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Short Communication

Semen characteristics of black bengal buck in relation to age, body weight and scrotal circumference

M.R. Islam[1], S. Afroz[2], M.A.M.Y. Khandoker[2], Q.S. Akter[2], A.H.M.S. Rahman[2]

[1]Animal Division, National Institute of Biotechnology (NIB), Atomic Energy Research Establishment (AERE), EPZ, Savar, Dhaka-1349

[2]Reproductive Biotechnology Laboratory, Department of Animal Breeding and Genetics, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh

Abstract

The experiment was conducted to find out semen quantity and quality in relation to age, body weight and scrotal circumference (SC) of Black Bengal buck, the most important goat breed in Bangladesh. Semen was collected from four bucks by the method of artificial vagina (AV). After collection semen was evaluated for volume per ejaculate (ml), mass motility (%), individual motility (%), morphology (% normal and abnormal sperm) and concentration (million/cc) in relation to age, body weight and SC. Age, body weight and SC of four different bucks were 34 to 45 months, 20.0 to 33.6 kg and 20.1 to 21.0 cm, respectively. The mean volumes per ejaculate were 0.4±0.03 to 0.5±0.22 ml and mass motility was found 76.0±1.5 to 78.0±1.1%. On the other hand individual motility in terms of progressive, oscillatory and rotatory were 65.7±0.1 to 68.0±1.3%, 5.0±0.6 to 6.1±0.4% and 4.0±0.4% to 5.4±0.3%, respectively. While the (normal and abnormal sperm) and sperm concentration were found to be 89.7±0.35 to 91.2±0.4% and 8.8±0.4 to 10.3±0.4% and 2434.0±52.8 to 2853.0±90.1 (million/ml), respectively.

Keywords: Black Bengal buck, Semen characteristics

Introduction

Goat farming and profitability is largely influenced by reproductive efficiency in terms of conception rate of doe. Male fertility as well as the semen characteristics or parameters are directly influenced by such factors as age, maturity, nutritional status, general health, endocrine balance and normality of sex organs (Peters, 2002), which further influence the conception rate. In any breeding program bucks are considered as half of the flock as because they are not only responsible for improving conception rate but also for establishing genetic merit of future kids. During selection of buck for breeding purpose attention is given about the growth rate, body weight, sexual characteristics and soundness of sexual organ of the buck. However, age of copulation and first ejaculation varies with breed, seasons, plane of nutrition and presence of teaser female (Prasad *et al.,* 1970; Singh and Senger, 1979; Tiwari, 1990). To maximize the amount and quality of spermatozoa, a selection of suitable donors from young males is necessary (Bongso *et al.,* 1982; Laboeuf *et al.,* 2003). Semen collection by artificial vagina (Perry, 1969; Terril, 1969; Watson, 1990; Herman *et al.*, 1994; Gordon, 2000) requires training of buck and may need the presence of an estrous female, at least initially (Miller, 1961; Tiwary, 1990; Mushtaq *et al.*, 1997; Shamsuddin *et al.*, 2000). For achieving good semen quality and sperm fertility, semen should be collected in a regular interval (Salamon, 1962; Jenning and Mc Weeney, 1976; Sullivan, 1978; Amann, 1986; Watson, 1990; Thwaites, 1995; Shamsuddin *et al.*, 2000). Higher concentration of sperm is obtained when semen was collected once daily (Sharma *et al.*, 1969). Once or twice daily collection for a certain period did not affect buck semen quality adversely (Prasad *et al.*, 1970; Foote, 1974; Smith *et al.*, 1977; Chahide *et al.*, 1978; Mittal, 1985; Pandey *et al.*, 1985;

Hafez, 1993; Amiri, 1997; Shamsuddin *et al.*, 2000). With so many things affecting fertility, it's a wonder that sterility is rarely a problem. Limited information is available on semen characteristics such as volume, sperm concentration, motility, total number of motile sperm cells/ejaculate, normal and abnormal spermatozoa of the Black Bengal buck. So, a deep understanding about the influence of age, body weight and scrotal circumferences (SC) on semen characteristics is necessary before selecting breeding bucks for mating or breeding purpose. From that standpoint the present study was therefore undertaken to uncover the effect of age, body weight and scrotal circumference (SC) on different semen characteristics of Black Bengal bucks.

