The Indian Journal of Animal Reproduction; 27 (2): 54-60; Dec. 2006

Research Article

Seminal characterization of some elite Ethiopian indigenous breeds of bull

HUNDERRA SORI[1], SHIV PRASAD[2], EMIRU ZEWDIE[3]

Faculty of Veterinary Medicine, Addis Ababa University, Ethiopia

Received : December 20, 2005
Accepted : November 12, 2006

ABSTRACT

The semen of six indigenous bulls consisting of Barca, Boran, Horro and Sheko breeds maintained at National Artificial Insemination Center (NAIC), Kaliti, Addis Ababa, (Ethiopia) were evaluated for physico-morphological parameters, biochemical parameters (GOT, GPT, ACP, ALP, total protein) and hormonal assay (testosterone). Semen was collected for thirteen weeks, once a week by using artificial vagina. The overall mean (SE) value for the semen characteristics studied were volume 4.84 (0.20) ml, mass motility 3.15 (0.11), individual motility 68.72 (1.37) %, concentration 1.54 (0.07) billions/ml, total count 7.35 (0.47) billions/ejaculate, viable number 5.10 (0.33) billions/ejaculate, total morphologically normal number 7.12 (0.45) billions/ejaculate, live percentage 79.73 (0.65), percent morphologic normal 94.70 (0.38), per cent normal acrosome 96.99 (0.60), GOT 1530.91 (60.15) u/l, GPT 131.99 (9.36) U/L, ALP 3333.98 (608.84) U/L, ACP 8003.68 (716.06) U/L, total protein 7.38 (0.26) gm/dl, testosterone 2.84 (0.3) ng /ml. It could be concluded that seminal attributes were comparable to normal fertile bulls of other breeds based on the evaluation of fresh semen parameters.

Key words: Ethiopia, NAIC, indigenous breeds physico-morphological, biochemical parameters, semen, spermatozoa

Reproductive performance is one of the major determinants of cattle productivity in any production systems. The contribution of the bull either through the natural mating or AI where each bull represents half of the genetic composition of its progeny (Blezinger, 1999) and many cows can be inseminated with the semen of a single bull (Faulkner and Pineda, 1980; Hafez, 1993), and its contribution in the production of meat and milk is of great importance which necessitates evaluation of the productive and the reproductive traits of bulls before extensive use (Coulter and Foote, 1979). 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 (Roberts, 1971; Hafez, 1993; Blezinger, 1999). No single measurement of seminal quality has been found as a reliable criterion for predicting fertility that necessitates incorporation of many useful measurements of seminal characteristics (Faulkner and Pineda, 1980).Objective of this study was to evaluate the semen physico-morphological and biochemical characteristics in Ethiopian indigenous bulls.

MATERIALS AND METHODS

The study was conducted at National Artificial Insemination Center (NAIC), which is located at Kaliti, Addis Ababa. The place is located at 38°45' 52" East longitudes and 8°54' 12" North latitude. Six indigenous bulls were selected for this 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).

Semen was collected once a week for thirteen weeks. In all of the cases semen was collected by using artificial vagina, and only the first ejaculate was used for the study purpose and a total of 67 semen samples

[1]DVM, Part of his M.Sc. thesis, AAU, Faculty of Veterinary medicine, Debrezeit,

[2]Professor Gynaecology and Obstetrics College of Veterinary Sciences, G.B.Pant University of Ag. and Tech., Pant Nagar, PIN. 263145. Distt. U.S. Nagar, Uttarakhand, India (for correspondence)

[3] DVM, AI specialist, from National Artificial Insemination Center, Addis Ababa, Ethiopia.

were collected and analyzed for physico-morphological analysis, from which 62 samples were used to analyze enzymes and protein and 40 samples for testosterone analysis.

Physical examination of the semen and the spermatozoa

Immediately following collection, the semen was kept at 34 °C (Bhosrekar, 1990) in water bath (IMV, L'AIGLE, France) and examined grossly (for appearance, volume, and presence of foreign materials such as dust or pus), microscopically (for mass activity and individual motility, live/dead count and morphology of the spermatozoa) and concentration, sperm total count, viable number (percent motile multiplied by total count) of the spermatozoa following the recommended procedures (Salisbury *et al.*, 1978; Roberts, 1971; Morrow, 1986; Bhosrekar, 1990; Garner, 1991; Hafez, 1993). Sperm cell concentration was determined by using calibrated spectrophotometer (IMV, Technologies France) and Dipura (HAMLITON micro lab ®500B).

