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Influence of breed, season and age on quality bovine semen used for artificial insemination

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The success or failure of artificial insemination starts with the quality status of semen used, hence, this study aimed to investigate the effects of breed, season and year on bovine semen quality of the National Bull Stud of Rwanda kept at Masaka bull station, Rwanda. A total of 1475 semen samples were collected bi-weekly from nine bulls of Holstein Friesian (n = 3), Inyambo (n = 3) and Jersey (n = 3) breed using an artificial vagina. Semen volume, colour, concentration, mass motility, live sperm percentage and post-freeze motility were evaluated. Libido of the bulls at the time of semen collection was scored. Ejaculate volume, mass motility, individual motility, density and post freeze motility significantly differed ($p < 0.05$) among seasons of the year, bull breeds and age/year of collection. Friesian bulls had superior ($p < 0.05$) semen volume (5.76±0.08 ml) to that of Jersey (4.29±0.09 ml) and Inyambo (3.37±0.1 ml). However, Friesians had an inferior ($p < 0.05$) lighter coloured semen, with only 52.7% of Friesian samples having the preferred cream colour, as compared to 65.2% of Jersey and 64.9% of the Inyambo semen samples. Year of collection (2011 or 2012) and in essence age of the bull negatively affected ($p < 0.05$) all the parameters studied, with semen volume dropping from 5.16 to 5.10 ml, colour lightened from 62.8 to 54% of the samples being cream; mass motility fell from 2.98 to 2.65, while live sperm percentage in ejaculates dropped from 65 to 63%. Of the eight parameters studied, only post-freeze motility was not affected by passage of time. Semen collected during the October to December period had the best quality characteristics, though collections in the long rains (March to May) had comparable mass motility and post-freeze motility. Semen volume (4.61 ml per bull ejaculate) and post-freeze motility (39%) were poorest in the long dry season (January to February). In conclusion, the Friesian breed should be promoted at the bull station. Most semen should be collected during the rain season, particularly the short rains (October to November). Bulls below three years of age should be of focus.

Key words: Cattle, mortality, seasonality, semen evaluation, pedigree bulls.

INTRODUCTION

Artificial insemination (AI) is the most valuable breeding management tool available to cattle breeders to improve the genetic potential of their herds. The optimal use of genetically superior bulls through artificial insemination is

highly dependent on precise seminal quality which allows for reasonable estimations of field fertility with normal or low-dose inseminations (Fuerst-Waltl et al., 2006; Christensen et al., 2011; Ahmed et al., 2016). There are factors determining the success of AI such as environmental seasonal variations and temperature (McDowell, 1972). In areas where seasonal variation in temperature occurs, sperm morphological characteristics were variable (Curtis, 1983; Mathevon et al., 1998; Brito, 2010). Mathevon et al. (1998) reported that the interaction between age and season could have a significant effect on the semen characteristics of Friesian bulls. seasonal factors, especially temperature, have an important effect on the spermatic production in most bulls, but the individual response to thermal stress is different. Detrimental effect on the spermatic parameters has been registered mostly in the warm seasons, during months with average temperatures of about 20°C. Numerous studies were dedicated to identification of the factors which affect the spermatic production and the quality of the semen. To date, some differences and even contradictions regarding the effect of the season on spermatic production were identified. Consequently, some studies (Mathevon et al., 1998 and Brito, 2010) demonstrated significant influence of season on the spermatic production, while some other studies did not detect any effect of season on the spermatic production (Brito et al., 2002; Girdhar, 2003; Vilakazi and Webb, 2004). Meanwhile, other researchers did observe the highest concentration and total number of spermatozoa occurs during the summer season (Stalhammar et al., 1989; Mathevon et al., 1998; Ghasemi and Ghorban, 2014).

Season of the year influences the secretion of luteinizing hormone (LH), the average concentration of testosterone in bulls, and the number of spermatozoa per ejaculate is consequently affected (Jimenez-Severiano et al., 2003). The study of Jimenez-Severiano (2003) also revealed that highest LH average values in young bulls were registered in spring and the lowest in the winter period and that average testosterone concentrations were also higher in spring and summer than in the cold season. The same effect was revealed in bulls of a beef breed from the tropical region, in which there was observed a depreciation of semen quality and a diminution of testicular dimensions in winter (Nichi et al., 2006). The optimal environmental temperature for spermatic production is estimated to range between 15 and 20°C (Parkinson, 1987). It is considered that not only the temperature registered in the day of semen collection affects the production, but also the temperature registered during the entire spermatogenesis period, until 70 days before collection. Furthermore, it was indicated that for young bulls, superior morphological characteristics were observed during the winter and spring as compared to summer and autumn. Dombo (2002) reported that the semen quality does not remain the same throughout the year, especially as the season changes and as the bull advances in age.

