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The effect of age on eemen quality of Holstein-friesian bulls

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Abstract

The aim of this research was to investigate the influence of the bull age on production and some semen quality traits. The semen was collected during 2014. The LSD test (Least Significant Difference) was used to compare the bulls of different age groups and the results of this test showed in some cases significant differences. This test showed that there was no significant difference in ejaculate volume between six-year-old and four-year-old bulls, but in two-year-old bulls a significant difference to older bull groups was determined. The results showed that there was a significant effect of the bull age ($p<0.001$) on the ejaculate volume and the number of doses per ejaculate.

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

Introduction

The production and quality of bull semen is strongly influenced by genetic factors such as breed and individual traits. However, sperm production and quality are also conditioned by other factors, such as diet, housing method and care of the bulls, body size and weight, age of the bull, environment conditions, frequency of ejaculation, and the skill to collect, preserve and store sperm (Fuerst-Waltl et al., 2006; Fiaz et al., 2010). The impact of bull age on semen quality will be discussed in more detail in this paper.

It is widely acknowledged that the age of a bull at collection affects semen characteristics (Brito et al., 2002a; Mandal et al., 2010), with older mature bulls having greater semen volume and quality than younger bulls (Brito et al., 2002a).

The quantity of ejaculate and the number of spermatozoa in them increase with age of the bull regardless of the season and the interval between collections. This is explained by the fact that the major factor affecting sperm production is testicular mass, which increases at least five years after puberty. All semen traits (ejaculate volume, total volume per day, sperm motility, sperm concentration per ml and per ejaculate) were significantly influenced ($P<0.01$) by bull age and season (Bhakat et al., 2011). The same authors state that the ejaculate trait values such as ejaculate volume and sperm concentration increase by the age of five, and then these values decrease. The highest fertility of bull has been observed at around 2-4 years of age and started declining once bull attained more than 4 years of age (Smith Thomas, 2009).

The age of the bull at semen collection affects the volume of the ejaculate, its concentration, and sperm motility (Mathevon et al., 1998). In general, the literature shows that all of these ejaculate characteristics increase as bulls age (Fuente et al., 1984; Siratskii, 1987). During the puberty, bull ejaculates contain an increased number of immature and abnormal sperm whose progressive motility is very poor. Bulls aged less than 1 year had the poorest semen production and sperm motility values for all parameters compared with bulls older than 1 year ($P < 0.01$) (Murphy et al., 2018).

As the bulls mature, their body weight increases, and the testicles also develop rapidly. This leads to a rapid decrease in the number of immature sperm and increase in the amount of semen and the number of mature sperm with good motility (Balić et al., 2012). The simultaneous development of the testis and accessory glands post puberty and during sexual maturation consequently leads to an increase in semen production (Almquist, 1978). Spermatogenous maturation results in the formation of mature sperm. Most bulls reach this condition from 3 to 5 years of age,

which again depends on breed, but also on diet, housing and care. After the sperm maturation is achieved, bulls maintain this level of semen production with minor variations over the next few years, followed by a gradual decline in the quantity and quality of semen as a result of atrophy of the seminal ducts (senile period). The reproductive performance of young bulls depends on the onset of puberty, and that period can vary greatly between breeds and within breeds (Barth et al., 2008). Under practical conditions, bulls are kept in production until they are 7-8 years old. After that, they are soon replaced by younger and better bulls. The exceptions are bulls of remarkable superiority over the population average, which are maintained in production as long as they produce sufficient semen.

Material and Methods

Research was carried out at the Livestock Center of PKB Corporation in Belgrade and included the results of 9 Holstein-Friesian bulls during 2014. Bulls were divided into three age categories: two-year, four-year and six-year bulls. The entire process of producing seed doses at the Center was monitored, beginning with the ejaculation of bulls and ending with the quality check of the semen after thawing. The ejaculates from majority of bulls were taken twice a week, usually on Mondays and Fridays. The ejaculate from bulls was taken once a day, while some bulls were used twice a day for collection. Collection of ejaculates was performed by an artificial vagina while another bull was used to induce a sexual reflex or jump.

A general examination of sperm involved an assessment of colour, odour and consistency. Ejaculates that were dirty or with blood/pus had to be discarded but recorded in the current year. If the sperm received postive evaluation in the general examination, the volume of ejaculate, density and motility of the sperm were evaluated. The ejaculate volume was determined volumetrically in a graduated sperm collector. Ejaculate density (sperm count in 1 ml of ejaculate) was measured using a photometer while motility was estimated based on the number of sperm exhibiting progressive motility. Observation was performed using a microscope (magnification 20 - 40x) and ratings were given for motility from 1 to 5.

The ejaculate that met the basic criteria was then diluted and divided into a number of doses, which were packed in paillette form. The most common was the mean degree of dilution (1:10 - 1:15) and the doses obtained had up to 20 million sperm. The most commonly used diluent was AndroMed, and the diluted semen was vacuum packed into 0.22mL straws and then sealed with ultrasound. Subsequently, the usual procedure of deep freezing semen doses was applied. The first control of deep-frozen semen was carried out 24 hours after freezing when the percentage of progressively motile spermatozoa was determined, which should be at at least 50% in a dose.