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

Seminal plasma was preserved at -20 °C until analysis of (GOT, GPT, ALP, ACP), total protein and testosterone. Alanine aminotransferase (ALT/GPT), Aspartate aminotransferase (AST/GOT), Alkaline phosphatase (AKP) and Acid phosphatase (ACP) analysis were carried out on Vitros 250 Chemistry system (Johnson Johnson, Ortho-clinical Diagnostic.Inc.100 Indiago Creek Drive Rochester, NY 14626-5101, USA) and total protein was carried out on SEAC ch 16 (Italy). Testosterone enzyme Immuno assay was made using testosterone enzyme Immuno assay kit (Linear Chemicals, S.L. 08390 Montgat, Barcelona, Spain).

Pooled mean was analyzed for semen characteristics by using the descriptive statistic, and bivariate correlation coefficient using SPSS (2002) statistical package, and the 95 % confidence interval of semen characteristics were analyzed using STATA (2001) statistical package.

RESULTS AND DISCUSSION

Total 67 semen samples were examined for physico-morphology, 62 samples for enzyme analysis and a random sample of 40 samples for testosterone are given as follows:

Physico-morphological analysis

The over all mean (SE) values of the semen physico-morphological analysis are given in Table 1.

In this study the mean (SE) semen volume was 4.84 (0.20). which is significantly lower ($P < 0.01$) than the semen volume reported in *Bos taurus* bulls (6.9 ml and 8.2 ml) in different years in Brazil and in *Bos indicus* in Brazil (Brito *et al.*, 2002), the value for the latter being 6.6 ml and 6.7 ml in different years. The semen volume reported by Ahsan *et al.* (2003) in Sahiwal bulls (3.64 ml) is significantly lower than this value. Such variability between reports on semen volume might be attributed to difference in age, breed, nutritional status, geographic location, season of the year the study covers, method of the semen collection procedure and frequency (Caroll *et al.*, 1963; Igboeli and Raka, 1971; Salisbury *et al.*, 1978; Tegegne *et al.*, 1992; Hafez, 1993; Blezinger, 1999). However, the range of values given for semen volume in the literature (Bhosrekar, 1990; Setchell, 1991; Sorensen, 1979; Hafez, 1993; Bearden and Fuquay, 2000) agrees well with the present.

In this study the mean (SE) value for spermatozoa

Table 1: Summary of semen physico-morphological characteristics of indigenous bull.

Parameters	Volume (ml)	Mass motility (0-5)	Individual motility (%)	Concentration (109/ml)	Total count +	Viable number +	TMN +	Live percentage	MN (%)	Normal acrosome (%)
Mean (SE)1	4.84 (0.20)	3.15 (0.11)	68.72 - 1.37	1.54 -0.07	7.35 (0.47)	5.1 (0.33)	7.12 (0.45)	79.73 (0.65)	94.70 (0.38)	96.99 (0.60)

Numbers in bracket indicate SE, TMN = total morphologically normal number, MN = percent morphological normal, + = billions/ejaculate

Table 2: Seminal plasma biochemical analysis.

Parameters[2]	GOT	GPT	ALP	ACP	Total protein (gm/dL)	Testosterone (ng/ml)
Mean (SE)[1]	1530.91 (60.15)	131.99 (9.36)	3333.98 (608.84)	8003.68 (716.06)	7.38 (0.26)	2.84 -0.3

[1] Numbers in bracket indicate SE, [2] all are in U/L unless specified.

Table 3: Morphologic abnormalities of spermatozoa.

Type of abnormalities[3]	Head abnormality	Midpiece[2] abnormality	Tail abnormality	Total abnormality	Major abnormality	Minor abnormality
Mean (SE)[1]	1.87 (0.15)	1.92 (0.22)	1.50 (0.18)	5.29 (0.38)	2.25 (0.19)	3.04 (0.28)

[1] Numbers in bracket indicate SE, [2] mid piece or body abnormality, [3] all are in percentage values.

