Haryana Vet. (June, 2022) **61(1),** 34-37

Research Article

INFLUENCE OF AGE ON SEMEN PARAMETERS AND SEMINAL PLASMA ANTIOXIDANTS IN NATIVE DOG SEMEN

S.N. ABEDIN*, V. LEELA, G. SUGANYA and N.A. KADER[1]
Department of Veterinary Physiology, Madras Veterinary College, TANUVAS, Chennai-600007, India
[1]College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, India

Received: 24.05.2021; Accepted: 10.11.2021

ABSTRACT

To investigate the influence of age on semen parameters and levels of seminal plasma antioxidants, the present study was carried out in 20 male dogs of different breeds and subsequently divided into 3 groups, *i.e.*, Group A (<3 years; n=6), Group B (3-6 years; n=8) and Group C (>6 to 9 years; n=6). The semen samples were analysed for sperm *in vitro* characteristics and the various antioxidants present in the seminal plasma were also quantified. Statistical analysis revealed a highly significant ($p<0.01$) difference in sperm motility, acrosome integrity, DNA integrity and GPx concentration when compared between the different age groups. Correlation analysis in the pooled samples revealed a significant ($p<0.05$) negative correlation of age with sperm motility, DNA integrity, acrosome integrity and GPx concentration.

Keywords: Seminal plasma, Dog, Age, Malondialdehyde, Antioxidants, Sperm characteristics

How to cite: Abedin, S.N., Leela, V., Suganya G. and Kader, N.A. (2022). Influence of age on semen parameters and seminal plasma antioxidants in native dog semen. *Haryana Vet.* **61(1)**: 34-37.

Semen quality and sperm fertility estimates in male dogs were largely extrapolations of research and clinical observation from other species. Additionally, age related effects on semen quality and fertility parameters have not yet been investigated in this species (Hesser *et al.*, 2017). Semen analysis necessitates the need for determining the fertilizing potential of a semen sample whether fresh, chilled or cryopreserved, using a rapid inexpensive procedure. Seminal plasma (SP) is a complex mixture fluid and can affect sperm morphology, motility, acrosome reaction and fertility. Semen analysis is a valuable diagnostic tool to assess the fertility status of the male. However, the prediction of potential fertility of a male on the basis of a single assay is not reliable. Each sperm cell consists of multiple sub cellular compartments with different functions, all of which must be intact for successful fertilization (Amann and Graham, 1993). In recent years, more attention has been given to evaluate sperm membrane integrity as it is of fundamental importance in the fertilization process, which was found to be positively correlated with sperm motility, viability and morphology (Abedin *et al.*, 2020). To protect the spermatozoa population from oxidative stress, the reproductive tract environment provides antioxidant protection. Therefore, the present study was performed to investigate the influence of age on semen parameters and antioxidants and to check for correlations, if any, in native dog semen.

MATERIALS AND METHODS

A total of 20 male dogs approaching for breeding soundness examination were selected for the present study and subsequently divided into 3 groups: Group A (<3 years; n=6), Group B (3-6 years; n=8) and Group C (>6 to 9 years; n=6). Semen was collected by digital manipulation technique. The pre-sperm and post-sperm fractions were discarded while the sperm rich second fraction was kept in water bath at 37 ºC for further evaluation. The samples were divided into two aliquots, one for estimation of microscopic characteristics and the second one for harvesting the SP for quantifying the levels of malondialdehyde (MDA) and antioxidant enzymes, *viz.* catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). Fresh ejaculates were immediately analyzed for progressive motility, viability, morphology, plasma membrane integrity and acrosomal integrity as per Kidd *et al.* (2001). DNA integrity status was evaluated by fluorescent staining by acridine orange (AO) dye (concentration - 0.19 mg/mL) as per Chohan *et al.* (2004). JC-1 cationic dye (Sigma Aldrich) was used for assessment of the sperm mitochondrial membrane potential (MMP) under fluorescent microscope (Selvaraju *et al.*, 2008). MDA and antioxidant enzymes namely CAT, GPx and SOD were also quantified by canine specific ELISA kits (Sincere Biotech, China). Statistical package for social sciences programme version 23 (IBM SPSS) was used for statistical analysis. Numerical data were expressed as mean±S.E.

