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## Basic characteristics assessment of seminogram in Chippiparai dogs

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### Abstract

The assessment of semen quality is a prerequisite for selection of male for propagation of superior germ plasm. The present study was aimed to provide the data on basic characteristics of semen in Chippiparai dog. The semen samples were collected from healthy Chippiparai male dogs. The physical qualities like volume, colour and consistency were examined immediately after the collection and the three fractions of semen were maintained at 37 °C until further analysis in the laboratory. The semen samples were analysed for motility, concentration, viability, morphology and membrane integrity of spermatozoa by the standard methods and techniques using the compound microscope. The study shows that the semen of Chippiparai dogs possess exemplary qualities with high percentage of progressively motile sperm, higher concentration of sperm, more percentage of live sperm with normal structure. The result of the study reports for the first time the preliminary data of seminogram in Chippiparai dogs. In conclusion, the best quality of Chippiparai dogs semen can be utilized to cater to the purpose of upholding this valuable indigenous breed of dog.

**Keywords:** Chippiparai dog, semen, reproduction, sperm viability

### Introduction

Dog was the first animal to be domesticated and it is closely associated with the humankind since the time immemorial (Freedman *et al.*, 2017) [4]. Over the years, the dog became a loyal guardian to his master besides being a companion animal. The people have the tendency of rearing an exotic dog breed not only as a matter of pride but also a symbol of social status. Indian dog breeds are in no way inferior to their foreign counterpart. One such noteworthy Indian dog breed is 'Chippiparai breed of dog'. It is well known for hunting and guarding traits (Karthickeyan *et al.*, 2015) [6].

The male animal contributes equally with its sexual partner in perpetuation of a species. The evaluation of semen quality plays a vital role in artificial insemination (AI) programme, breeding soundness examination and diagnostic purposes (Martinez, 2004) [9]. The semen analysis predicts the fertilizing capacity of spermatozoa (Mann and Lutwak Mann, 2012) [10]. The assessment of semen is perhaps the most widely used measure of semen quality (Lopate, 2009) [8]. The determination of sperm concentration is one of the most important parameters for the evaluation of semen sample for fertility (Rijsseleare *et al.*, 2005 [12]; Bjorndahl, 2013 [2]). The sperm with normal morphology is positively correlated with fertility (Alm *et al.*, 2006) [1].

The studies on semen gain currency in the recent trend of research. Since no information is available on the seminogram of Chippiparai dogs, the study was undertaken with the objective of finding the basic characteristics of semen in Chippiparai dogs.

### Materials and Methods

#### Semen collection

Semen samples were collected from Chippiparai male dogs (n = 6) aged between 2- 4 years by digital manipulation technique in the presence of an estrus bitch (Fig. 1 & 2). Under ideal conditions, the male dog is allowed to display courtship behavior with an estrous bitch until a partial erection occurs. Then the dog's penis is gently massaged through the prepuce with the gloved hand. The prepuce is quickly retracted over the bulbus glandis and firm constant pressure is applied on the penis behind the bulbus glandis by squeezing the penis between index finger and thumb. Once the penis attains complete erection, the dog ejaculates the semen in three different fractions (Fig. 3) such as first fraction containing prostatic and urethral fluid,

second sperm rich fraction and third post ejaculatory prostatic fluid (Kutzler, 2005) [7]. They were collected separately in clean and sterile graduated beakers.



**Fig 1:** Sexual arousal of male dogs before semen collection



**Fig 2:** Semen collection by digital massage method



**Fig 3:** Three fractions of collected semen

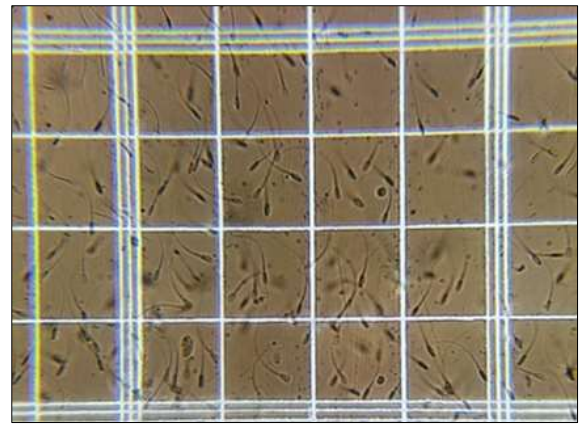
#### Examination of physical qualities of semen