Table 4: Different head abnormalities of spermatozoa.

Head and neck abnormalities[2]	Acrosome defect	Narrow at base	Abnormal contour	Undeveloped form	Detached abnormal	Pear shaped	Small abnormal	Abaxial implantation
Mean (SE)[1]	3.0 (0.60)	0.37 (0.09)	0.12 (0.04)	0.74 (0.15)	0.21 (0.09)	0.05 (0.02)	0.05 (0.03)	0.57 (0.11)

[1] Numbers in bracket indicate SE, [2] all are in percentage values.

mass motility was 3.15 (0.11). Ahsan *et al.* (2003) reported the mass motility of spermatozoa in Friesian-Sahiwal cross and Sahiwal bulls as 1.25 and 1.36 ,respectively which are significantly lower ($P < 0.01$) than the present value, and Veeraiah *et al.* (1999) reported the mass motility of spermatozoa as 2.89 in Ongole bulls which is significantly lower ($P < 0.05$) than the present observation. The mass motility of spermatozoa reported by Dhami *et al.* (1998) as 3.43 in Friesian bull and Adamou *et al.* (1996) as 3.85 in Borgou bull is significantly higher ($P < 0.01$) than the present value. On the other hand some researchers reported mass motility of spermatozoa as 2.96 (Shelke and Dhami, 2001) in Gir, which does not have significant difference with the present value.

The mean (SE) individual motility of spermatozoa of indigenous bulls in this study was 68.72 (1.37) %. The individual motility of spermatozoa reported by Ahsan *et al.* (2003) as 50.5 % and 60.55 % respectively in Friesian-Sahiwal cross and Sahiwal bulls, individual motility reported by Andrabi *et al.* (2002) as 55.0 % in Friesian-Sahiwal cross bulls are significantly lower ($P < 0.01$) than the present value. On the other hand, the individual motility of spermatozoa reported by Veeraiah *et al.* (1999) as 76.55 % in Ongole bulls, individual motility reported by Omar (1997) as 79.33 % in Zambian short horn zebu and individual motility reported by Adamou *et al.* (1996) as 75.7 % in Borgou bulls show strong significant difference with the present value ($P < 0.01$). The reports of Shelke and Dhami (2001) on individual motility of spermatozoa as 67.89 % in Gir and Hector and Oscar (1998) in dual-purpose Mexico bulls agree with the present value. It has been known than 40-75 % (Sorensen, 1979; Hafez, 1993) and 50-80 % (Bearden and Fuquay, 2000) of the semen of bulls has been found motile.

Present study found that the mean (SE) spermatozoa concentration of semen was 1.54 (0.07) billions/ml. This value is in line with spermatozoa concentration reported in *Bos indicus* in Brazil as 1.65 billion/ml (Brito *et al.*, 2002), concentration reported by Veeraiah *et al.* (1999) in Ongole bulls and that reported by Rana and Dhami (2003) in Gir as 1.61 billion/ml. On the other hand the present value is significantly higher ($P < 0.01$) than spermatozoa concentration reported by Hector and Oscar (1998) in Mexican dual purpose bulls,

Dhami *et al.* (1998) in Friesian bull, Omar (1997) in Gambian short horn zebu, and Adamou *et al.* (1996) in Borgou bull who reported spermatozoa concentration of 0.08, 0.95, 1.09, and 1.19 billion/ml respectively. 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 works report could be due to variation in genotype, nutrition, age, management, semen collection frequency and technique (Caroll *et al.*, 1963; Igboeli and Raka, 1971; Salisbury *et al.*, 1978; Tegegne *et al.*, 1992; Hafez, 1993; Blezinger, 1999; Andrabi *et al.*, 2002).

