

E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(4): 1704-1707

© 2020 JEZS

Received: 07-05-2020

Accepted: 09-06-2020

SN Abedin

Department of Veterinary
Physiology, College of
Veterinary Science, Assam
Agricultural University,
Guwahati, Assam, India

V Leela

Department of Veterinary
Physiology, Madras Veterinary
College, TANUVAS, Chennai,
Tamil Nadu, India

G Suganya

Department of Veterinary
Physiology, Madras Veterinary
College, TANUVAS, Chennai,
Tamil Nadu, India

K Loganathasamy

Department of Veterinary
Physiology, Madras Veterinary
College, TANUVAS, Chennai,
Tamil Nadu, India

NK Roy

Department of Livestock
Production and Management,
College of Veterinary Science,
Assam Agricultural University,
Guwahati, Assam, India

Corresponding Author:**SN Abedin**

Department of Veterinary
Physiology, College of
Veterinary Science, Assam
Agricultural University,
Guwahati, Assam, India

Correlation and association of hypo osmotic swelling test (HOST) on conventional semen parameters in dogs

SN Abedin, V Leela, G Suganya, K Loganathasamy and NK Roy

Abstract

The hypo-osmotic swelling test (HOST) has been established as a simple reliable technique for determining the functional integrity of sperm membrane. Sperm cells subjected to hypo-osmotic solution generally respond with various degrees of coiling in the tail region. No swelling is evidenced if the sperm membrane is damaged. The percentage of swollen spermatozoa determines the HOS reacted sperms. A total of 20 semen samples from dogs of different breeds coming for breeding soundness evaluation were collected for the present investigation. The fresh semen samples were subjected to HOST with 150 mOsmol/L sodium citrate and fructose solutions. The average values of motility, viability, morphology and HOST in the present study were 76.3%, 69.1%, 63.5% and 67.4% respectively. Statistical analysis depicted a highly significant positive correlation ($P < 0.01$) between HOST positive spermatozoa (%) with sperm motility (%), viability (%) and sperm morphology (%). Linear regression model delineated a statistically significant ($P < 0.001$) influence of HOST positive spermatozoa (%) on percent sperm motility, viability and sperm morphology. In conclusion, HOST technique being simple, rapid and practically inexpensive and conducive of determining the fertility potential of a dog can be suitably used for early screening of semen samples.

Keywords: Hypo-osmotic swelling test, dog, semen, HOST, correlation

1. Introduction

Dogs represent a significant role in society with considerable impact on global economy. In this context, studies focusing on new advances in reproductive biotechnologies such as artificial insemination (AI) and semen cryopreservation are the need of the hour (Thomassen and Farstad, 2009) [19]. In the equation for evaluation of male fertility potential, semen qualitative and quantitative parameters should be considered (Salisbury and Van Demark, 1961) [14]. Different laboratory assays have been developed so as to evaluate the percentage of progressively motile, viable and sperm cells with abnormal morphology. Several other assays have also been demonstrated that evaluate the fertilizing capacity of the sperm by determining the ability of the sperm to undergo normal capacitation in the female tract, ability of the sperm to bind to the zona pellucida and also to bind and penetrate either homologous or heterologous oocytes *in vitro*. In comparison to other domestic animals, semen elements that affected pregnancy rates in bitches have not yet been sufficiently studied. Despite major advances in use of chilled semen in dog breeding programmes and AI, the resultant level of predictability and the odds of fertile mating for dogs was not sufficiently understood (Kustritz, 2007) [11]. Active functional membrane during fertilization was found out to be essential for sperm to fertilize the ovum. Fertilization failure was reported if plasma membrane was physically intact but biochemically/functionally inactive (Jeyendran *et al.*, 1984) [8]. Due to high significance of the plasma membrane in the process of fertilization, the routine evaluation of the functional integrity of the plasma membrane has got utmost importance in the semen evaluation process. The eosin-nigrosine staining simply measures the structural integrity of the plasma membrane but the hypo-osmotic swelling test (HOST) whereby sperm cells are incubated in a hypo-osmotic solution adequately measures the functional integrity of the plasma membrane. Sperm tail swelling in the presence of hypo-osmotic medium reflected normal water transport across the sperm membrane, suggesting normal membrane integrity and functional activity (Selvaraju *et al.*, 2008) [15]. In dog spermatozoa, the hypo-osmotic swelling was found to be positively correlated with sperm motility (Kumi-Diaka, 1993) [10], with sperm motility and viability (Rodriguez-Gil *et al.*, 1994) [13], or not correlated at all with motility, morphology or viability

(England and Plummer, 1993) [5]. The HOST has been adapted to test the functional membrane integrity of spermatozoa from several domestic species, including the dog (Kumi-Diaka, 1993; England and Plummer, 1993 and Rodriguez-Gil *et al.*, 1994) [10, 5, 13]. A relationship between HOST and sperm characteristics in dog spermatozoa has not yet been established. So the present study has been carried out to check out the effectiveness of HOST in dog semen and its correlation with conventional semen evaluation parameters which are routinely used for evaluation of fertility potential.