The semen quality properties analyzed were: ejaculate volume (in mL), sperm concentration (in 106/mL), sperm motility in the native ejaculate (score 1 to 5), degree of sperm dilution (sperm: diluent ratio), number of doses from one ejaculate and sperm motility 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 bull age on semen quality, and the least significant difference test (LSD - test) was used to test the significance of differences in average values between different age groups. The number of ejaculates whose quality parameters were analyzed was not the same for all traits tested. The minimum values that the semen had to satisfy in order to be used for artificial insemination were: ejaculate volume - 2mL, sperm concentration - 800 x 106/mL, motility score - 4 (75-80% progressively motile sperm), motility after thawing - 50 %. Statistical processing of the obtained data was performed with the software package "STATISTICA 6.0 StatSoft, 2001".

Results and discussion

Tables 1 and 2 show the average values for semen traits tested depending on the age of the bull. All bulls from which the data were collected were divided by age into three groups: two-year-olds (2 bulls), four-year-olds (3 bulls) and six-year-olds (4 bulls). The analysed data showed that the age of the bull had a significant effect on the volume of ejaculates and the number of doses but not on semen quality traits.

Table 1. Effect of bull age on some semen quality traits

Age, year	Ejaculate volume, Average	Std. error	N	Dilution ratio Average	Std. error	N	No. of doses Average	Std. error	N
2	4.6	0.15	156	16.4	0.38	81	299.0	13.81	81
4	5.8	0.19	103	17.0	0.51	45	474.9	18.53	45
6	5.3	0.23	67	15.3	0.62	32	397.5	22.33	31
Fexp.	11.948***		326	2.456n.s.		158	30.000***		157

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

Table 2. Effect of bull age on some semen quality traits

Age, year	Concentration (10^6/ml), Average	Std. error	Motility, Average	Std. error	N	Post thawing motility Average (%)	Std. error	N
2	1092.1	39.30	3.36	0.07	156	51.65	1.24	79
4	1076.7	48.37	3.39	0.09	103	49.44	1.64	45
6	997.9	59.97	3.30	0.11	67	51.72	2.05	29
Fexp.	0.886n.s.		0.218n.s.			326		153
						0.645n.s.		

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

The average ejaculate volume values were higher in 4-year and 6-year-old bulls than in 2-year-old bulls. These values are expected as two-year-old bulls have yet to achieve full sperm maturation. Also, older bulls have higher body weight, which means more developed testicles and other genital organs, which contributes to producing ejaculates of greater volume than in younger bulls. Analysis of the data using the F test revealed that the age of the bull significantly affected the volume of the ejaculate. This allowed the LSD test to be conducted to examine the significance of differences between bulls of different ages (Table 3). The results showed that was no significant difference between six-year-old and four-year-old bulls. The two-year-old bulls had lower ejaculate volume.

Table 3. Effect of bull age on ejaculate volume (LSD test)

Bulls' age	Two-year	Four-year	Six-year
Two-year		***	*
Four-year	0.000002		n.s.
Six-year	0.022210	0.076180	

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

Another trait that was significantly influenced by the bull's age is the number of doses per ejaculate. This result was expected because the number of doses depends on the volume of ejaculates.

The LSD test showed a significant differences between bulls of different ages on the number of doses per ejaculate (Table 4). Two-year-old bulls gave significantly lower (p<0.001) doses in relation to older bulls. Four-year-old bulls gave a significant more (p<0.01) number of doses in relation to six-year-old bulls.

Age of bull significantly affected all traits (ejaculate volume, sperm concentration, percentage of viable spermatozoa in the ejaculate, total spermatozoa per ejaculate) (P<0.01 to P<0.001) except motility score (Fuerst-Waltl et al., 2006). The same authors state that the ejaculate volume and total number of spermatozoa increased with age of bull while sperm concentration was lower in higher age classes.

Table 4. Effect of bull age on number of doses per ejaculate (LSD test)

Bulls' age	Two-year	Four-year	Six-year
Two-year		***	***
Four-year	0.000000		**
Six-year	0.000245	0.008510	

$^{***}(p<0.001)$; $^{**}(p<0.01)$; $^{*}(p<0.05)$; $^{n.s.}(p>0.05)$

For other tested ejaculate properties (sperm concentration, sperm motility in native ejaculate, degree of dilution, and sperm motility after thawing), the F test results showed that there was no significant effect of bull age on the manifestation of these traits.

Conclusion

Based on the obtained results it could be conclude that the bull age have a strong effect on volume of ejaculate and semen quaquality traits.

Especially great differences were found in bulls, with a 4 year age difference. The results are expected, as with the maturation of the bull, an increase in body weight, including testes, results in higher volume ejaculates and better quality. The four-year-old bulls had the highest volume of ejaculate with the high semen quality with greatest potential in semen production.

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