Many efforts have been made to improve milk production in Rwanda and a crossbreeding programme is being implemented on a large scale (Girdhar, 2003; MINAGRI, 2011). This programme is aimed to maximally depend on the use of artificial insemination both for small-scale/smallholder/zero grazer units, and for large scale dairy farms. The artificial insemination programme in Rwanda relies on both imported and in-country produced bovine semen, with a plan to produce most of the semen domestically. In tropical regions, semen output, quality and mating behaviour of bulls can vary from season to season, depending mainly on availability and quality of feed and climatic conditions. Therefore, this study aimed to study the influence of breed of the bull, season of semen collection and age of the bull on bovine semen quality of Friesian, Jersey and Inyambo bulls kept at Masaka Bull Station, Rwanda.

MATERIALS AND METHODS

Experimental animals and housing

A total of 1475 semen samples were collected from the National Bull Stud of Rwanda from three Holstein Friesian bulls (946 semen samples), three Jersey bulls (371 semen samples) and three Inyambo bulls (174 semen samples) maintained at the semen production facility at Masaka Bull Station. At the onset of the study, the breeder bulls were in good health status and were aged between three and seven years. Data recorded over a period of two years were used for semen quality evaluation. Bulls were housed individually in pens, which were constituted into two large sheds/barns. The sheds were designed conventionally, to provide sufficient natural cross-ventilation and to minimize heat stress. Each bull pen was facilitated with a water point and a feeding manger. The pens were provided with floor litter in form of dry grass to increase the comfort of the bulls.

Feeding and health management

The feeding requirements of animals were calculated according to their body weight, with daily feed Although the production system and marketing are offering on dry weight basis of 3% of body weight. The feeding regime per bull consisted of Napier grass and 5 to 6 kg of protein-energy concentrate mixture.

Water was provided *ad libitum* to the bulls via a water point. Regular vaccination was carried out; twice a year in June and December against hemorrhagic septicemia; and foot and mouth

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Table 1. Mean temperature recorded by Rwanda meteorological service.

Seasons	Maximum temperature (°C)	Average temperature (°C)	Minimum temperature (°C)
Long rain (March-May)	25	20	15
Long dry (June-September)	27	22	15
Short rain (October-November	26	21	15
Short dry (December-February)	26	21	15

Source: Rwanda meteorological service.

disease in February and September; and once a year in April against black quarter. Preventive measures against external and internal parasitic infestation were also undertaken three times a year. Multivitamins were given once a month by intramuscular injection.

Climate

The season condition of the study area was considered as indicated in Table 1.

Semen collection and processing

Semen collection was undertaken twice a week. Bulls to be used on a particular day went through preparatory measures including showering, drying and cleaning, brushing, grooming and half an hour exercise. The bulls were then allowed to mount a teaser bull and the semen was collected with the help of a pre-warmed (42 to 45°C) artificial vagina. Ejaculate volume was recorded directly from the graduated semen collection tube. Each ejaculate was examined for mass motility, individual motility and sperm concentration and ejaculates having less than 50% sperm motility were discarded. The fresh semen was then diluted depending upon microscopic evaluation of sperm concentration at 32°C with tris, citrate, fructose, egg yolk. All collections were performed early in the morning between 6.00 and 8.00 h. After collection, the tubes containing semen were immediately transferred from the collection room to the laboratory and placed in a water bath at 36°C to maintain the temperature of sperm cells. The characterizations of semen require laboratory tests and depend on norms and standards fixed by the management of the station. After the initial observations, the semen was diluted using andromed extender (AndroMed®, Tiefenbach, Germany).