Materials and Methods

Four adult Black Bengal bucks were selected to produce semen with 80% morphologically normal spermatozoa with a satisfactory motility and concentration. The bucks aged between 34-45 months. The body weight and scrotal circumference (SC) of bucks were 20.0 to 33.6 kg and 20.1 to 21.0 cm, respectively. The bucks were reared isolated from does.

The bucks were trained to ejaculate in artificial vagina (AV) by homo sexual mounting. Semen was collected twice a week. After collection, semen was kept at 37°C in water-bath. After collecting the semen volume of the ejaculate was measured by reading the graduated tube in milliliter or ml. For observation of mass motility, one drop of semen was mixed with tris buffer and was placed on a clean prewarmed slide (37°C) and covered with a cover slip. The motility was determined by eye-estimation on the basis of the movement of spermatozoa at higher magnification (40x). Just after collection the semen was diluted with buffer solution and inclined slowly. When examined under microscope, movement of the sperm was observed with care. Motility was observed by following types of movement: progressive/forward, oscillatory and rotatory movement. In case of normal and abnormal sperm count (%) two drops of buffer was placed on a clean, dry glass slide and one drop of mixed semen was added in buffer. Covering with another slide spread the buffer with semen. Then the slide was removed by pulling parallel gently. The slides were stained with Rose Bengal stain for 4-5 minutes, then it was rinsed with distilled water to remove excess stain. Then the smear was dried in the air. The slide was placed on the stage of microscope and counted in high magnification. A total of 333 sperms were counted for normal and abnormal sperm (Fig. 1) by using random fields on different parts of the slide. The concentration of spermatozoa (million/cc) was determined by using haemocytometer. The concentration of spermatozoa per ml of semen was expressed as million/ml.

The data generated from this experiment were entered in Microsoft Excel worksheet, organized and processed for further analysis. Descriptive statistics were performed to calculate mean, standard error and percentage. One-way ANOVA test was performed to obtain the difference in sperm motility. Statistical analyses were performed using SPSS (Statistical Package for Social Sciences, SPSS inc. 1999, Microsoft Corporation 1998) windows package.

Results and Discussion

Semen characteristics in relation to age, body weight and scrotal circumference (SC) of different Black Bengal bucks are summarized in Table 1.

Table 1. Semen characteristics of different Black Bengal bucks in relation to age, body weight and scrotal circumference (SC)

Buck no.	Age (months)	Body weight (kg)	SC (cm)	Volume per ejaculate (cc) (mean±SE) (n)	Mass Motility (%) (mean±SE)	Individual motility (%) (mean±SE)			Morphology (mean±SE)		Sperm concentration (million/cc) (mean±SE)
						Progressive	Oscillatory	Rotatory	% Normal sperm	% Abnormal sperm	
1	45	33.6	21.0	0.45±0.22 (10)	76.50±1.50	65.70±0.99	5.80±0.59	5.00±0.83	89.72±0.35	10.28±0.35	2434.00±52.81
2	34	20.0	20.1	0.44±0.22 (10)	76.00±1.45	65.70±1.59	5.70±0.50	4.60±0.34	90.23±0.59	9.77±0.59	2708.00±75.83
3	40	28.0	20.4	0.43±0.03 (10)	77.00±1.33	68.00±1.27	5.00±0.56	4.00±0.39	91.16±0.36	8.84±0.36	2549.00±62.87
4	38	23.0	20.1	0.43±0.03 (10)	78.00±1.11	66.50±1.59	6.10±0.35	5.40±0.34	90.67±0.30	9.33±0.30	2853.00±90.12
F-Value				NS	NS	NS	NS	NS	NS	NS	NS