Immediately after collection, the semen was evaluated to assess its physical qualities like volume, colour and consistency besides storing it in a water bath at 37°C until further analysis. The volume of semen was directly measured from the graduated semen collection cup and beaker. The colour was assessed by observing it in the collection cup and was classified as cloudy, white and milky white. The consistency was assessed by passing the semen sample through the pipettes and was graded as creamy to watery.

#### Microscopic examination of semen

The basic characteristic tests such as mass/individual motility, sperm concentration, viability, abnormality and plasma membrane integrity were assessed by standard methods using the compound microscope. A drop of semen was kept on a clean, pre-warmed microscopic slide and examined under microscope to assess the mass motility of sperm. The

individual motility was assessed by placing a drop of semen was kept on a microscopic slide, covered with a clean cover slip and examined under microscope (Martinez, 2004) [9]. The concentration of spermatozoa in the semen was assessed by Neubauer haemocytometer method (Bjorndahl, 2013) [2] (Fig. 4). The sperm viability and morphology were assessed by Eosin-Nigrosin staining technique and examined under microscope (100 X). The sperm with pink head was counted as dead sperm and the white head was taken as live sperm (Fig. 5). The membrane integrity was assessed by the hypo-osmotic swelling test (HOST) was performed (Jeyendran *et al.*, 1984) [5] by mixing 1 ml of hypo-osmotic solution (fructose – 13.5 g and sodium citrate – 7.35g dissolved in 1000 ml of distilled water with osmolality of 150 mosm/l) in a test tube with 0.1 ml of the semen and incubating the mixture for 30 minutes at 37°C. Thereafter, a drop of mixture was placed on a clean, pre-warmed microscopic slide and observed under the microscope (40 X). The spermatozoa with curled tails are counted as HOST reactive sperm (Fig. 6).



**Fig 4:** Enumeration of spermatozoa by hemocytometer method



**Fig 5:** Live and dead spermatozoa



**Fig 6:** HOST reactive sperms with curled tails

## Results and Discussion

The macroscopic examination of semen revealed the appropriate physical characteristics of dog semen. The result of the physical qualities of dog semen was presented in Table. 1. The volume of the semen in all three fractions varied among the animals. The obtained value of first fraction was 1.5 to 2.25ml, the sperm rich second fraction varied from 2.5 to 4.0 ml and the last fraction with the clear prostatic fluid was varying from 18.0 to 35.0 ml. Volume is not an indicator of semen quality in dogs. However, the measurement of semen volume is important in the calculation of total number of spermatozoa in the sample (England and Allen, 1989) [3]. The visual examination of the semen revealed the clear and watery first and third fraction that indicates less or no sperm. The grayish milky appearance with opaqueness of the second fraction gives an idea of the sperm concentration. The turbidity or opacity of semen provides an indication of higher concentration of sperm (Root Kustritz, 2007) [13].

**Table 1:** Physical qualities of semen in Chippiparai Dog

S. No.	Parameters	1 <sup>st</sup> Fraction	2 <sup>nd</sup> Fraction	3 <sup>rd</sup> Fraction
1.	Volume (ml)	1.5 – 2.25	2.5 – 4.0	18.0 – 35.0
2.	Colour	Turbid/Cloudy	Thin Milky	Translucent
3.	Consistency	Watery	Viscous	Watery