Characteristics of semen after collection

The collected semen was firstly examined with naked eyes to observe the colour and was then confirmed from a published colour chart; and then the volume was read off from a graduated semen collecting tube. The semen was then transferred to a water bath at 33-36°C. Microscopic evaluation was done using a microscope (Minitube, Germany), for mass activity. Individual motility and determination of concentration was then done with a calibrated photometer (Minitube, Germany). Mass motility was then tested by placing a small drop of semen (approximately 5 µl) on a slide using a microscope at 10x magnification. Another small drop was deposited on the slide and carefully placing a cover slip over it and then was visualized at 40x to observe individual motility. A score was given on a hedonic scale of 0 to 4 for the mass motility and percentage for individual motility by each of three independent observer technicians. The scores were not significantly different and were then summed up and mean computed.

To continue the process, the minimum mass motility score needed was 3, and 60% for individual motility, also the colour was scored on a scale of 1 to 3 (Javed et al., 2000). Score 3 was given to semen with a high concentration of sperm cells, and this was characterized by a creamy consistence, and also marked as good semen. Score 2 was given to semen with tolerable concentration of sperm cell, and was characterized by a milky consistence. Score 1 was given to semen with a very low concentration of sperm cells; and characterized by a watery consistence. Score 0 was given to ejaculate without sperm cells and characterized by a clear watery consistence. The semen with score 0 and 1 was discarded.

Data analysis

The data were typed into spreadsheets of the MS-Excel software. Preliminary analysis was conducted using Chi-square procedure of statistical analysis system (SAS, 2004), to determine whether there were significant differences between the levels of the class variables. This was because the number of animals used as the samples were few and possibly, the small sub-class numbers could hinder the effects of the independent variables to be quantified. On finding significant differences (Table 2), further analyses particularly, least square means were then computed using the generalized linear models of statistical analysis system (SAS, 2004) and were separated using Duncan's multiple range test. The results were presented as means ± standard error of the mean (S.E.M.). Data on scrotal circumference was not collected hence correlation with semen volume could not be done.

RESULTS

The results showed that ejaculate volume, mass motility, individual motility and post freezing motility significantly differed ($p < 0.05$) among the three cattle breeds studied (Table 2). Friesian bulls had superior ($p = 0.001$) semen volume to that of Jerseys and Inyambo. However, Friesian bulls had an inferior ($p= 0.003$) semen colour score (3.12) to that of Jersey (3.31) and Inyambo (3.29), but the latter two did not differ (Table 2). Only post freeze motility was not affected by time factor. The short rain season (October to November) gave the best semen quality characteristics but was not significantly ($p=0.838$) better than the long rains except in mass motility and post-freeze motility. Semen volume (4.61) and post-freeze motility (38.51%) were poorest in the long rainy season (March to May) (Table 3).

The results of the study indicated a significant interaction between breed and season in the effect on ejaculate volume ($p = 0.003$), libido score ($p = 0.0014$),

Table 2. Least square means of various semen quality parameters as influenced by breed.

Parameter	n	Bull breed				
		Friesian (n = 946)	Jersey (n = 371)	Inyambo (n = 174)	GLM p-Value	χ^2 p-Value*
Volume (ml)	1468	5.76±0.08[a]	4.29±0.09[b]	3.37±0.1[c]	***	<0.001
Libido score	1463	3.14±0.01[a]	3.97±0.01[b]	2.81±0.03[c]	***	<0.001
Semen color (%)					*	<0.001
Creamy	854	52.7	65.2	64.9		
Milky	456	31.9	25.3	34.5		
Watery	62	4.3	5.4	0.6		
Yellowish	99	11.1	4.1	0		
Semen concentration (10^9 spz/ml)	1471	3.12±0.03[b]	3.31±0.05[a]	3.29±0.07[a]	***	<0.001
Mass motility (%)	1360	2.67±0.03[c]	3.05±0.04[a]	2.90±0.06[b]	***	<0.001
Individual motility (%)	1400	62.04±0.6[b]	68.14±0.9[a]	66.01±1.04[a]	***	<0.001
Live-dead Percentage (%)	1375	35.96±0.5[a]	29.37±0.7[b]	34.12±1.05[a]	***	<0.001
Post freezing motility (%)	762	40.43±0.5[b]	43.45±0.9[a]	42.20±1.1[a]	**	0.0012

[abcd] Values within the same column with different superscripts differed significantly { ***(p<0.001); **(p<0.01); * p<05)}. The Chi-square analysis was done at preliminary stage, to justify further ANOVA analysis.

Table 3. Influence of season on semen quality.