2. Materials and Methods

The present study was conducted in 20 male dogs of different breeds brought to Veterinary College Teaching Hospital, Chennai for semen evaluation. All the experimental animals were in good general condition with normal sexual libido. The genitalia were also examined and dogs with no pathological alterations in the genitalia were included in the study. Semen was collected by digital manipulation technique as described by Linde-Forsberg *et al.* (1999) [12]. The pre-sperm and post-sperm fractions were discarded while the sperm rich second fraction was kept in water bath at 37°C for evaluation of sperm *in vitro* characteristics. Ejaculates were analysed for motility, viability and morphology according to procedure described by Kidd *et al.* (2001) [9]. For studying the plasma membrane integrity and to get the maximum reactive spermatozoa, different osmolar solutions (100, 150, 200 and 250 mOsmol/L) with sodium citrate and fructose were used. The hypo-osmolar solution of 150 mOsmol/L was used in the experiment since visible changes in the sperm tail were eminent at this concentration. Briefly, 1mL of 150 mOsmol/L solution was taken and mixed with 50 µL of semen sample and incubated at 37 °C for 60 minute. One drop of the mixture

was taken and placed on a clean grease free slide and covered with coverslip. This slide was observed at 400X under the microscope magnification and 200 sperms were counted (Figure 1a & 1b). Spermatozoa with swollen tail were counted as percentage of HOST positive (Jeyendran *et al.*, 1984) [8]. The values recorded as % of HOST positive spermatozoa and progressive motility (%), live-dead spermatozoa (%) and morphologically normal spermatozoa (%) were subjected to Pearson's linear correlation method (Steel and Torrie, 1980) [18]. $P < 0.001$ was considered highly significant.

3. Results and Discussion

The average values of motility, viability, morphology and HOST in the present study were 76.3%, 69.1%, 63.5% and 67.4% respectively. Pearson correlation (Table - 1) revealed that HOST positive sperm percentages were highly significantly positively correlated ($P < 0.001$) with sperm motility (Figure 2), viability (Figure 3) and morphology (Figure 4) percentages. Linear regression analysis (Table - 2) delineated a significant influence of HOST spermatozoa (%) on percent sperm motility ($\beta = 0.918$, $t = 10.098$, $P < 0.001$); viability ($\beta = 0.901$, $t = 9.034$, $P < 0.001$) and sperm morphology ($\beta = 0.901$, $t = 9.066$, $P < 0.001$) respectively.

Table 1: Pearson's correlation (r) of HOS positive sperm (%) with different semen parameters in dogs

Variables	HOS positive sperm (%)	
	r	P value
Sperm motility (%)	+0.918	0.000***
Sperm viability (%)	+0.901	0.000***
Sperm morphology (%)	+0.901	0.000***

***- $P < 0.001$

Table 2: Regression analysis depicting relationship of HOST with sperm motility, viability and morphology

Semen Variables	Degree of association with HOST					
	B value	t value	β	R ²	F value	P value
Motility	5.403	10.098	0.918	0.843	101.975	0.000***
Viability	-10.250	9.034	0.901	0.811	81.610	0.000***
Sperm morphology	-24.707	9.066	0.901	0.812	82.186	0.000***

***- $P < 0.001$



Fig 1a: Functional membrane intact sperm x 400 (HOST positive)



Fig 1b: Functional membrane non intact sperm x 400 (HOST negative)

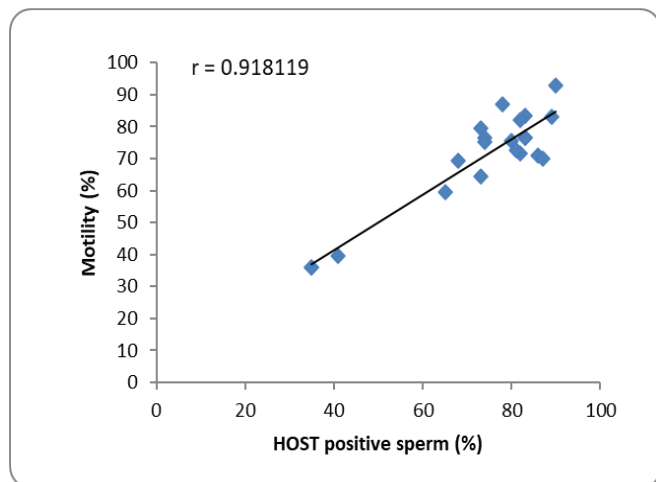


Fig 2: Significant correlation between HOST (%) and motility (%) of spermatozoa in fresh semen.

