSD test to determine the significance of differences between bulls (Table 4). The results of this test showed that in 27 of the 36 cases of mutual comparison there was no significant difference between the bulls while in the remaining 9 cases of the mutual comparisons there was a significant difference between the bulls. Particularly prominent was the bull 5, which showed a very high difference (p <0.001) compared to most of the other bulls.

Table 4. Effect of bull on ejaculate volume, sperm concentration, sperm motility in native ejaculate, sperm dilution and number of doses per ejaculate (LSD test)

Bull	Ejaculate volume	Sperm concentration	Sperm motility in native ejaculate	Sperm dilution	Number of doses
1	(2,3,5,10)***	(2,3,5,8,10)***	(2,3,5,8,10)***	(3,5)***	10***
2	(1,4,5,8,9,10)***	(1,4,7,9)***	(1,4,7,9)***	5***	(5,8)***
3	(1,4,5,9,10)***	(1,4,5,7,10)***	(1,4,7,10)***	(1,7)***	5***
4	(2,3,5,7,10)***	(2,3,5,8,10)***	(2,3,5,8,10)***	/	/
5	(1,2,3,4,7,8,9,10)***	(1,3,4,7,8,9)***	(1,4,7,9)***	(1,2,7,8,9,10)***	(2,3,10)***
7	(1,4,5,9,10)***	(2,3,5,8,10)***	(3,5,8,9,10)***	(3,5,10)***	10***
8	(2,5,10)***	(1,4,5,7,10)***	(1,4,7)***	5***	(2,10)**
9	(2,3,5,7,10)***	(2,3,10)***	(2,5,7,10)***	5***	10***
10	(1,2,3,4,5,7,8,9)***	(1,3,4,7,8,9)***	(1,3,4,7,9)***	(5,7)***	(1,5,7,8,9)***

***(p<0.001)

Analysis of the effect of the bull on the number of doses per one ejaculate showed that there was a significant effect on this trait. Since the significant influence of the bull on the number of doses has been confirmed, an LSD test followed to examine the significance of the differences between the bulls (Table 4). Based on this test, it

can be concluded that the bull 10 differed significantly from all other bulls. These differences were generally very highly significant ($p<0.001$) and this only confirmed the results in Table 3 where it is evident that this bull had by far the best average number of doses per ejaculate. However, if we exclude the bull 10 and pay attention only to the remaining bulls, the results show that in most cases there were no significant differences between them.
Sperm motility after thawing is the last and most important property tested. The values of this trait determine whether doses of one ejaculate will be used for insemination. The average values for this trait in these nine bulls ranged from 42.5 to 54.3%, but sperm motility after thawing is the only trait found that was not under significant effect of the bull.
It can be concluded that the bull significantly affected the volume of the ejaculate, sperm concentration, sperm motility in the native ejaculate, the degree of dilution and the number of doses per ejaculate, and had no significant effect on the sperm motility after thawing. These are the expected results as the motility of sperm after thawing is more influenced by the freezing and thawing process.

Conclusion

Based on the results of research conducted at the Livestock Center of PKB Corporation on the impact of various factors on the production and quality of bull sperm, the following conclusions can be drawn:
- analysis of the data showed that the ejaculates on average met the basic criteria with less or more variation of traits,
- the influence of the bull on the tested traits – it can be concluded that the bull significantly affects the volume of the ejaculate, the concentration of sperm, the motility of the sperm in the native ejaculate, the degree of dilution and the number of doses per ejaculate, and has no significant effect on the motility of the sperm after thawing.
- some semen quality traits are medium to high in regard to heritability, and appropriate selection on these traits could lead to progress in semen production.

Uticaj bika na kvalitet semena bikova holštajn-frizijske rase

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Rezime

Cilj ovog istraživanja je da se ispita uticaj bika odnosno genotipa na posmatrane osobine kvaliteta semena i da se ustanovi da li postoje značajne razlike između bikova u pogledu proizvodnje semena. Istraživanjem je obuhvaćeno 9 bikova holštajn-frizijske rase iz Centra za stočarstvo PKB Korporacije. Analiziran je uticaj bika na određena svojstva semena (zapremina ejakulata, koncentracija spermatozoida, pokretljivost spermatozoida u nativnom ejakulatu, razređenje sperme, broj doza od jednog ejakulata, pokretljivost spermatozoida posle odmrzavanja) i podaci su prikupljeni tokom 2014 godine. U ovom periodu sakupljeno je ukupno 326 ejakulata od 9 bikova i utvrđene su prosečne vrednosti za ispitivana svojstva i analiziran je uticaj bika na ta svojstva. Rezultati su pokazali da postoji veoma značajan uticaj bika ($p<0.001$) na stepen razređenja sperme i broj doza od jednog ejakulata. Značajan uticaj ($p<0.01$) je utvrđen kod osobina zapremina ejakulata, koncentracija spermatozoida, pokretljivost spermatozoida u nativnom ejakulatu dok je kod pokretljivosti spermatozoida posle odmrzavanja utvrđeno da ne postoji značajan uticaj ($p> 0.05$) bika. LSD test *(Least Significant Difference)* je korišćen za međusobno poređenje bikova i rezultati ovog testa su pokazali u većini slučajeva značajne razlike između bikova.

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

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