Parameters	Season[ϕ]				*P.* value
	Long rain season (n = 274)	Long dry season (n = 380)	Short rain season (n = 401)	Short dry season (n = 389)	
Volume (ml)	4.61±0.15[b]	5.31±0.12[a]	5.01±0.09[a]	5.28±0.13[a]	***
Libido score	3.05±0.04[b]	2.97±0.02[c]	3.0±0.09[c]	3.22±0.02[a]	***
Semen color (%)					
Cream (%)	59.5	53.5	67.5	49.7	
Milky (%)	35.6	34.4	21.2	36.6	
Watery (%)	4.9	3.8	5.0	3.1	
Yellowish (%)	-	7.0	6.3	9.8	
Semen concentration (10^9spz/ml)	3.14±0.08[b]	3.11±0.04[b]	3.36±0.04[a]	3.07±0.05[b]	***
Mass motility (%)	2.71±0.06[b]	2.58±0.04[c]	2.86±0.04[a]	2.94±0.04[a]	***
Individual motility (%)	65.29±1.17[a]	63.79±0.78[b]	62.09±0.98[a]	65.85±0.78[a]	**
Live-dead Percentage (%)	34.70±1.17[ab]	36.06±0.79[a]	32.7±0.74[c]	33.97±0.78[ab]	*
Post freezing motility (%)	38.51±1.41[b]	42.45±0.76[a]	42.85±0.78[a]	39.68±0.96[b]	**

[abcd] Values within the same column with different superscripts differed significantly { ***p<0.001); **(p<0.01); * p<05)}. [ϕ]A: Long rain season (March-May); B: Long dry season (June-September); C: Short rain season (October–November); D: Short dry season (December- February).

semen concentration, mass motility (p = 0.0014) and post freeze motility (p = 0.0009). Age of the bulls negatively affected (p=0.025) all the parameters studied, with semen collected during 2011 possessing better attributes than that collected in 2012. While the semen volume dropped with age between 2011 and 2012, from 5.16 to 5.10 ml, semen colour also lightened from a 3.28 hedonic scale score to a 3.11 score. Over the test period, semen mass motility score reduced from 2.98 to 2.65, while the proportion of live sperm in ejaculates dropped from 65 to 63% (Table 4).

DISCUSSION

Normal reproduction in male livestock is measured by the production of semen with normal and adequate spermatozoa, as well as the desired ability to mate. These sexual functions (sexual development, production of spermatozoa and desired ability to mate) are under the

Table 4. Influence of year on semen quality.

Parameter	Years		
	2011 (n = 568)	2012 (n = 823)	P-Value
Volume (ml)	5.16±0.09	5.10±0.08	
Libido Score	3.27±0.02[a]	2.95±0.01[b]	***
Semen colour (%)			*
Cream (%)	62.8	54.0	
Milky (%)	21.5	37.5	
Watery (%)	6.5	2.4	
Yellowish (%)	8.2	5.7	
Semen concentration (10^9 spz/ml)	3.28±0.04[a]	3.11±0.03[b]	***
Mass motility (%)	2.98±0.03[a]	2.65±0.03[b]	***
Individual motility (%)	65.08±0.8[a]	63.12±0.5[b]	**
Live-dead %	30.44±0.6[b]	36.76±0.5[a]	**
Post freezer motility (%)	40.50±0.8[a]	41.93±0.5[a]	*

[abcd] Values within the same column with different superscripts differed significantly { ***(p<0.001); **(p<0.01); * p<05)}.

control of gonadotrophin hormones such as testosterone, LH and FSH. These gonadotrophins are influenced to a larger extent by a combination of environmental fctors such as temperature, nutrition and animal management practices (Vilakazi and Webb, 2004). Temperature is one of the important factors affecting reproduction. Periods of high temperature damage the spermatogenic cells and lead to testicular degeneration, reduction in the efficiency of spermatogenesis and hence poor semen quality (Vilakazi and Webb, 2004). Season factors include temperature, photoperiod, humidity and feed quality (Andrabi et al., 2002; Barth and Waldner, 2002; Menon et al., 2011). Differences in the quantity of feed or in feed composition, environmental temperature, humidity and seasonal variation do affect semen output (McDowell, 1972; Castillo et al., 1987; Soderquist, 1996; Koivisto et al., 2009).

