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Research Article

Evaluation of semen in Ethiopian Indigenous bulls (Horro bulls)

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ABSTRACT

Semen ejaculates (n = 38) were collected at weekly interval from three Horro bulls kept at Kaliti Artificial insemination (AI) center, Ethiopia for thirteen weeks to evaluate the ejaculate for its physico-morphological and biochemical parameters. The overall mean (SE) value for volume 5.28 (0.24) ml, mass motility 3.17 (0.12), individual motility 69.36 (1.76 %), concentration 1.34 (0.06) billions/ml, total count 7.03 (0.41)billions/ejaculate, viable number 4.85 (0.29)billions/ejaculate, total morphologically normal number 6.64 (0.42) billions/ejaculate, live percentage 81.47 (1.2), morphologically normal per cent 94.1 (0.5), per cent normal acrosome 98.23 (0.33), GOT 1475.1 (69.96) U/l, GPT 150.8 (19.24) U/l, ALP 2674.3 (202.45) U/l, ACP 4503 (309.7) U/l, total protein 7.3 (0.3) g/dl, testosterone 1.21 (0.18) ng/ml, head abnormality 1.81 (0.20) %, midpiece abnormality 2.60 (0.35) %, tail abnormality 1.50 (0.18) %, total abnormality 5.90 (0.51) %, major abnormality 3.02 (0.29) %, minor abnormalities were 2.9 (0.33 %).During the study it was found that semen of Horro bulls has very less numbers of the different types of spermatozoa morphologic abnormalities compared to semen features set for normal fertile bulls.

Key words: Ethiopia, Horro bull, physico-morphological, biochemical, semen, spermatozoa

Failure of many bulls to consistently and efficiently breed has been reported to be associated with the production of poor quality semen, seasonal changes in semen quality, high incidence of abnormal spermatozoa and problems in sexual behaviors that reduce the fertility of the bull (Hafez, 1993). Semen examination has been known to have great diagnostic value in determining the cause, severity, and degree of testicular or accessory gland pathology or infertility, as well as in estimating the fertility of male, as there has been found a definite correlation between testicular pathology, disease of the reproductive tract and accessory gland, and the semen characteristics and fertility (Donald, 1980).

No single measurements of seminal quality have been found as a reliable criterion for predicting fertility that necessitates incorporation of many useful measurements of seminal characteristics within the limits of practicality (Donald, 1980). In Ethiopia, currently the Horro bulls are kept at artificial insemination center for genetic conservation and for artificial insemination. But the information on semen characteristics and fertility of these bulls is lacking. The main objective of this study is therefore, to evaluate the semen physico-morphological and biochemical characteristic in Horro breed bulls kept at AI center, Kaliti, Ethiopia.

MATERIALS AND METHODS

The study was conducted at National Artificial Insemination Center (NAIC), which is located at Kaliti, Addis Ababa, Ethiopia. The place is located at 38° 45' 52" East longitudes and 8° 54' 12" North latitude. Three Horro were selected for this particular study. All the bulls were kept intensively under the same management conditions being given 2 kg concentrate and 9 to 10 kg hay per day, mineral lick every 1.5 to 2 months during dry period (1.25 kg/ bull) and green fodder at the time of availability.

Semen was collected weekly for thirteen weeks from first week of october to end of december following the optimal recommended procedures (Bhosrekar, 1990).

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Semen was collected by using the artificial vagina and only the first ejaculate was used for the study. A total of 38 semen samples were collected and analyzed for physico-morphological analysis.

Physical examination of the semen and the spermatozoa

Immediately following collection, the semen was examined for appearance, volume, and presence of foreign materials, mass and individual motility, live/dead count, spermatozoa morphology, concentration, sperm total count, viable number (per cent motile multiplied by total count) and abnormality of the spermatozoa following the standard procedures. Proportions of live spermatozoa were determined on smears prepared from fresh semen by the Eosin-Nigrosin technique. Sperm dimension measurements were done by measuring the head, midpiece and tail of the spermatozoa.

Biochemical analysis of enzymes, total protein and testosterone in seminal plasma

All the ejaculate were centrifuged at 3000 rpm for 10 minutes and seminal plasma was separated from spermatozoa and preserved at -20 °C until analysis. Alanine aminotransferase (ALT/GPT) and Aspartate aminotransferase (AST/GOT analyses were carried out by method described by Bergmeyer and Harder (1985a and 1985b), Alkaline phosphatase (ALP) analysis was carried out as per method described by Tietz (1987), Acid Phosphatase (ACP) analysis was carried out by Hillmann calorimetric method (Hillmann, 1971), Total protein analysis was carried out as per method described by Peters (1968) and Testosterone enzyme immuno assay was made using testosterone enzyme immuno assay kit (Linear Chemicals, S.L. 08390 Montgat Barcelona, Spain).

