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Age and Season Related Changes in Semen Quality of Horro Bulls

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Abstract

With the overall objective of selection and evaluation of bulls based on their actual fertility performances, a total of 119 semen ejaculates were collected for seven months from 19 Horro bulls, aged between 36 and 74 months. The bulls were divided into two age groups (36-51and 61-74 months). Depending on the availability of feeds, the experimental period (August-February) was also classified into two seasons, viz wet season (August-November) and dry season (December-February). The semen ejaculates were collected monthly using artificial vagina (A.V.) method and were subjected to evaluation by inspection and microscopic examination. Data were analysed by General Linear Model Procedure (SAS, 1996) using fixed effect model. Simple correlation analysis was used to determine the interrelationship of age and the seminal traits considered. The seasonal differences in most of the semen traits were statistically not significant except in mass motility ($p<0.05$). But the differences due to age were statistically significant in ejaculate volume ($p<0.01$) and mass motility ($p<0.05$). There was positive correlation between age and volume ($r=0.37$; $p<0.001$) and age and live sperm counts ($r=0.36$; $p<0.001$). Higher volume of semen was positively and significantly related with mass motility ($p<0.001$), concentration ($p<0.05$) and total sperm production ($p<0.001$). There is also significant correlation between sperm motility and concentration and total sperm production ($p<0.001$). This study revealed that the bulls' actual fertility performances are of paramount importance in selection and evaluation programme of breeding bulls. However, as the study was not exhaustive across all seasons and age future research should include these and other factors that likely affect semen quality.

Keywords: semen quality, reproduction, age, season, and Horro bulls

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Introduction

The process of selection and use of breeding bulls is very important if optimum fertility is to be obtained and depends on whether they are intended for natural mating or artificial insemination (A.I.). In all cases, however, breeding bulls must be superior not only in their genetic potential, but also in their semen qualities (Perera, 1999). This is because the quality of semen reflects the actual performance of the bull and the efficiency of breeding herd. The major portion of progress in genetic improvement is currently made through selection of appropriate breeding bulls, because sires have more offspring than cows and their breeding value can be more accurately determined. Bulls with high genetic merit and high fertility will enhance genetic progress and improve cowherd fertility. On the other hand, the male with reduced fertility poses serious problems and causes economic losses to breeders and the artificial insemination industry.

Attempts made to develop an accurate and objective test for assessing the potential fertility of a bull on the basis of some specific characteristics of a given semen sample have not been successful (Raja and Rao, 1983). The consensus of opinion prevailed was that a combination of semen characteristics such as ejaculate volume, mass motility, concentration and liveability of spermatozoa and the magnitude of incidence of spermatozoa abnormality will serve the purpose of a single sure test to select bulls of high fertility from a mixed farm (Maule, 1962; Raja and Rao, 1983). There are different views on the effects of season and temperature on semen quality and fertility of a bull. Several workers (Sane *et al*., 1982; Sekoni *et al*., 1988; Juinudeen and Hafez, 2000) reported seasonal differences in sperm quality and fertilizing ability of semen. Working on Sahiwal bull semen, Ulfina, (2002) noted significant age differences in some of the semen characteristics. Since semen characteristics are known to vary, it is essential to determine the normal values for each breed or genotype. Therefore, this study was carried out to assess the effect of age and season on the semen characteristics of indigenous Horro bulls.

Materials and Methods

The study was carried out at Bako Agricultural Research Centre, western Oromia, Ethiopia from August 2001 to February 2002. The centre is situated at an altitude of 1650 m above sea level and located at 9^0 07' North and $37^0$05'

East. The rainfall pattern of the farm is uni-modal amounting to 1200 mm and extends from March to October. The mean annual temperature is 21^{o}C.