The sperm rich second fraction was subjected to the assessment of basic characteristics and the results are summarized in Table.2. The mass and individual motility showed more than 70% and more than 60% respectively in the second fraction of semen. This is supported by findings of Lopate (2009) [8] who reported the normal percentage of progressively motile spermatozoa is 70% or greater in the dog. A progressively motile sperm achieves higher fertilizing ability (Rijsselaere *et al.*, 2003) [11]. The sperm concentration was ranging from 425million to 628million/ ml of semen. The higher concentration of semen correlates with the fertility in dogs (England and Allen, 1989) [3]. The sperm viability and morphology were above 71% and 89% respectively. The finding of this study is in accordance with Shipley (1999) [14] who stated that the percentage of live spermatozoa with normal morphology should be at least 70%. The hypo-osmotic swelling test showed more than 82% of HOST reactive sperm that indicates the spermatozoa have more fertilizing ability as their membrane integrity was intact (Jeyendran *et al.*, 1984) [5]. Hence, the semen sample is suitable for cryopreservation.

**Table 2:** Basic Characteristics of spermatozoa of Chippiparai Dog

S. No.	Parameters	Range	Mean±S.E
1.	Mass Motility	70-80%	75.00± 3.00
2.	Individual Motility	60-80%	73.00± 5.00
3.	Sperm concentration	425- 628 million/ml	533.50± 29.78
4.	Live spermatozoa	71-84%	77.50± 2.09
5.	Sperm Morphology	89-94%	92.17± 0.69
6.	Hypo-osmotic swelling test	82-92%	87.94± 1.57

## Conclusion

From this study, it is obvious that the semen of Chippiparai dog is exemplary quality in terms of fertilizing ability of spermatozoa. This information will be useful in successful breeding programme so as to augment the dwindling population of valuable indigenous Chippiparai dogs.

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## References

1. Alm K, Peltoniemi O, Koskinen E, Andersson M. Porcine field fertility with two different insemination doses and the effect of sperm morphology. *Reproduction in Domestic Animals* 2006;4(1):210-213.
2. Bjorndahl L. Methods for sperm concentration determination. Springer Science & Business Media LLC 2013;1:3-13.
3. England GCW, Allen WE. Semen characteristics and fertility in dogs. *Veterinary Record* 1989;125-139.
4. Freedman, Adam H, Wayne, Robert K. "Deciphering the Origin of Dogs: From Fossils to Genomes". *Annual Review of Animal Biosciences* 2017;5(1):281-307.
5. Jeyendran RS, Vandervan HH, Palaez MP, Crabo BG, Zaneveld LJD. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *Journal of Reproduction and Fertility* 1984;70:219-228.
6. Karthickeyan SMK, Ravimurugan T, Hisham A Sivaselvam SN. Chippiparai breed of dogs in Tamil Nadu: An assessment of physical and performance characteristics. *Indian Journal of Veterinary Sciences and Biotechnology* 2015;10(3):45-49.
7. Kutzler MA. Semen collection in the dog. *Theriogenology* 2005;64(3):747-754.
8. Lopate C. Advanced in canine semen evaluation techniques. *Clinical Theriogenology* 2009;1(1):169-194.
9. Martinez ALP. Canine fresh and cryopreserved semen evaluation. *Animal Reproduction Science* 2004;82:209-224.
10. Mann TC, Lutwak Mann. Male Reproductive Function and Semen: Themes and Trends in Physiology, Biochemistry and Investigative Andrology 2012,25-69.
11. Rijsselaere T, Van Soom A, Maes D, Kruif A. Effect of technical settings on canine semen motility parameters measured by the Hamilton-Thorne analyzer. *Theriogenology* 2003;60(8):1553-1568.
12. Rijsselaere T, Van Soom A, Tanghe S, Coryn M, Maes D, Kruif A. New techniques for the assessment of canine semen quality: A review. *Theriogenology* 2005;64:706-719.
13. Root Kustritz MV. The value of canine semen evaluation for practitioners. *Theriogenology* 2007;69:329-331.
14. Shipley C. Breeding soundness examination in the boar. *Swine Health and Production* 1999;7:117-120.