In this study the mean (SE) spermatozoa total count was found to be 7.35 (0.47) billions/ejaculate. This value agrees well with spermatozoa total count reported in *Bos taurus* in Brazil (Brito *et al.*, 2002) as 8.2 billions/ ejaculate, but was significantly lower ($P < 0.01$) than the spermatozoa total count reported by same authors in *Bos indicus* as 11.4 billions/ejaculate. The latter variability might be attributed to various factors like age and management condition of the bull, season the study covers (Caroll *et al.*, 1963; Hafez, 1993; Blezinger, 1999). The present value agrees with the sperm total count set for the normal fertile bull (Bhosrekar, 1990; Setchell, 1991; Hafez, 1993; Bearden and Fuquay, 2000).

The spermatozoa mean (SE) live percentage was 79.73 (0.65) %. This value is in line with the spermatozoa live percentage reported by Shelke and Dhami (2001) in Gir as 80.13 %, but is significantly higher ($P < 0.01$) than live percentage reported by Rana and Dhami (2003) in Gir, Ahsan *et al.* (2003) in Sahiwal and Friesian-Sahiwal cross bulls. The spermatozoa live percentage reported by Dhami *et al.* (1998) in Friesian bulls as 87.35 % and Veeraiah *et al.* (1999) in Ongole bulls as 82.17 % was significantly higher ($P < 0.01$) than the present value. However, the spermatozoa live percentage observed in these bulls agrees well with live percentage recommended for normal fertile bulls.

The mean (SE) percent normal spermatozoa observed in this study were 94.70 (0.38). This value agrees well with the percentage morphological 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 (Faulkner and Pineda, 1980; Hafez, 1993).

The mean (SE) percent normal acrosome observed was 96.99 (0.60) %. This value is significantly higher ($P < 0.01$) than the value reported for normal acrosome by Veeraiah *et al.* (1999) in Ongole bulls and Rana and Dhami (2003) in Gir who respectively reported 92.33 % and 84.8 %. The viable number of spermatozoa observed in these Ethiopian indigenous bull was 5.10 (0.33) billions/ejaculate. This agrees well with viable number of spermatozoa reported in *Bos taurus* as 4.9 billions/ejaculate but is significantly lower ($P < 0.01$) than the viable number of spermatozoa reported in *Bos indicus* as 6.7 billions/ejaculate in Brazil (Brito *et al.*, 2002). Ejaculate characteristics of a bull in general has been known to be affected by a number of factors: age, genotype, nutrition, season the study covers, ejaculation frequency and method of semen collection, knowledge of the investigator (Caroll *et al.*, 1963; Hafez, 1993; Blezinger, 1999).

Seminal plasma biochemical analysis

The mean (SE) value in the seminal plasma GOT, GPT, ALP, ACP, total protein and testosterone level are given in Table 2.

The mean (SE) seminal plasma levels of GOT, GPT, ALP, ACP, total protein and testosterone were 1530.91 (60.15) u/l, 131.99 (9.36) u/l, 3333.98 (608.84) u/l, 8003.68 (716.06) u/l, 7.38 (0.26) gm/dl, and 2.84 (0.30) ng/ml. This seminal plasma GOT level in these bulls is significantly higher ($P < 0.01$) than GOT level reported in crossbred bulls by Singhal *et al.* (1976) who reported seminal plasma GOT level as 545.1 u/l, but found lower than GOT level reported by Saxena and Tripathi (1978) in crossbred bulls who reported seminal plasma GOT level as 4825 u/l and GOT level reported by Pandit and Garg (1983) in crossbred bulls who reported the seminal plasma GOT level as 2068.8 u/l. The seminal plasma GPT level reported in seminal plasma of crossbred bulls by Singhal *et al.* (1976) and Saxena and

