STUDY ON PHYSICO-MORPHOLOGICAL SEMINAL CHARACTERS OF GERMAN SHEPHERD CANINE BREED

K. RAY*, A. K. JHA¹, P. BISWAS AND S. BASU

*Department of Veterinary Gynaecology and Obstetrics
Faculty of Veterinary and Animal Sciences
West Bengal University of Animal and Fishery Sciences
37, K B Sarani, Kolkata -700 037, W. B., India*

The present study was undertaken with six numbers of German Shepherd dogs from different locations of Belgachia, Kolkata, West Bengal. Semen samples were collected biweekly in the morning (8:00 hr) by massage method aseptically from each dog for six occasions. A total of 36 ejaculates were collected. After collection, all semen samples were pooled, and all the macroscopic characters and physico-morphological seminal characteristics were evaluated. The volume of semen, pH, sperm concentration, individual motility, live percentage of spermatozoa, positive for hypo-osmotic swelling test (HOST) and total sperm abnormality percentage were 5.516 ± 0.166 cu mm., 6.383 ± 0.031 , $274.333 \pm 2.667 \times 10^6/\text{mL}$, $76.333 \pm 2.728\%$, 84.166 ± 1.990 , 80.166 ± 2.522 and 14.83 ± 2.386 , respectively. In the study all the dogs were found fertile.

Key words: German Shepherd dog (GSD), Physico-morphological character, Semen

Dog has not only a tremendous mental healing power and guarding ability but also gradually becoming one of the biggest income generating animal. Dog should receive maximum love, affection and more importantly proper health care management and treatment. Attention should also be given for production of optimum quality breed consisting of those characteristics which are essential for fulfilling the human needs.

Semen evaluation and its preservation are the two vital requisite for successful artificial insemination (AI). Use of chilled extended semen for AI in the canine has become increasingly popular over the last 2 decades. Semen parameters such as semen volume, sperm concentration, motility percentage of spermatozoa, percentage of live and dead spermatozoa and sperm morphology in the whole ejaculate are frequently examined to assess the quality of

*Corresponding Author

¹Regional Agricultural Research Station, Parwanipur, Bara, Nepal

semen. The functional integrity of the sperm plasma membrane is crucial for the viability and fertilizing ability of spermatozoa (Hafez, 1993). The hypo-osmotic swelling test (HOST) by using distilled water has been effectively used to assess the functional integrity of sperm plasma membrane (Lomeo and Giambersio, 1991).

Spermatozoa must have good motility in order to reach the oviduct and to penetrate the investments around the oocyte. An intact plasmalemma is a prerequisite for maintaining membrane potential, ionic micro environment and pH. Furthermore, spermatozoa cannot survive in the female reproductive tract and bind to the zona pellucida and the vitelline membrane without having an intact plasmalemma. Damage to acrosome can also result in infertile spermatozoa, since acrosomal enzymes are needed for penetration of the zona pellucida and fusion with the oocyte (Bedford, 1970; Roberts, 1986).

To evaluate the fertility of a male dog, several methods have been proposed. Although the evaluation of conception rates remains the ultimate test to assess male fertility, this method is time-consuming and causes birth of unwanted puppies (Oettle, 1993). Therefore, alternative methods to assess the fertilizing capacity of canine spermatozoa *in vitro* are required and for this reason HOST is performed as *in vitro* fertility test.

In the present study, an attempt has been made with the canine species of German

Shepherd dog breed. Evaluation of semen characteristics of this breed was taken into consideration.

MATERIALS AND METHODS

The present study was undertaken in the Department of Gynaecology and Obstetrics, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, Kolkata. The semen was collected for ten weeks from six German Shepherd dog breeds for study under this experiment. The animals were selected from different locations of Belgachia, Kolkata, West Bengal. The ages of experimental dogs were 3 to 4 years and the body weights were 25 kg to 32 kg. Soon after selection, a thorough history of those animals was taken. It included injuries, illness, treatments, management practices and owner observations related to development of reproductive problem, behavior and mating experiences. Semen was collected by digital manipulation (Macpherson and Panner, 1967; Linde-Forsberg, 1991; Kutzler, 2005). Under ideal conditions, this procedure was performed in the presence of an estrous bitch.