RESULTS AND DISCUSSION

The comparison between different sperm and seminal plasma parameters between dogs of different age groups has been depicted in Table 1.

Age-adjusted analysis of this study data showed significant ($p<0.01$) differences in motility (%) parameter

*Corresponding author: sayedna14@gmail.com

between young (73.67 ± 4.03), middle (75.60 ± 4.84) and older age (44.20 ± 2.47). Haidl *et al.* (1996) studied the influence of age on different sperm parameters and reported that there was no differences in sperm function with age, except for decreased sperm motility in the older group. This could be due to reduction in metabolism as the animal age advances, which in turn is reflected on the motility of the spermatozoa. Age-dependent alterations of the epididymis may also cause alterations in sperm mitochondrial functioning, which is paramount for sperm motility.

Current study showed non-significant ($p>0.05$) differences in sperm viability between the different age groups. A trend towards decline in semen quality in horse stallions after 11-14 years of age has been described by Dowsett and Knott (1996). In dogs, unlike in humans, studies relating to age are scarce and age has not been shown to consistently affect seminal parameters.

The total number of spermatozoa with normal morphology did not differ significantly ($p>0.05$) between different age groups. Current results associate closely to the findings of Oettle (1993) that reported no statistical difference between the age of dogs when compared against the morphological abnormalities and concluded that fertility status of a dog may be affected at any age. Furthermore, disturbances in androgen secretion, a decrease in the number of sertoli cells, modification in testicular microvascularization, increased fibrosis and thickening of the basal membrane which accounted for decrease in cellular oxygenation might confer the spermatozoa with various degrees of abnormalities.

Age related variability in the sperm plasma membrane integrity when studied between different dog breeds did not reach a statistical significant ($p>0.05$) difference in this study. Current results are in close association with the findings of Dobranic *et al.* (2005) who reported that functionally intact plasma membrane value was 67.66 percent in nine sexually mature dogs aged between 1 to 5 years.

Current study depicted a significant ($p<0.01$) decrease in acrosome integrity in aged dogs (58.78 ± 7.08) than in younger (68.17 ± 6.76) and middle (67.51 ± 3.65) aged subjects. This could also be linked to the increase in oxidative stress associated with ageing. Ahmad *et al.* (2011) studied age related changes in the acrosome integrity in eight different age groups in Holstein Friesan bulls and reported that the percentage of spermatozoa with intact acrosome did not change significantly with age.

The present study showed significant ($p<0.05$) decrease in the DNA integrity status in older dogs (65.98 ± 1.45) when compared with younger (81.05 ± 4.97) and middle aged (78.10 ± 3.08) dogs. Although the highest percentage of spermatozoa with intact DNA was observed in younger age group than in middle and older age group. Winkle *et al.* (2009) in their reports didn't detect any significant correlations between age and conventional semen parameters or sperm DNA damage.

Literatures on canine subjects are scanty regarding age related changes in the sperm MMP. The present study showed non-significant ($p>0.05$) effect of age on the sperm MMP in dogs of different age groups.

Age related variation of MDA concentration in current study did not show any significant ($p>0.05$) difference between young, middle and older aged dogs. This corresponds to the findings of Ahmed *et al.* (2018) that reported no significant difference in the seminal plasma MDA concentration between aged and young bulls. In present study, hypothesis was framed that lipid peroxidation in the seminal plasma would increase with age of dog. However, based on current results, it appeared that overall lipid peroxidation in canine SP was independent of age of the animal. Increased lipid peroxidation in the SP may not be an universal feature of aging. Therefore, based on the MDA concentration in the SP it was proposed that lipid peroxidation occurs independently of age and could adversely affect the spermatozoal properties (Kadirvel *et al.*, 2009).