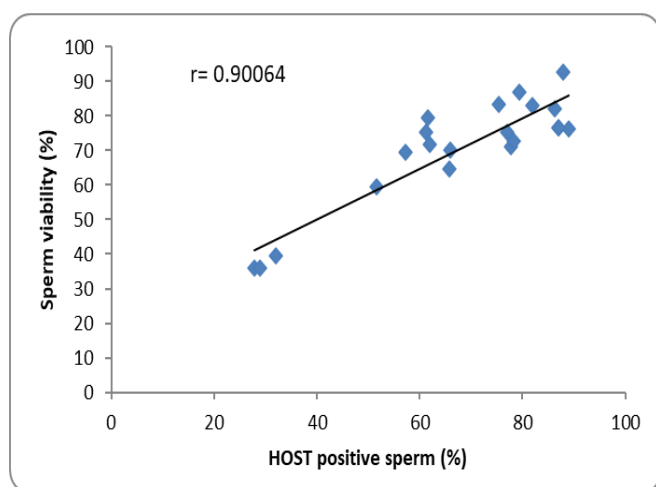


Fig 3: Significant correlation between HOST (%) and viability (%) of spermatozoa in fresh semen.

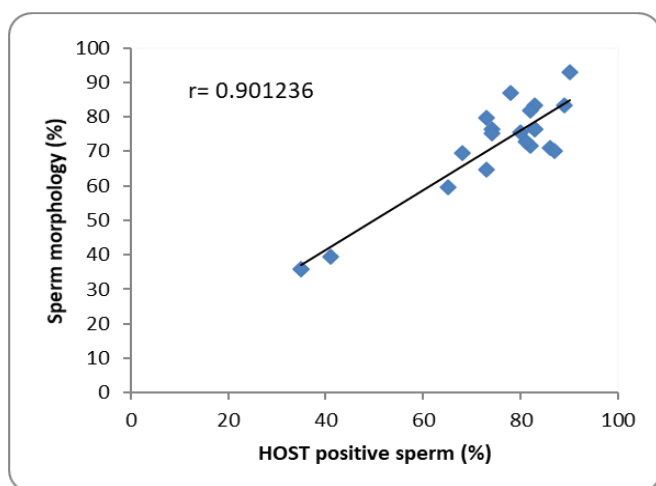


Fig 4: Significant correlation between HOST (%) and morphologically normal (%) of spermatozoa in fresh semen.

The most frequently used parameters in semen lab for initial screening of a semen sample for fertilizing potential is sperm motility, viability and morphology. Simple, rapid tests to determine the functional integrity of the sperm membrane could be conducive of the quality of semen sample for better fertilizing potential. The basic objective in semen evaluation has always been to find out the physiological properties of

sperm that causes infertility (Hammerstedt, 1996) [6]. The sperm plasma membrane was involved in metabolic exchanges with the surrounding medium which necessitates the need to study the integrity of the plasma membrane (Sofikitis *et al.*, 1992) [16]. The process of capacitation, acrosome reaction and the binding of spermatozoa to the oocyte surface membrane required a biologically active and intact plasma membrane and HOST evaluates the biochemical activity of an intact membrane. Biologically active spermatozoa when exposed to hypo osmotic solution undergoes swelling due to influx of water and subsequent increase in volume to establish a state of equilibrium between the fluid compartment within the spermatozoon and the extracellular environment (Jeyendran *et al.*, 1992) [7]. Several authors also quoted HOST as a suitable assay to test male fertility (Stanger *et al.*, 2010 and Baiee *et al.*, 2017) [17, 1]. The findings of the present study showed highly significant positive correlation between HOST positive spermatozoa (%) with sperm motility (%), viability (%) and sperm morphology (%) in dog semen. High correlations between HOST positive spermatozoa, morphologically normal and progressively motile spermatozoa were expected since previous studies has already established that sperm motility partially depends on the functional integrity of the sperm plasma membrane and partially on other sperm biochemical metabolism (Jeyendran *et al.*, 1984) [8]. A good correlation between HOST positive spermatozoa percentage and percentage of live sperm ($r = +0.65$) were reported by Cabrita *et al.*, (1999) [2] after staining with Hoechst 33258 dye, which is in agreement with our results ($r = +0.90$). Dobranic *et al.*, (2005) [4] reported a significant strong positive correlation in dog between HOST and supravital staining and motility $r = +0.94$ and $r = +0.82$ respectively. Our study depicted a significant correlation of $r = +0.90$ and $r = +0.91$ between HOST and sperm viability and motility respectively and are linear to the studies of them. Strong correlation between structural integrity of sperm plasma membrane and HOST positive sperms ($r = +0.81$) were demonstrated in case of bulls to see the effectiveness of supravital staining and HOST by Correa and Zavos, (1994) [3]. Our regression analysis result shows the valuable association between HOST and conventional semen evaluation parameters.