In the area where there is marked seasonal variation in environmental temperature, bull semen quality tends to be lower during summer or in tropical terms, warm months (Curtis, 1983; Vilakazi et al., 2004) as this results in thermal stress. Thermal stresses cause testicular degeneration, abdominal scrotal thermogram and hence lower the semen output (McDowell, 1972; Curtis, 1983). Significant seasonal variations are also observed in the incidence of sperm head abnormalities and total sperm abnomalities; and appear to be strongly associated with dry seasons (spring and summer) as compared to rain seasons. The results of this study indicated that a higher percentage of normal sperm occured during short rainy season and this was in agreement with Mathevon et al. (1998) where short rain (spring) season was also associated with higher percentage of normal sperm regardless of the age and breed of the bull.

In this study, age significantly affected semen characteristics. Previous studies also reported that the bulls aged 36 to 48 months were found to produce sperm of better morphology than bulls of 72 months age and older (Vilakazi and Webb, 2004). This succinctly shows the importance of bull age on influencing semen morphological traits. Several researchers have attributed similar observations to the scrotal circumference, the regulation balance mechanism, fat deposition in the brain and reproductive tissues which affect semen production and quality (McDowell, 1972; Salisbury et al., 1978; King, 1993; Coe, 1999; Mamabolo, 1999; Brito et al., 2002). Dairy bulls reach puberty at the age of 12 months (Bearden and Fuquay, 1997; Vilakazi and Webb, 2004) and attain maturity at the age of 3 to 4 years (Almquist, 1982). These studies found that bulls recorded higher sperm defects prior to maturity. This is probably due to the fact that in younger bulls, the testicles are still developing and hence the semen in the ejaculate is of low quality (Coulter and Foote, 1979; Vilakazi and Webb, 2004). Lower semen outputs in older bulls may be associated with the degenerative changes in seminiferous tubule (Coe, 1999), fat deposition which may take place in scrotum (Salisbury et al., 1978; King 1993) and the break down of body tissues, particularly, testicular tissues (King, 1993) with advancement in age. Fat deposition as a bull progresses in age may take place around the scrotum. This may affect semen quality by reducing the heat radiation capacity from the scrotal neck (Brito et al., 2002). Indeed, supportive findings by Vilakazi and Webb (2004) reported that the semen quality starts to deteriorate in bulls older than 72 months.

This deterioration may be associated with the accumulative hazards of life including non-specific infections, nutritional stress, diseases and accidents, which all combine to cause the direct positive relationship

between semen quality and age to disappear (Salisbury et al., 1978; Vilakazi and Webb, 2004; Christensen et al., 2011). Its therefore very interesting for us to report that age as a factor is a necessary evil and paradoxical, in that at a young age, low age is disadvantageous and leads to poor quality semen, in mid-age, the age effect becomes positive, with bulls producing high grade/quality semen, before the bulls age effect becomes negative, causing the production of abnormal and sub-standard semen. Studies that have combined the effects of age and season have found that younger bulls recorded poor semen morphology during winter, while old bulls showed poor morphology during long dry season (Vilakazi and Webb, 2004; Koivisto et al., 2009; Ghasemi and Ghorban, 2014).

The current study hence recognizes that breed, age and season and their interactions are important sources of variation in semen quality. This implies that for a successful artificial insemination programme, semen collection should be done at the younger age, from two years to a maximum of 5 years for all breeds. It is therefore recommended that age, breed and season should be given urgent attention in any bull management system employed in Rwanda in order to obtain the best semen quality.

Indeed, when breed effects are kept out of the picture, past studies (Vilakazi and Webb, 2004) showed that the summer season is associated with very poor semen quality and is not ideal for semen collection from bulls that are five years or older. Manipulation of temperature in dairy cattle through the provision of management practices to reduce the effect of heat stress on bull reproduction is hereby suggested as a primary tool in optimising the quality of semen harvested in AI dairy bulls in Rwanda.

CONCLUSION AND RECOMMENDATIONS

In conclusion, Friesian bulls had better semen quality, the short rainy season had the best semen quality, as was the semen collected earlier. Hence, the Friesian breed should be promoted at the bull station ahead of the Jersey. Most semen should be collected during the rainy season, particularly the short rains. Particularly, bulls below three years of age should be of focus. It can be recommended that the relationship between the nutrition, reproduction physiology and management should be given a high priority in dairy production systems and further studies in this regard should be undertaken.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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