Nineteen Horro bulls kept for breeding purpose (aged between 36 and 74 months) were used. They were allowed to graze together on natural pasture during the day (9:00AM-5:00PM) and received concentrate supplements in-group feeding pen during the night, at the rate of 2 kg/head/day. The formulation of the concentrate supplementation was noug (*Guizotia abssynica*) seed cake (49%), maize grain (49%), common salt (1%) and bone meal (1%). After a preliminary period of training the bulls, semen was collected by artificial vagina (A.V.) method from the bulls at monthly intervals over a period of 7 months. A total of 119 ejaculates for colour and volume and 112 ejaculates for mass motility, concentration and total sperm production were examined. The following semen traits were assessed.

Colour and consistency, ejaculate volume and mass motility

The scoring method of Hafez and Hafez (2000) was used for the physical appearance of semen and a 1 to 5 scoring was given by judging as clear (watery), milky, thin creamy, creamy or thick creamy, respectively. The volume of ejaculate semen was read to the nearest 0.1ml directly from the graduated collecting tube. Mass motility was evaluated as per method described by Tomar *et al.,* (1996). Briefly, a drop of thick layer of neat semen was placed on a clean slide and examined under low power (10X) objective microscope. Normal bull semen exhibits a wave like motion when viewed in reflected light. On the basis of swirling current, semen was rated into eleven categories and numerical grading from 0 to 5 was given accordingly.

Sperm concentration and total sperm production per ejaculate

The concentration of spermatozoa was determined by Haemocytometer method. Sperm concentration estimate by using Haemocytometer was made by diluting semen samples 1000 times with 4 % (NaCl) saline solution, in serial dilution method. A pinch of eosin dust (0.05%) was added to the saline solution to give background to spermatozoa. A Haemocytometer cover slip was placed over the ruled field of Neubar's chamber and a drop of diluted semen was allowed to run under the cover slip without floating and allowed to settle for one minute. The spermatozoa in four corner and one middle secondary squares, i.e. 80 tertiary squares were counted under 400 X magnification using a phase contrast microscope. The sperm concentration is expressed in million per millilitre of semen. Total sperm production per ejaculate is a product of

sperm concentration and volume of ejaculates. This figure gives the total number of cows that could be inseminated per breeding bull per year.

Live sperm counts and abnormality of spermatozoa

Percentage of live sperms was determined from eosinophilic reaction using eosin-nigrosin stain. Eosin-nigrosin stain was prepared by the method described by Bloom (1950) and Hancock (1951). The composition of the stain was; 100 mg eosin (B), 500 mg nigrosin and 10 ml distilled water. One drop of semen sample was mixed with 2 to 3 drops of eosin-nigrosin stain on a clean slide. About 200 spermatozoa were assessed in different fields. Live spermatozoa appear unstained and dead stain pink against a brownish purple background. Percentage of live spermatozoa was calculated as:

$$\text{Live \%} = \text{No. of unstained spermatozoa} / \text{Total No. of spermatozoa}$$

The type of abnormal sperm and their incidence was estimated from the slides prepared with eosin-nigrosin stains for eosinophilic sperm counts. The types of abnormality (head, mid piece and tail) were summed up and reported as total abnormality of spermatozoa.

Statistical analysis

The data on different semen characteristics were analysed by GLM Procedure (SAS, 1996) using fixed effect model taking season and age group as fixed effects. Simple correlation analysis was used to determine the interrelationship between age and the seminal traits considered.

Table 1. Least squares means (±SE) for semen characteristics of Horro bulls in different seasons and age groups

Effects	Color and consistency	Volume (ml)	Mass motility	Concentration (million)	Total sperm production (million)
N	119	119	112	112	112
Overall	3.53±0.22	4.23±0.12	4.01±0.11	714.65±38.72	3199.87±230.20
Season	NS	NS	*	NS	NS
Wet season (Aug-Nov)	3.48±0.12	4.35±0.24	3.82±0.18	781.32±57.12	3647.25±338.96
Dry season (Dec- Feb)	3.76±0.43	4.19±0.26	4.16±0.19	628.83±59.14	2742.23±351.90
Age group	NS	**	*	NS	NS
G_1 (36-51 month)	3.64±0.18	3.66±0.24	4.31±0.18	781.80±55.31	3071.22±330.13
G_2 (52-74 month)	3.32±0.20	4.87±0.26	3.67±0.19	628.35±60.77	3318.26±361.31

*=p<0.05; **=p<0.01, **=p<0.001; NS = not significant

Results

Least squares means, analysis of variance and correlation coefficients of semen characteristics of Horro bulls are presented in Tables 1, 2 and 3, respectively.