Tripathi (1978) significantly differ ($P < 0.01$) from the present value who reported seminal plasma GPT levels as 322.2 u/l and 212 u/l, respectively. The seminal plasma ALP and ACP level reported by Aguirre *et al.* (1988) as 954.2 u/l and 1268.1 u/l respectively, ACP level reported by Reddi and Raja (1980) as 4065.8 u/l in buffalo bulls were significantly lower ($P < 0.01$) than the present value. The level of enzymes in seminal plasma varies based on the level of initial damage to the spermatozoa, or subsequent damages due to freezing or dilution (Roberts, 1971; Mann and Lutwak-Mann, 1981; Dhami and Kodagali, 1990). In this particular study the semen was not frozen or diluted prior to the sampling for enzyme analysis. The present value for seminal plasma total protein agrees well with the seminal plasma total protein reported by Hafez (1993), Faulkner and Pineda (1980a) and Setchell (1991). Normally 3 to 8 gm/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 well with bovine seminal plasma testosterone level reported by Gunjam and Amann (1976) as 2.87 ng/ml, but differs significantly ($P < 0.01$) from seminal plasma testosterone level reported by Tuli *et al.* (1991) and Javed *et al.* (2000) who reported the seminal plasma testosterone level as 1.41 and 0.97 ng/ml respectively. Testosterone level in serum of bulls has been found to vary with factors like age and level of sexual stimulation of bull (Salisbury *et al.*, 1978; Faulkner and Pineda, 1980) but the cause of variability in seminal plasma testosterone level of bulls needs further study.

Different morphologic abnormalities of spermatozoa.

The mean (SE) values of different morphologic abnormalities in different bulls (Table 3).

Different head abnormalities of spermatozoa

In the study conducted to characterize the head abnormalities using the William stain, in the indigenous bull the mean (SE) values are given in Table 4.

The mean (SE) head, mid piece (body), tail abnormality, total abnormality, major abnormality and minor abnormality observed for these bulls were 1.87 (0.15) %, 1.92 (0.22) %, 1.50 (0.18) %, 5.29 (0.38) %, 2.25 (0.19) %, 3.04 (0.28 % respectively. Previous reports on spermatozoa abnormalities reported values which have strong significant difference with present values ($P < 0.01$) like total morphologic abnormalities 15.54, 27.15, 15.41, 11.74, 9.26, 22.5 percent respectively in Gir (Shelke and Dhami, 2001), Friesian Sahiwal cross (Ahsan *et al.*, 2003), Ongole (Veerai *et al.*, 1999), Friesian (Dhami *et al.*, 1998) and Gir (Rana and Dhami, 2003). Such differences could be attributed to several factors, which affect ejaculate characteristics. On the other hand, the present observed values were lower than the maximum recommended spermatozoa abnormality value for normal fertile bull. The spermatozoa of normal fertile bull has been recommended not to contain more than 20 % total abnormality, and individual head, mid piece and tail abnormality of 10 % or more (Faulkner and Pineda, 1980; Hafez, 1993). In line with this the different head and neck abnormalities observed were appreciably low in Ethiopian indigenous bulls.

The spermatozoa have been known to have head and tail, the latter consisting of neck, midpiece, principal piece and end piece (Roberts, 1971; Salisbury *et al.*, 1978; Prasad and Sinha, 1985; Garner, 1991; Hafez, 1993; Bearden and Fuquay, 2000). The spermatozoa dimensional measurement results in this study agree well with reports of Bhosrekar (1990) and Sullivan (1978) on head length and tail length who reported the spermatozoa head length ranging from 8 to 10 µm and spermatozoa tail length ranging from 45 to 50 µm, but had strong significant difference in mid piece length reported by Faulkner and Pineda (1980b) and Ortavant *et al.* (1961) who reported the mid piece length as 13 µm and 18.84 µm respectively.

This study evaluated the semen physico-morphological and biochemical characteristics of indigenous bulls. Based on the physico-morphologic and biochemical parameters of fresh semen analyzed from these indigenous bulls, it was observed that most of the semen attributes lie within the normal level set for the normal fertile bulls.

ACKNOWLEDGEMENT

Authors are thankful to Dean, Graduate Studies, Faculty of Veterinary Medicine, Addis Ababa University, Ethiopia for providing funds to carry out the research work. Thanks are also due to Director, National Artificial Insemination Centre, Kality for providing facilities for semen collection and evaluation. Support from the Ministry of Education is duly acknowledged.

REFERENCES

Adamou, N., Diaye, M., Jondel, R. and Adjovi, A. (1996). Borgou bull semen characteristics and potential use in artificial insemination. Revenue d'Elevage et de Medicine Veternariredes pays Tropicaux, **49**: 174- 177.