The parameters for study with fresh semen were volume, pH, sperm motility, sperm concentration, percentage of live spermatozoa, percentage of different types of abnormalities of head, mid piece and tail, and hypo-osmotic swelling test (HOST). The first (pre-sperm) fraction is small in volume and contains few to no

spermatozoa. The second (Sperm rich) fraction comes from the epididymes and testes. The third (prostatic) fraction consists solely of prostatic fluid and also contains few to no spermatozoa. The volumes of the first and third fractions, especially the later, are variable, and the volume of the third fraction was controlled by the person during collecting the sample, as they choose to collect more or less of the cell free prostatic fluid. Volume is not an indicator of semen quality in dogs. However the volume measurement is part of the calculation of total number of spermatozoa in the sample, which is one indicator of semen quality. The pH of dog semen was measured by pH paper in the pH range from 5.0-8.0 (Freshman, 2002). The mass activity of the spermatozoa was carried out and gradation was done on the basis of character of waves as expressed by Seager and Platzz (1977) and individual motility was observed followed by grading 0-5 as advocated by Herman and Swanson (1941) and finally expressed as the percentage of total motile or progressively motile spermatozoa. Sperm concentration was determined by haemocytometer technique (Andersen, 1975). Live percentage of spermatozoa was done using eosin-nigrosin stain as described by Andersen (1975). To determine the percentage of different types of abnormalities of head, mid piece and tail, Rose-Bengal stain was used (Castelo *et al.*, 2010). The HOST was done as per Jeyendran *et al.* (1984) where at least 100 spermatozoa were examined under phase contrast microscope (Olympus Optical Co.,

Tokyo, Japan; CX21i-LED, Binocular Version).

Data was analyzed by the general linear model of the SPSS software version 20. The means were compared using Duncan's multiple range tests. The probability of $P < 0.05$ was considered to be statistically significant. Correlation was done through Pearson correlation study in SPSS.

RESULTS

Mean semen volume of German Shepherd dogs was found to be 5.516 ± 0.116 cu mm. Mean of semen pH was 6.383 ± 0.031 . Mean of sperm concentration of German shepherd dogs was $274.333 \pm 2.667 \times 10^6$ /mL. Progressive motility of the sperm was estimated as $76.333 \pm 2.728\%$. Live percentage of spermatozoa was $84.166 \pm 1.990\%$. Mean value of hypo-osmotic swelling test (HOST) was showed 80.166 ± 2.522 . Total sperm abnormality percentage was 14.830 ± 2.386 .

DISCUSSION

Despande *et al.* (1970), reported semen volume of Alsatian and Golden Retriever were 5.9 mL and 5 mL respectively, which was similar to our experiment. Correa *et al.* (1997) also recorded semen volume of breed like Grey Hound was 7.5 mL. Daiwadnya *et al.* (1995) in their study on evaluation of dog semen reported that, in 48 ejaculates, the average volume was 2.57 mL.

The value of pH of the present findings was corroborated with the finding of Daiwadnya

et al. (1995), who reported it as 6.57. The pH of the second fraction (sperm rich) of domestic canine ejaculates was approximately 6.2 (Rota *et al.*, 1995). In agreement with measured ejaculate pH, Foote and Leonard (1964) found extender at pH 6.6 to be superior to both 5.9 and 7.3 for the maintenance of whole ejaculate canine sperm motility stored at 5°C. While some investigators have titrated their extenders to a pH of 6.8 (Foote and Leonard, 1964) and 6.7 (Rota *et al.*, 1995, 1999), others have maintained extender at a more neutral pH of 7.0 (Smith and Graham, 1984), 7.1 (Thomas *et al.*, 1993) and 7.4 (Hay *et al.*, 1997). In two studies comparing various extender pH, maximum canine sperm motility was maintained in the range of pH 7.0-8.5 when held at room temperature or at a pH of 7.3 when held at 39°C (England, 1992). Furthermore, England (1992) determined extender pH 7.3 to be the least deleterious on sperm morphology over time compared to pH 3.3, 5.3 and 9.3, and therefore deemed the best overall extender pH.