Colour and consistency, ejaculate volume and mass motility

Horro bulls were found to produce watery, yellowish, milky and creamy semen. On average, the bulls gave milky to creamy colour with the mean value of 3.5±0.22. The overall mean volume of semen per ejaculate was 4.23±0.12 ml. The seasonal difference in volume of semen was not significant. But the mean value recorded for age group 2 was significantly higher (P<0.01) than bulls in-group 1. The mean mass motility recorded was 4.01±0.11. A significant (p<0.05) difference in mass motility was observed between both the two age groups and season of collection.

Sperm concentration and total sperm production per ejaculate

The average sperm concentration in the current study was 714.65±38.72 million per ml of semen. No significant variation in sperm concentration was observed either between seasons or age groups. The mean value of total sperm production was found to be 3199.88±230.20 million per ejaculate of semen. As this is essentially the same trait with sperm concentration, there was no significant variation observed in both seasons and age groups. Although not significant, it was evident from the result of correlation analysis that the total sperm production per ejaculation was higher in older bulls (r=0.09).

Table 2. Analysis of variance and level of significance of semen characteristics of Horro bulls

Source	df	Color and consistency	Ejaculate volume	Mass motility	Sperm concentration	Total Sperm Production
Age group	1	1.6090	43.1637**	3.33550*	$6.49x10^{18}$	$1.24x10^{19}$
Season	1	3.1295	0.80735	11.2829*	$6.53x10^{18}$	$6.64x10^{18}$
Error		0.9164	3.9406	2.0099	$1.88x10^{17}$	$6.647x10^{18}$
Error df		116	116	109	109	109

*=P<0.05, **=p<0.01

Live sperm count and sperm abnormality

The mean percentage of live sperm in the ejaculate was 92.0 % (range from 78 to 100%). The average percentage of sperm abnormality was 22.6% (range from 10 to 35%). Neither age nor season of collection was found to exert significant effect on the occurrence of abnormalities of spermatozoa in the

ejaculate. Similarly the correlation analysis showed positive and non-significant relation between age and abnormality of spermatozoa.

Table 3. Pearson correlation coefficients of semen quality traits in Horro bulls

	1	2	3	4	5	6	7
Age =1	-						
Vol =2	0.37***	-					
Mot =3	-	0.25**	-				
Conc =4	-	0.19*	0.50***	-			
Tsp =5	-	0.65***	0.39***	0.79***	-		
Live =6	0.36***	-	-	-	-	-	
Tabp =7	0.006 NS	-	-	-	-	-0.64***	-

Vol=volume, Mot=motility, Conc=concentration, TSP=total sperm production, Live=live sperm counts, Tabp=percentage of total abnormalities, cons = consistency

*=p<0.05, **=p<0.01, **=p<0.001, NS = not significant (p>0.05)

Discussion

Colour and consistency, ejaculate volume and mass motility

The physical appearance of semen at the time of collection has due importance in judging the quality of semen because it is the number of sperm cells in a given volume of semen that affects its appearance. Horro bulls were found to produce watery, yellow, milky and creamy semen. On average the bulls were giving milky to creamy colour with the mean value of 3.9±0.23. Neither seasonal nor age group differences were significant. Similar results were reported on Indian Sahiwal bulls (Ulfina, 2002).