Analysis of the data were done using descriptive statistics, percentage values, confidence interval and one sample t-test on SPSS (2002) statistical package and STATA (2001) statistical package.

RESULTS AND DISCUSSION

The various physico-morphological and biochemical parameters of Horro bulls semen are presented in Table 1. The mean (SE) volume of semen of Horro bulls observed in this study was 5.28 (0.24) ml which agrees well with the semen volume reported by Dhami *et al.* (1998) and Shelke and Dhami (2001). On the other hand the present value is siginificantly ($P < 0.01$) lower than the semen volume reported by Brito *et*

Table 1: Physico-morphological and biochemical parameters of Horro bulls' semen.

Parameters	Mean (SE)
Volume (ml)	5.28 (0.24)
Mass motility (0-5)	3.17 (0.12)
Individual Motility (%)	69.36 (1.76)
Concentration (10^9/ml)	1.34 (0.06)
Total count (billions/ ejaculate)	7.03 (0.41)
Viable number (billions/ ejaculate)	4.85 (0.29)
TMN (billions/ ejaculate)	6.64 (0.42)
Live percentage	81.47 (1.2)
MN (%)	94.1 (0.5)
Normal acrosome (%)	98.23 (0.33)
Head length (µm)	9.22 (0.03)
Head breadth (µm)	4.63 (0.01)
Midpiece length (µm)	13.43 (0.05)
Tail length (µm)	48.17 (0.14)
GOT (U/l)	1475.09 (69.96)
GPT (U/l)	150.82 (19.24)
ALP (U/l)	2674.27 (202.5)
ACP (U/l)	4503 (309.7)
Total protein (g/l)	7.3 (0.29)
Testosterone (ng/ml)	1.21 (0.18)

TMN = Total morphologically normal, MN = morphologically normal.

Table 2: Different morphological abnormalities of spermatozoa in Horro bulls.

Type of abnormalities	Mean (SE)
Head abnormality (%)	1.81 (0.2)
Mid piece (body) abnormality (%)	2.6 (0.35)
Tail abnormality (%)	1.5 (0.18)
Total abnormality (%)	5.92 (0.51)
Major abnormality (%)	3.02 (0.29)
Minor abnormality (%)	2.9 (0.33)
Acrosome defect (%)	1.76 (0.33)
Narrow at base (%)	0.44 (0.11)
Abnormal contour (%)	0.05 (0.03)
Undeveloped (%)	0.66 (0.15)
Detached abnormal (%)	0.22 (0.1)
Pear shaped (%)	0.05 (0.03)
Small abnormal (%)	0.01 (0.01)
Abaxial (%)	0.49 (0.1)
Others (%)	0.03 (0.02)

TMN = Total morphologically normal, MN = morphologically normal.

al. (2002) in Bos indicus and in *Bos Taurus*. The semen volume observed in this report is siginificantly higher than ($P < 0.01$) the reports of Veeraiah *et al.* (1999), Adamou *et al.* (1996) and Ahsan *et al.* (2003). Such variability between reports on semen volume might be attributed to difference in age, breed, nutritional status, geographical location, season of the year the study covers, method and frequency of semen collection However, the range of values given for semen volume in the literature agrees well with the present result.

The mean (SE) mass motility of the spermatozoa of Horro bulls observed in this study was 3.17 (0.12) which agrees well with the reports of Shelke and Dhami (2001) and Dhami *et al.* (1998) who reported the spermatozoa mass motility as 2.96 in Gir and 3.43 in Friesian, respectively.

In this study the mean (SE) individual motility of Horro bulls was 69.36 (1.76) %. The individual motility reported by Hector and Oscar (1998), Shelke and Dhami (2001) and Rana and Dhami (2003) as 68.6 %, in dual purpose Mexican breeds, 67.87 % in Gir and 71.5 % in Gir, respectively agrees well with the present value. But the individual motility reported by Adamou *et al.* (1996) in Borgou, Omar (1997) in Zambian zebu and Veeraiah *et al.* (1999) in Ongole as 75.7 %, 79.33 % and 76.53 %, respectively were higher ($P < 0.01$) than the present value. The spermatozoa individual motility reported by Ahsan *et al.* (2003) as 60.55 % and 50.5 % in Sahiwal and Friesian-Sahiwal crossbred bulls was significantly lower than ($P < 0.01$) the present result.

The mean (SE) spermatozoa concentration observed in this study was 1.34 (0.06) billions/ml which agrees well with the spermatozoa concentration reported by Veeraiah *et al.* (1999) as 1.44 billions/ml in Ongole and Shelke and Dhami (2001) as 1.22 billions/ml. Wide ranges have been known for normal fertile bull spermatozoa concentration as 800 to 2000 (Hafez, 1993), 1000 to 3000 (Bearden and Fuquay, 2000) million per milliliter of semen. The variability of spermatozoa concentration with different workers reports could be due to variation in genotype, age, management, semen collection frequency and techniques.