Aguirre, S. M., Capaul, E. G., Luca, L. D., Cinque, M. and De-Luca-L, A. D.(1988). Total and none prostatic acid phosphatase and alkaline phosphatase activity in bull and Ram semen. Veterinaria-Argentina, **45**: 382-387.

Ahsan, U. H., Miraz, R. H. and Zahid, I. A.(2003).Semen characteristics of crossbred (Friesian x Sahiwal) and Sahiwal young bulls maintained under subtropical conditions of Punjab. Pakistan Veterinary Journal,**23**: 100-102.

Andrabi, S. M. H., Naheed, S, Khan, L. A. and Ullah, N.(2002). Semen characteristics of crossbred (Friesian x Sahiwal) bulls at Live-stock Research Station, National Agricultural Research Center. Islamabad. Pakistan Vet J, **22**: 181-187.

Bearden, H. J. and Fuquay, J. W. (2000).Applied Animal Reproduction. 5th ed. Upper Saddle, New Jersey: Prentice Hall Inc pp 138-147.

Bhosrekar, M. R.(1990). Semen Production and Artificial Insemination. 1st ed. India: BAIF Development Foundation pp 4-205.

Blezinger,S.(1999).Cattle Today: Many Factors Affect Bull Performance. http://www.cattletoday.com

Brito, L. F. C., Silva, A. E. D. F., Rodrigues, L. H., Vieira, F. V., Deragon, L. A. G., Kastelic, J. P.(2002). Effect of environmental factors, age and genotype on sperm production and semen quality in *Bos indicus* and *Bos taurus* in Brazil. Anim. Reprod. Sci., **70**: 181-190.

Caroll, E., Ball, L. and Scott, J.(1963). Breeding soundness in bulls, J American Vet. Med. Assoc., **142**: 1105-1111.

Coulter,G.H. and Foote, R. H.(1979). Bovine testicular measurements as indicator of reproductive performance and their relation to productive traits in cattle. Theriogenology, **11**: 297-311.

Dhami, A. J. and Kodagali, S. B.(1990). Enzyme leakage and fertility of buffalo spermatozoa in relation to the quality of semen ejaculates and extenders. Theriogenology, **34**: 853-864.

Dhami, A. J.,Greesh, M. and Sahni, K. L.(1998).Seasonal influence on the quality and freezablity of semen of Friesian and Murrah buffalo bulls. Indian J. Anim. Reprod., **19**: 55-58.

Faulkner, L. C. and Pineda, M. H.(1980a). Male reproduction. In: Donald, M. C. (ed.): Veterinary Endocrinology and Reproduction. 3rd ed. Philadelphia: Lea and Febiger pp 235-273.

Faulkner, L. C. and Pineda, M. H.(1980b). Artificial insemination. In: Donald, M.C. (ed): Veterinary Endocrinology and Reproduction. 3rd ed. Philadelphia: Lea and Febiger pp 330-366.

Garner, D. L.(1991). Artificial Insemination. In: Cupps, P. T. (ed.): Reproduction in Domestic Animals. 4th ed. San Diago, California: Academic Press Inc pp 251-274.

Gunjam, V. K. and Amann, R. P.(1976). Steroids in fluids and sperm entering and leaving the bovine epididymis, epididymal tissue, and accessory sex gland secretions. Endocrinology, **99**: 1618-1630.

Hafez, E. S. E.(1993). Reproduction in Farm Animals. 6th ed. Philadelphia: Lea and Febiger pp 405-439.

Hector, C. R. and Oscar, G. C. M. (1998). Reproductive aspects of crossbred bulls in a dual purpose cattle project in Mexico. In: Ostensson, K. and Vale, W. G. (ed): Animal Reproduction and Biotechnology for Latin American proceeding of a conference pp 33-38.

Igboeli, G. and Raka, A. M.(1971). Seasonal changes in the ejaculate characteristics of Agoni (short horn zebu) bulls. J. Anim. Sci., **33**: 651-654.

Javed,M.T, Abrar, K. and Mumtaz, A.(2000). Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal bulls and their relationship with other semen parameters. Veternarski archive, **70**: 141-149.

Lindasay, D. R., Entwistle, K. W., Winantea, L. (1982): Reproduction in Domestic Livestock in Indonesia. Australian Universities of International Development Program (AUIDP), Australia.