Figures in the parenthesis indicate the total number of observation
NS= Non-significant
SE= Standard error

Among four bucks, the highest age and body weight was recorded in buck 1 (45 months and 33.6kg), followed by buck 3 (40 months and 28.0kg), 4 (38 months23.0kg) and 2 (34 months and 20.0kg) respectively. The highest scrotal circumference recorded in buck 1 (21.0cm), followed by buck 3 (20.4 cm) and buck 2 and 4 (20.1 cm). Average values for scrotal circumferences ranged between 20.1 to 21.0 cm, with less variation among the bucks, although the age and body weight of different bucks varied widely. A satisfactory potential breeding buck should have suitable scrotal circumference because SC positively correlates with sperm concentration per milliliter and total spermatozoa per ejaculates (Bongso *et al.,* 1982). The SC observed in the present study varied from 20.1 to 21.0 cm, which collaborates with the results of Igboeli (1974) obtained the mean scrotal circumference of 20.9±0.3 cm for native Zambian bucks. To maximize the amount and quality of spermatozoa, a selection of suitable donors from young males is necessary (Laboeuf *et al.,* 2003). The mean scrotal circumference of a Black Bengal buck at puberty is 14 cm to 16 cm when sexually matured (Shamsuddin *et al.*, 2000).

Volume per ejaculate (cc): The highest volume of semen was recorded in buck 1 (0.45±0.22 cc), followed by buck 2 (0.44±0.22 cc) and 3 and 4 (0.43±0.03 cc) though the differences were not reached in significant level (Table 1). Average semen volume (cc) obtained in this study ranged from 0.43±0.03 to 0.45±0.22 cc, with a small variation among different bucks, which might be due to the SC of four different bucks, as the SC in different bucks did not varied widely. But, semen production of animal largely depends on several factors such as age, maturity, nutritional status, general health, endocrine balance and normality of sex organs (Peters, 2002). The findings of the present study was more or less similar to the findings of Singh *et al.* (1985) obtained average ejaculate volume of 0.46 cc in Black Bengal buck. Marx *et al.* (1975) reported average ejaculate volume of 1.27 ml, which was much greater than the results of the present study and Khan (1999) reported average ejaculate volume of 0.27±0.12 ml, which was lower than the value of the present study, these differences could be due to the individual variation of bucks and seasonal variations, as seasonal variations affects sexual glands (Karatzas *et al.*, 1997; Karagiannidis *et al.,* 2000). Semen volume of buck was recorded to be larger in amount to some workers during the breeding seasons because the accessory glands are more active during that time (Karatzas *et al.*, 1997, Karagiannidis *et al.,* 2000). Furthermore, the values obtained in this study were more or less similar with many other investigators (Bakshi *et al.*, 1987; Vilar *et al.,* 1993). The variation of semen volume per ejaculate in the present study also might be due to the variation in age, body weight and SC of the bucks. However, age of copulation and first ejaculation varies with breed, seasons, plane of nutrition and presence of teaser female (Prasad *et al.,* 1970; Singh and Senger, 1979; Tiwari, 1990). Jenning and Mc Weeney (1976)

and Shamsuddin *et al.* (2000) found that repeated ejaculation may not change sperm motility and percentage of abnormal spermatozoa but semen volume, sperm concentration and number of total sperm per ejaculate decline.