The mean (SE) spermatozoa live percentage observed in this study was 81.47 (1.2) % which was significantly ($P < 0.01$) lower than values reported by Dhami *et al.* (1998) as 87.35 % in Friesian bull, and Omar (1997) as 89.47 % in bovine bulls and higher than values reported by Rana and Dhami (2003) and Ahsan *et al.* (2003) as 71.85 % in Gir, and 72.22 % in Sahiwal and 74.22 % in Friesian-Sahiwal crossbred bulls.

The mean (SE) per cent normal spermatozoa observed in this study was 94.1 (0.5) % which agrees well with the per cent normal spermatozoa recommended for normal fertile bull. The proportion of ejaculated spermatozoa that contain normal spermatozoa of 80 % or more has been known not to be associated with lowered fertility (Donald, 1980; Hafez, 1993). The mean (SE) per cent normal acrosome observed in the present study was 98.23 (0.33) % which was significantly ($P < 0.01$) higher than the value reported for normal acrosome by Rana and Dhami (2003) as 84.8 % in Gir.

The mean (SE) incidence of head, midpiece, tail, total, major and minor spermatozoa defects observed in this study are given in table 2. The spermatozoa of normal fertile bull has been recommended not to contain more than 20 % total morphologic abnormality, and individual head, midpiece and tail abnormality of 10 % or more (Donald, 1980; Hafez, 1993). In line with those ideas the different spermatozoa abnormalities observed in this study was appreciably low.

The mean (SE) seminal plasma levels of GOT, GPT, ALP, ACP, total protein and testosterone observed in this study were 1475.09 (69.96) U/l, 150.82 (19.24) U/l, 2674.27 (202.3) U/l, 4503 (309.70 U/l, 7.3 (0.29) g/dl and 1.21 (0.18) ng/ml, respectively. The GOT level reported by Singhal *et al.* (1978) as 545.1 U/l is significantly ($P < 0.01$) lower than the present value but the GOT level reported as 4825 U/l and 2068.8 by Saxena and Tripathi (1978) and Pandit and Garg (1980), respectively in crossbred bulls is significantly higher than ($P < 0.01$) the present value. The seminal plasma GPT level reported as 322.2 U/l and 212 U/l by Singhal *et al.* (1978) and saxena and Tripathi (1980) in crossbred bulls is significantly higher ($P < 0.01$) than the present value. The ACP and ALP level reported by Aguirre *et al.* (1988)

as 1268.1 U/l and 954.2 U/l in bovine bull and ALP level reported as 3427.8 U/l by Reddi and Raja (1980) are significantly lower and higher ($P < 0.01$) than the present value, respectively. The level of enzymes in seminal plasma varies based on the level of initial damage to the spermatozoa or subsequent damages to the spermatozoa following dilution or freezing (Roberts, 1971; Mann and Lutwak-Mann, 1981; Dhami and Kodagali, 1990). The present value for seminal plasma total protein agrees with the seminal plasma total protein reported by Hafez (1993) and Donald (1980). Normally 3 to 8 g/dl of the total protein has been known to be found in bovine seminal plasma (Setchell, 1991). The seminal plasma testosterone level observed in this study agrees with the previous work on seminal plasma testosterone level in seminal plasma of buffalo bull by Tuli *et al.* (1991) and Javed *et al.* (2000) who reported seminal plasma testosterone value as 1.41 ng/ml and 0.97 ng/ml, respectively. The seminal plasma testosterone level reported by Gunjam and Amann (1976) as 2.87 ng/ml is significantly ($P < 0.01$) higher than the present value. Testosterone level in serum of bulls has been found to vary based on age and level of sexual stimulation of bulls (1978; Donald, 1980) but the cause of variability in seminal plasma testosterone level of bulls needs further study.

The spermatozoa dimensional measurements observed in the Horro bulls in this study agrees with the former reports of Salisbury *et al.* (1978) in spermatozoa head lenghth, head breadth and tail length who reported the spermatozoa head length, head breadth and tail lenghth as ranging from 8-10 μm, 4-5 μm and 45-50 μm, respectively. It also agrees with reports of Prasad and Sinha (1985) in spermatozoa head length, reports of Ortavant *et al.* (1969) in the spermatozoa tail length. The present value of midpiece length was lower than reports of Ortavant *et al.* (1969) who reported spermatozoa midpiece length as 14.84 μm.

It could be concluded from this study that physicomorphological and biochemical characteristics of Horro bulls' semen is very much comparable to any other breedable semen from different breeds. The sperm abnormalities are appreciably less than other reports. Based on this study it could be advised to use liquid semen of Horro bulls for breeding purpose in Ethiopia.

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