Mann, J. and Lutwak-Mann, C.(1981). Male Reproductive Function and Semen, New York: Springerverlag pp 19.

Morrow,A.(1986).Current Therapy in Theriogenology.2nd ed. USA: W. B. Sounders Company pp 125-152.

Omar, M. A. M.(1997). Source and breed variations in semen quality of South African bulls. Egyptian J Physiol Sci., **21**: 13-19.

Ortavant, R., Courot, M. and Hochereau, M.T. (1961). Spermatogenesis and morphology of spermatozoon. In: Cole, H. H. and Cupps, P. T. (ed): New York and London: Academic Press.

Pandit, R. K. and Garg, U. K. (1983). Freezablity, GOT release and fertility of crossbred bull semen. Indian J. Anim. Prod., **4**: 36-38.

Peters, T.J.R. (1968). Proposals for standardization of total protein assay. Clin. chem., **14**: 1147-1152.

Prasad, A. and Sinha, A. K.(1985). Essential of endocrinology and reproductive physiology. New Delhi: Allied publishers pp 121-147.

Rana, C. M. and Dhami, A. J.(2003). Sephadex filtration-measure improving the quality of Gir (*Bos indicus*) and Jafarabadi (*Bubalus bubalis*) bulls. Indian J. Anim. Sci., **73** : 607-611.

Reddi, N. M. and Raja, C. K. S. V.(1980). Seasonal variations in acid and alkaline phosphatase contents in surti buffalo bull

semen. Kerala J. Vet. Sci., **11**: 48-50.

Roberts, S. J.(1971). Veterinary Obstetrics and Genital Disease, 2 ed. Indian: CBS publishers and Distributors pp 612-750.

Salisbury, G. W., Van Demark, N. L., Lodge, J. R.(1978). Physiology of Reproduction and Artificial Insemination of Cattle. 2ed. ed. San Francisco: W. H. Free man pp 385-479.

Saxena, V. B. and Tripathi, S. S. (1978). Studies on the physico-morphological attributes and preservability of semen of crossbred bulls. Indian J. Anim. Sci., **48**: 865-869.

Setchell, B. P.(1991). Male reproductive organs and semen. In: Cupps, P. T. (ed): Reproduction in Domestic Animals. 4th ed. San Diago: Academic Press pp 221-249.

Shelke,V.B.and Dhami,A.J. (2001).Comparative evaluation of physico-morphological attributes and freezability of semen of Gir cattle (*Bos indicus)* and Jafarabadi buffalo (*Bubalus bubalis*) bulls. Indian J. Anim. Sci., **71**: 319-324.

Singhal, J. P., Kaker, M. L. and Reirian, M. N.(1976). A note on enzymatic activity of semen transaminases in crossbred bulls. Hisar Agricultual University J. Res., **6**:164-166.

Sorensen Jr, A. M. (1979). Animal Reproduction Principles and Practices. New York: McGraw-Hill Publishing Compa pp 85-233.

SPSS. (2002). SPSS 11.5 for windows, standard version, SPSS INC. http://www.spss.com.

STATA. (2001). Intercooled stata 7.0 for windows. 702 University Drive East: Stata Corporation. stata@stata.com

Sullivan, J. J. (1978). Morphology and motility of spermato In: Salisbury, G. W., Van Demark, N. L., Lodge, J. R. (ed) Physiology of Reproduction and Artificial Insemina Cattle. 2ed ed. San Francisco: W. H. Free man pp 286-32

Tegegne, A., Entwist, K. W. and Mukasa-Mugerwa, E.(1992) Nutritional influence on growth and onset of puber in Boran and Boran-Friesian crosses bulls. Theriogeno **35**: 1005-1016.

Tuli, R. K., Lohan, J. S. and Singhal, S. P. (1991). Testosterone progesterone hormone in buffalo bull seminal plasma and their correlation with seminal characteristics. Indian J. Dairy Sci., **44**: 587-589.

Veeraiah, G., Naidu, K. V. and Rao, K. B.(1999). Studies on semen characteristics of Ongole bulls. Indian Vet. J., **76**: 585-560