Mass motility (%): Comparatively highest value of mass motility was found in buck 4 (78.00±1.11%), followed by buck 3 (77.00±1.33%), 1 (76.50±1.50%) and 2 (76.00±1.45%) again the differences were in an insignificant level (Table 1). This might be due to the small number of population and as well as observation studied in the present research. Mass motility of sperm obtained 76.00% to 78.00%, which supports the findings of Khan (1999) obtained motility of 73.87%. Koh (1976) found 85.00% motility which, also collaborates with the results of the present study although the values found higher in the former study, that might be due to animal variation as because in the former study crossbred goat were used as experimental animal. Several factors such also affects on sperm motility such as seasonal variations, nutrition, management, frequency of semen collection, collection interval, temperature and pressure of AV and physical condition of the animal (Nazir, 1988). Furthermore, results of the present study also support the findings of Bakshi *et al.* (1987) with a small deviation of the values. Findings of Vilar *et al.* (1993), Karatzas *et al.* (1997), Khan (1999), Bhuskat *et al.* (2000), Shamsuddin *et al.* (2000) and Biswas (2001) also supports the results of the present study. Sperm motility is also affected by collection interval as because prolonged collection interval, spermatozoa in the epididymis degenerate resulting inferior quality semen (Amann, 1986; Watson, 1990).

Individual motility (%): In case of individual motility, comparatively highest progressive motility was found in buck .3 (68.00±1.27%), followed by buck 4 (66.50±1.59%), 2 (65.70±1.59%) and 1 (65.70±0.99%) and the differences were insignificant. Oscillatory and rctatory movement was observed higher in buck 4 (6.10±0.35% and 5.40±0.34% respectively), followed by buck 1 (5.80±0.59% and 5.00±0.83%), 2 (5.70±0.50% and 4.60±0.34%) and buck 3 (5.00±0.56% and 4.00±0.39% respectively). In case of Individual motility satisfactory results (Table 1) with little variation among different bucks were found in the present study, although supporting works in this regards are not available. But individual motility observed in the present study further supports the role of SC and volume per ejaculate, because these are the important criteria for the quality of semen and they determine the rate of conception (Nazir, 1988).

Normal and Abnormal sperm (%): Sperm morphology also varied among the bucks with little variation (Table 1). Comparatively higher percentage of normal sperm was found in buck 3 (91.16±0.36%), followed by buck 4 (90.67±0.30%), 2 (90.23±0.59%) and 1 (89.72±0.35%). Comparatively lower percentage of abnormal sperm was found in buck 3 (8.84±0.36%), followed by buck 4 (9.33±0.30%), 2 (9.77±0.59%) and 1 (10.28±0.35%). Average normal spermatozoa found in the present study were 89.72 to 91.16%, which is in agreement with Singh *et al.* (1985) obtained 86.37 to 91.07% normal spermatozoa. The finding of the present study also collaborates with the results of Khan (1999) and Marx *et al.* (1975). For achieving good fertility, semen should be collected in a regular interval. High collection frequencies of semen cause spermatozoa with acrosomal defects; however, detached heads were evidenced at prolonged sexual rests, which resulted in poor quality semen (Sullivan, 1978). At prolonged collection interval, spermatozoa in the epididymis degenerate resulting inferior quality semen (Amann, 1986; Watson, 1990).