The value of sperm concentration in the present study was corroborated with the findings of Hancock and Rowland (1949) who reported average concentration of sperm in 19 ejaculates of dog was 275 million/mL. Harrop (1955) reported less average concentration of spermatozoa (125 million/mL) on 100 ejaculates belonging to several breeds of dogs. Daiwadnya *et al.* (1995) in their study on evaluation of dog semen reported that in 48 ejaculates, the

average sperm concentration was 264 million / mL. On contrary, Rota *et al.* (1995) found the total number of spermatozoa was 916 million/mL.

As the canine ejaculate consists of three fractions, volume and sperm concentration of the sperm-rich fraction will depend on the amount of prostatic fluid collected. Therefore, the total number of spermatozoa in the ejaculate is one of the parameters indicative of semen quality. Total sperm number in normal ejaculates, which may range from 300 to 2000 million (Johnston *et al.*, 2001), is highly variable between breeds, as sperm production depends on testicular size.

The motility ratings in different breeds varied widely as reported by the different workers. Rota *et al.* (1995) reported that the average motility of canine semen was 78.6% and Hendrikse and Antonisse (1984) found average motility was 71.38%. The percentage of total motile spermatozoa in normal canine ejaculates was between 70% and 90% (Johnston *et al.*, 2001), although it may be lower after prolonged periods of sexual rest. It has been proposed that fertile dogs should have at least 70% of total sperm motility (Lentner, 1982).

The percentage of live spermatozoa of the present study was in the agreement of Daiwadnya *et al.* (1995), who reported that in 48 ejaculates, the average live sperm percentage 82.81. The value of HOST in the present study partially corroborates the findings of the other workers. Rota *et al.*

(1995) observed the average value of HOST positive sperm was 93.6%.

Wong and Dhaliwal (1985) and Daiwadnya *et al.* (1995) found the average percentage of morphologically abnormal spermatozoa as 9% based on 8 ejaculates and 10.08 from 48 ejaculates respectively which was less than our findings of 14.830 ± 2.386 . Hendrikse and Antonse (1984) found, for 2024 ejaculates from dogs aged 1 to 9 years representing 121 breeds, the percentage of abnormal spermatozoa was 14.07 which support our findings. Motility decreased with age, while the proportion of abnormal spermatozoa increased significantly.

The prime necessity for professional dog breeders is to recognize the dog in respect

of its fertility as well as pedigree selection for hybridization practice to maintain the trend of further enhancing the production of good quality puppies. In this context, present findings can help to get much more information on different aspect of semen parameters to encourage the future research on enhancement of quality and preservation process of German Shepherd dog semen.

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REFERENCES

- Andersen K, 1975. Insemination with frozen dog semen based on a new insemination technique. *Zuchthygiene*, 10(1): 1-4
- Bedford JM, 1970. Sperm capacitation and fertilization in mammals. *Biol Reprod*, 2(Suppl): 128-158
- Castelo TS, Bezerra FSB, Lima GL, Alves HM, Oliveira IRS *et al.*, 2010. Effect of centrifugation and sugar supplementation on the semen cryopreservation of captive collared peccaries (*Tayassu tajacu*). *Cryobiology*, 61: 275-279
- Correa JR, Pace MM and Zavos PM, 1997. Relationships among frozen-thawed sperm characteristics assessed via the routine semen analysis, sperm functional tests and fertility of bulls in an artificial insemination program. *Theriogenology*, 48(5): 721-731
- Daiwadnya CB, Hukeri VB and Sonawane SA, 1995. Studies on evaluation of dog semen. *Livest Adv*, 20(11): 34-37
- Despande BR, Velhankar DP, Hadi MA, Gujrathi KG, Hukeri VB *et al.*, 1970. *Bom Vet Coll Mag* XVII: 27
- England GCW, 1992. The cryopreservation of canine semen. Thesis, University of London
- Foote RH and Leonard EP, 1964. Influence of pH, osmotic pressure, glycine and glycerol on survival of dog sperm in buffered yolk extenders. *Cornell Vet*, 54: 78-89
- Freshman JL, 2002. Semen collection and evaluation. *Clinical Tech Small Anim Pract*, 17(3): 104-107
- Hafez ESE, 1993. Reproduction in farm animals. Semen evaluation, Lea and Febiger, pp 405-423