The overall mean volume of semen per ejaculate (4.23±0.12 ml) reported in this study is higher than the value reported by Raja and Rao (1983) and Mathew (1974) for crossbred bulls and Ulfina (2002) for both indigenous Indian Sahiwal and crossbred bulls. The seasonal difference in volume of semen was not significant (P>0.05). But the mean value recorded for age group 2 was significantly higher (P<0.01) than bulls in-group 1. This is in agreement with earlier report by Ulfina (2002) who found significantly higher values of ejaculate volume for older age group than the younger bulls.

Mass motility has been considered essential to provide spermatozoa transport through the female reproductive tract and is essential for fertilization. Normal bull semen exhibits a wave like motion when viewed in reflected light. This activity is observed when a thick layer of neat semen is viewed on a microscopic slide without a cover slide. The mean mass motility

recorded in this study was 4.01±0.11. A significant ($p<0.05$) difference observed between the two age groups in mass motility is in agreement with the report of Ulfina (2002) for Indian Sahiwal bulls. The mass motility of spermatozoa was significantly influenced by season of collection, which is at variance with earlier observation by Rao and Rao (1978) in crossbred bulls. According to Tomer *et al.,* (1966), humid hot season does not appear to be conducive for the production of semen with high mass motility. But the humidity of Bako Agricultural Research Center was moderate throughout the experimental period, as it excludes the peak months of dry and rainy season, and this might be the reason for the production of semen with high mass motility inboth season.

Sperm concentration and total sperm production

Accurate determination of the number of spermatozoa per milliliter of semen is extremely important, as it is a highly variable semen characteristic. The number of sperms per unit volume of semen varies from zero in complete azoospermia to over three billion sperms per ml in occasional very dense samples. When combined with volume of the ejaculate, this quantity of the spermatozoa determines how many females can be inseminated, each with the optimal number of sperm cells. The average sperm concentration recorded in this study was (714.65±38.72 million per ml of semen) lower than most of the reports available in this regard (Mathur *et al*., 2002; Shanmugavel and Singh, 2002; Ulfina, 2002) for Friswal and Sahiwal Indian bulls, but higher than the reports of Madrid *et al.,* (1993) and Usmani *et al.,* (1993) in crossbred and Holstein Friesian bulls, respectively. No significant variation in sperm concentration was observed either between seasons or age groups. Different to our findings, Ulfina (2002) noted a significantly higher sperm concentration for older age groups for Indian Sahiwal bulls.

The mean value of total sperm production was found to be 3199.88±230.20 per ejaculate of semen. As this is essentially the same trait with sperm concentration, there was no significant variation observed in both seasons and age groups. Although not significant, it was evident from the result of correlation analysis that the total sperm production per ejaculation was higher in older bulls ($r=0.09$).

Live sperm count and sperm abnormality

The mean percentage of live sperm in the ejaculate was 92.0 % (range from 78 to 100%). This is higher than the values reported by Raja and Rao (1983)

and Ulfina (2002) for Friswal and Sahiwal bulls, respectively. The average percentage of sperm abnormality was 22.6% (range from 10 to 35%). This value is by far higher than the sperm abnormality reported by Raja and Rao (1983). Hafez and Hafez (2000) reported a typical decline in fertility when abnormal sperm cells exceed 20%. Since there was variation between bulls, it requires a systematic evaluation and selection of bulls before using them for breeding purposes. Neither age nor season of collection was found to exert significant effect on the occurrence of abnormalities of spermatozoa in the ejaculate. This inference is in agreement with that of Rao and Rao (1978) in crossbred bulls. But the correlation analysis showed positive and non-significant relation (r=0.006) between age and abnormality of spermatozoa.

Conclusion

In general, the overall values founf in the present study indicate that most of the semen traits considered are in agreement with the standard fertility parameters in the literature. Significant age and season variation was observed in some semen characteristics of Horro bulls. Hence it can be evident from the study that the bulls' actual fertility performances are of paramount importance in selection and evaluation programme of bulls for breeding purpose. However, as the study was not exhaustive across all seasons and age groups future reserach should include these and other factors that likely affect semen quality.

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