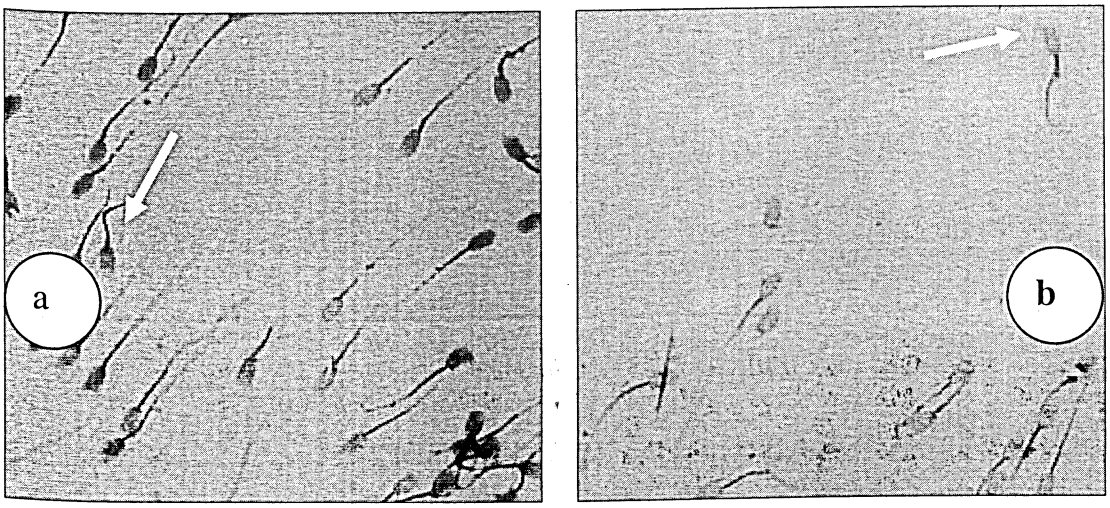

Fig. 1. Indicator in the representative photograph showing a) Normal and b) abnormal sperm

Sperm concentration (million/cc): Highest sperm concentration ($x10^6$) of semen was found in buck 4 (2853.00±90.12), followed by buck 2 (2708.00±75.83), 3 (2549.00±62.87) and 1 (2434.00±52.81) and differences among the bucks were found insignificant (Table 1). In the present study the average sperm concentration varied from 2434.00×10^6/ml to 2853.00×10^6/ml in different bucks. The result of the present study collaborates with the results of Evans and Maxwell (1987), Karatzas *et al.* (1997), Ibrahim (1997) and Shamsuddin *et al.* (2000). The findings of the study also collaborates with the results of Singh *et al.* (1985) obtained sperm concentration of 2619.58 to 2910.33×10^6/ml. Khan (1999) obtained sperm concentration of 3777.93×10^6/ml which was higher than that of the results of the present study. These differences could be due to the individual variation of bucks and seasonal variations, as seasonal variations affect sexual glands (Karatzas *et al.*, 1997; Karagiannidis *et al.*, 2000). Singh *et al.* (1985) found average sperm concentration ($\times10^6$/ml) of 2619.58, which further supports the findings of the present study. There are several factors, which affects the semen characteristics such as seasonal variations, nutrition, management, frequency of semen collection, collection interval, efficiency of semen collector, presence of dummy animal, training of the animal, temperature and pressure of AV and physical condition of the animal (Nazir, 1988). On the other hand, higher concentration of spermatozoa is possible to achieve by maintaining temperature and pressure of AV (Perry, 1969). In case of bucks, the quality semen with less variation in total number of spermatozoa per ejaculate is obtained during the breeding season (Corteel, 1977). Frequency of ejaculation is important for sperm concentration as because, four to eight daily ejaculations have been found to significantly decrease semen volume, sperm motility, sperm concentration and number of spermatozoa per ejaculation in ram (Thwaites, 1995). Higher concentration of sperm is obtained when semen was collected once daily (Sharma *et al.*, 1969).

Conclusion

Semen parameters of Black Bengal bucks varied among different bucks with varying age, body weight and scrotal circumferences although the parameters were in an insignificant level. Whether the differences found among the bucks in relation to age, body weight and scrotal circumferences reflected in the fertility results after AI is yet unknown and needs to be further studied.

References

Amann, R.P. 1986. How a bull works. *Proceedings of Eleventh Technical Conference on Artificial Insemination and Reproduction, National Association of Animal Breeders.* pp 6-18.

Amiri, Y. 1997. Effects of collection frequency, ejaculate number and diluents on the survival and morphology of buck spermatozoa. MS thesis submitted to the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Bakshi, S.A., Patil, V.K., Srivas, A.K., Jagtap, D.Z. and More, B.K. 1987. Studies on semen evaluation and fertility rate of Angora and 7/8 Angora bucks. *Livestock Adviser.* 12: 13-18.

Bhuskat, S.N., Takarkhede, R.C., Mangle, N.S. and Ali, S.Z. 2000. Comparative study of some semen attributes of different breeds of bucks. *Ind. Vet. J.* 77: 963-965.