- Hancock JL and Rollinson DHL, 1949. A seminal defect associated with sterility of Guernsey bulls. *Vet Rec*, 61: 742-743
- Harrop AE, 1955. Some observations on canine semen. *Vet Rec*, 67: 494
- Hay MA, King WA, Gartley CJ, Leibo SP and Goodrowe KL, 1997. Effects of cooling freezing and glycerol on penetration of oocytes by spermatozoa in dogs. *J Reprod Fertil*, 51(suppl): 99-108
- Hendrikse J and Antonisse HW, 1984. Evaluation of dog sperm. *Tijdschr Diergeneeskde*, 109: 171-174
- Herman HA and Swanson EW, 1941. Variation in dairy bull semen with respect to its use in artificial insemination. *Missouri Agric Exp Sta Res Bull*, 326: 1-82
- Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Crab OBG and Zanevald LJD, 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil*, 70: 219-228
- Johnston SD, Root Kustritz MV and Olson PNS, 2001. *Canine and Feline Theriogenology*, WB Saunders Co., Philadelphia, pp287-306
- Kutzler MA, 2005. Semen collection in the dog. *Theriogenology*, 64(3): 747-754
- Lentner C, 1982. Statistical significance tests. In: *Geigy scientific tables*. Basle, Switzerland: Ciba Geigy Ltd, pp192-202
- Linde-Forsberg C, 1991. Achieving canine pregnancy by using frozen or chilled extended semen. *Vet Clin North Am Small Anim Pract*, 21: 467-485
- Lomeo AM and Giambersio AM, 1991. Water test: a simple method to assess sperm-membrane integrity. *Int Androl*, 14: 278-282
- Macpherson JW and Panner P, 1967. Canine reproduction: I. Reaction of animals to collection of semen and insemination procedures. *Can J Comp Med Vet Sci*, 31(3): 62-64
- Oettle EE, 1993. Sperm morphology and fertility in dog. *J Reprod Fertile Suppl*, 47: 257-260
- Roberts SJ, 1986. Fertilization and transport of the ovum, sperm and zygote. In: Roberts SJ (ed), *Veterinary Obstetrics and Genital Diseases (Theriogenology)*. Woodstock, Vermont, pp422-427
- Rota A, Iguer-ouada M, Verstegen J and Linde-Forsberg C, 1999. Fertility after vaginal or uterine deposition of dog semen frozen in a tris extender, with or without Equex STM paste. *Theriogenology*, 51: 1045-1058
- Rota A, Strom B and Linde-Forsberg C, 1995. Effects of seminal plasma and three extenders on canine semen stored at 4°C. *Theriogenology*, 44: 885-900
- Seager SW and Platz CC, 1977. Collection and evaluation of canine semen. *Vet Clin North Am*, 4: 765-773
- Smith FO and Graham EF, 1984. Cryopreservation of canine semen: technique and performance. 10th Int Cong Anim Reprod, 2: 216, 944-960
- Thomas PGA, Larsen RE, Burns JM and Hahn CNA, 1993. Comparison of three packaging techniques using two extenders for the cryopreservation of canine semen. *Theriogenology*, 40(6):1199-1205
- Wong WT and Dhaliwal GK, 1985. Observations on semen quality of dogs in the tropics. *Vet Rec*, 116(12): 313-314