4. Conclusion

With advanced technological developments, though there are several improved and advanced laboratory techniques for undermining the fertility potential of a semen sample but the panic seems to be in developing economies and rural areas where a short, rapid, cheap and practically inexpensive test will solve the dilemma. The results of the present study showed that HOS positive sperm percentages were significantly correlated with sperm motility, viability and morphology. So, in areas where laboratory services are defunct, HOST can be suitably used for early screening of semen samples in dogs.

5. References

1. Baiee FH, Wahid H, Rosnina Y, Ariff OM, Yimer N, Salman H et al. Hypo-Osmotic swelling test modification to enhance cell membrane integrity evaluation in cryopreserved bull semen. *Pertanika Journal of Tropical Agricultural Science*. 2017; 40(2):257-268.
2. Cabrita E, Alvarez R, Anel E, Herraez MP. The hypo osmotic swelling test performed with coulter counter: a

- method to assay functional integrity of sperm membrane in rainbow trout. *Animal Reproduction Science*. 1999; 55:279-287.
3. Correa JR, Zavos PM. The hypo osmotic swelling test: Its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology*, 1994; 42:351-360.
 4. Dobranic T, Samardzija M, Cergolj M, Prvanović N. Determination of membrane integrity on dogs sperm. *Veterinarski Arhiv*. 2005; 75:23-30.
 5. England GCW, Plummer JM. Hypo-osmotic swelling of dog spermatozoa. *Journal of Reproduction and Fertility/Supplement*. 1993; 47:261-270.
 6. Hammerstedt RH. Evaluation of sperm quality: Identification of the subfertile male and courses of action. *Animal Reproduction Science*. 1996; 42:77-87.
 7. Jeyendran RS, Van der Ven HH, Zaneveld LJD. The hypoosmotic swelling test: an update. *Archives of Andrology*. 1992; 29(2):105-116.
 8. Jeyendran RS, Van Der Ven HH, Perez-Pelaez M, 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. *Reproduction*. 1984; 70(1):219-228.
 9. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertility and Sterility*. 2001; 75(2):237-248.
 10. Kumi-Diaka J. Subjecting canine semen to the hypo-osmotic test. *Theriogenology*. 1993; 39(6):1279-1289.
 11. Kustritz MR. The value of canine semen evaluation for practitioners. *Theriogenology*, 2007; 68(3):329-337.
 12. Linde-Forsberg C, Strom-Holst B, Govette G. Fertility data from 327 artificial inseminations with frozen-thawed dog semen comparing vaginal and intrauterine deposition: a retrospective study. *Theriogenology*. 1999; 52(1):1-23.
 13. Rodriguez-Gil JE, Monserrat A, Rigau T. Effects of hypoosmotic incubation on acrosome and tail structure on canine spermatozoa. *Theriogenology*, 1994; 42(5):815-829.
 14. Salisbury GW, Van Demark NL. Physiology of reproduction and artificial insemination of cattle. *Canine Veterinary Journal*. 1961; 20(12):361.
 15. Selvaraju S, Ravindra JP, Ghosh J, Gupta PSP, Suresh KP. Evaluation of sperm functional attributes in relation to *in vitro* sperm zona pellucida binding ability and cleavage rate in assessing frozen thawed buffalo (*Bubalus bubalis*) semen quality. *Animal Reproduction Science*, 2008; 106(3, 4):311-321.
 16. Sofikitis NV, Miyagawa I, Zavos PM. Capacitation/acrosome reaction and outcome of hypoosmotic swelling test in human sperm. *Molecular Andrology*. 1992; 4:363-368.
 17. Stanger JD, Vo L, Yovich JL, Almahbobi G. Hypo-osmotic swelling test identifies individual spermatozoa with minimal DNA fragmentation. *Reproductive Biomedicine Online*. 2010; 21(4):474-484.
 18. Steel RGD, Torrie JH. *Principles and Procedures of Statistics: A biometrical approach*. Edn 2, McGraw Hill Inter Book Co. Tokyo, Japan, 1980.
 19. Thomassen R, Farstad W. Artificial insemination in canids: a useful tool in breeding and conservation. *Theriogenology*. 2009; 71(1):190-199