Biswas, D. 2001. Effects of glycerol percentages on the motility of frozen thawed buck (Capra hircus) spermatozoa. MS thesis submitted to the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Bongso, T.A., Jainudeen, M.R. and Zaharah, A.S. 1982. Relationship of scrotal circumference to age, body weight, and onset of spermatogenesis in goats. *Theriogenology.* 18: 513.

Chahide, A.A., EL-Azab, A.I. and Rakha, A.M. 1978. Some factors affecting sexual activity on buffalo bulls. *Egyptian J. Vet. Sci.* 15:1-7.

Corteel, J.M. 1977. Production, storage and insemination of goat semen. In Management of Reproduction in sheep and goats. *Sheep Industry Development Program Symposium* pp. 41-47.

Evans, G. and Maxwell, W.M.C. 1987. *Salamon's artificial insemination of sheep and goats.* Bulterworths, Sydney, Boston, London, Durtan, pp. 107-141.

Foote, R.H. 1974. *Artificial insemination. In: Reproduction in farm animals.* 3rd edition ESE Hafez (ed.). Lea & Febiger, Philadelphia, pp. 409-431.

Gordon, I. 2000. *Introduction to Controlled Breeding in Goats in: Controlled Reproduction in Sheep and Goats.* Cab International. pp. 351-373.

Hafez, E.S.E. 1993. *Reproduction in farm animals,* 6th edition, Lea & Febiger, Philadelphia, pp. 405-429.

Herman, H.A., Mitchell, J.R. and Doak, G.A. 1994. Extender and extension of semen. *In: The artificial Insemination and Embryo Transfer of Diary and Beef cattle.* Herman II. A. (edt). 8th Edition. Interstate Publishers, Inc. Danville, Illions, pp 101-116.

Ibrahim, S.A. 1997. Seasonal variations in semen quality of local and crossbred ram raised in the United Arab Emirates. *Anim. Reprod. Sci.* 49: 161-167.

Igboeli, G., 1974. A comparative study of the semen and seminal characteristics of breeds of goats. *Agril. forest. J. Zambia.* 40: 132-137.

Jennings J.J. and Mc Weeney, J. 1976. Effects of frequent ejaculation on semen characteristic of ram semen. *Vet. Record.* 98: 230-233.

Karagiannidis, A., Varsakeli, S. and Karatzas, G. 2000. Characteristics and seasonal variations in the semen of Alpine, Saanen and Damascas goat bucks born and raised in Greece. *Theriogenology.* 53: 1285-1293.

Karatzas, G., Karagiannidis, A., Varsaleli, S. and Brikas, P. 1997. Fertility of fresh and frozen thawed goat semen during the non-breeding season. *Theriogenology.* 48: 1049-1059.

Khan, R.A. 1999. A quantitative study on semen characteristics of Black Bengal buck. MS thesis submitted to the Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Koh, S.H. 1976. Semen characteristics of crossbred goats. *Animal Breeding Abstract.* 44: 424.

Laboeuf, B., Restall, B. and Salamon, S. 2003. Production and storage of goat semen for artificial insemination. *INRA-Productions-Animales.* 16(2): 91-99.

Marx, D., Klempp, K. and Bohmm, S. 1975. Gynaecomastia in a buck. Sexual activity, semen quality and milk quality. *Zuchthygiene.* 10: 125-135.

Miller, S.J. 1961. Ram management for artificial insemination. *In: Proceedings of the conference on artificial breeding of sheep in Australia.* Robert, EM (ed.) University of New South Wales Press, Sydney, pp. 163-167.

Mittal, J.P. 1985. Libido and semen quality of Jamuna Pari bucks under arid condition. *Ind. Vet. J.* 62: 179-182.

Mushtaq. A., Memon, M.A., Mickelsen, W.D. and Goyal, H.O. 1997. Examination of the reproductive tract and evaluation of potential breeding soundness in the Buck. *In: Current Therapy in Large Animal Theriogєnology,* Youngquist, R.S. (edt) 1st edition, WB Saunders, Philadelphia. pp. 485-488.

Nazir, 1988. Semen evaluation and sperm morphology-monography on reproductive pattern of Riverine buffaloes and recommendations to improve their reproductive performance at small farmer level. PARC, Islamabad, Pakistan.

Pandey, R.P., Sinha, S.N., Singh, B.K. and Akhtar, M.N. 1985. Characteristics of semen and fertility in semen and Barbari bucks. *Ind. J. Anim. Sci.* 55: 773-774.

Perry, E.J. 1969. Factors influencing the quality and quantity of semen. *In: The artificial insemination of farm animals.* Perry EJ (ed.) 4th edition. Oxford and IBH publishing Co. Calcutta, Bombay, New Delhi, pp. 76.

Peters, K.J. 2002. Evaluation of goat populations in tropical and subtropical environments. http://kinne.net/fertbuck.htm

Prasad, P., Roy, A. and Pandey, M.P. 1970. Effect of age on semen quality and development of sex libido in Barbari males. *J. Res. Sci.* 19: 23-30.

Shamsuddin, M., Amiri, Y. and Bhuiryan, M.M.U. 2000. Characteristics of buck semen with regard to ejaculate numbers, collection intervals, dilution and preservation periods. *Reprod. Domestic Anim.* 35: 53-57.

Sharma, R.P., Tiwari, S.B. and Roy, A. 1969. Effect of frequency of semen collection from ram on seminal attributes and fertility. *Ind. J. Anim. Sci.* 39: 15-19.

Singh, D.H., Sinha, M.P., Singh, C.S.P., Singh, R.A. and Singh, K.K. 1985. Comparative study on seminal quality of pure and crossbred bucks. *Ind. Vet. Med. J.* 9: 50-58.

Singh, S.N. and Senger, O.P.S. 1979. Annual reports on PL 480 project on goats. RBS College, Bichpuri, Agra, India, pp. 260-267.

Smith, P.A., Boland, M.P. and Godon, I. 1977. Studies in ram semen collection for use in AI during the breeding season. *J. Dept. Agric. Fish.* 14: 56-65.

SPSS, Windows for version-10.0. Release on 27.10.1999 (Microsoft Corp. 1998). Trends SPSS Inc., Michigan Avenue, Chicago, IL. 19-182.

Sullivan, J.J. 1978. Morphology and motility of spermatozoa. *In: Physiology of Reproduction and Artificial Insemination of Cattle.* Salisbury, G.M., VanDemark, K. I. and Lodge, J. R. (eds.) Freman and Company, Sanfranciscio, USA, pp. 286-320.

Terril, C.F. 1969. Sheep and goats. *In: The artificial insemination of farm animals.* Perry E. J. (ed.) 4th edition, Oxford and IBH publishing CO. Calcutta, Bombay, New Delhi, pp. 215-243.

Thwaites, C.J. 1995. The comparative effects of under nutrition, exercise and frequency of ejaculation on the size and tone of the testes and on semen quality in the ram. *Anim. Reprod. Sci.* 37: 299-309.

Tiwari, S.B. 1990. Libido and semen ejaculation in goats. Central Institute for Research on goats. Makhdoom, Farah, Mathura, (UP) India, pp. 61-65.

Vilar, A.C., Barnabe, V.H., Birgel, F.H., Barnabe, R.C. and Visintin, J.A. 1993. Testis and semen characters in goats reared in a semi-arid area in Pariaba State. *Revista Brasileira de Reproducao Animal.* 17: 23-32.

Watson, P.F. 1990. Artificial insemination and the preservation of semen. *In: Marshall's physiology of reproduction.* Vol. 2. Reproduction in the male. Lammong GE (ed.) 4th edition. Churchill Livingstone. Edinburgh, London, Melbourne and New York, pp. 747-869.