Current results depicted non-significant ($p>0.05$) differences in the CAT concentration with age when compared among different subjects. Waheed *et al.* (2013) described the activity of CAT in different age groups of Arabian horses and reported no significant differences when compared among different age.

A highly significant difference existed in current study in the GPx concentration when studied between different age groups. Results pertaining to this study are in alignment with the findings of Kelso *et al.* (1997) that reported marked reduction in the GPx activity in the SP of bulls with advancing age.

Age related variability in the concentration of SOD was not evident in this study and coincides with the reports of Waheed *et al.* (2013) that found non-significant differences in the SOD activity in different age groups of Arabian horses.

Correlation between different seminal parameters and antioxidants with age (Table 2)

Correlation analysis revealed a significant ($p<0.05$) negative correlation of age with sperm motility ($r = -0.544$, $p= 0.011$) and DNA integrity ($r = -0.486$, $p = 0.026$).

Table 1

Comparison of different sperm and seminal plasma parameters between dogs of different age groups

Variables	Groups			F value
	Group A (n=6) (Mean ± S.E)	Group B (n=8) (Mean ± S.E)	Group C (n=6) (Mean ± S.E)	
Semen Parameters				
Volume (mL)	0.86±0.12	1.32±0.13	1.28±0.16	2.630^{NS}
Concentration (millions/mL)	294.55±73.43	323.78±48.41	207.15±22.81	1.018^{NS}
Sperm motility (%)	73.67±4.03^{a}	75.60±4.84^{a}	44.20±2.47^{b}	7.382**
Sperm viability (%)	65.58±8.64	70.86±5.78	63.63±9.33	0.259^{NS}
Normal morphology (%)	64.46±8.94	63.58±6.25	54.00±11.27	0.385^{NS}
Functional membraneintegrity (%)	74.33±8.29	70.16±4.10	66.19±5.26	0.341^{NS}
Acrosome integrity (%)	68.17±6.76^{a}	67.51±3.65^{a}	58.78±7.08^{b}	7.547**
DNA integrity (%)	81.05±4.97^{a}	78.10±3.08^{a}	65.98±1.45^{b}	3.639*
Mitochondrial membrane potential (%)	87.34±2.41	80.58±4.85	72.54±4.53	1.844^{NS}
Seminal plasma parameters				
MDA concentration (ng/mL)	68.44±1.81	66.34±1.78	68.69±2.41	0.441^{NS}
CAT concentration (ng/mL)	26.44±4.21	23.66±1.55	23.08±0.62	0.450^{NS}
GPx concentration (ng/mL)	23.24±0.85^{b}	23.65±0.51^{b}	8.70±3.50^{a}	24.891**
SOD concentration (ng/mL)	10.81±0.78	9.95±0.48	9.14±0.54	1.455^{NS}

Means bearing different superscript between groups differ significantly at 1% and 5 % level of significance
*- Significant ($p<0.05$)
**- Highly Significant ($p<0.01$)
NS- Not Significant

Table 2

Pearsons correlation (r) of the semen variables in different age groups

Variables	Age (in years)							
	Group A (n=6)		Group B (n=8)		Group C (n=6)		Pooled (n=20)	
	r	p value	r	p value	r	p value	r	p value
Volume (mL)	0.182	0.730^{NS}	0.390	0.265^{NS}	0.156	0.802^{NS}	0.370	0.098^{NS}
Concentration (millions/mL)	0.072	0.892^{NS}	-0.661	0.038*	0.983	0.003**	-0.319	0.158^{NS}
Sperm motility (%)	-0.593	0.215^{NS}	0.205	0.569^{NS}	-0.052	0.934^{NS}	-0.544	0.011*
Sperm viability (%)	-0.374	0.465^{NS}	-0.074	0.840^{NS}	0.719	0.171^{NS}	0.010	0.964^{NS}
Normal morphology (%)	-0.478	0.338^{NS}	0.143	0.693^{NS}	0.578	0.307^{NS}	-0.078	0.737^{NS}
Functional membrane integrity (%)	-0.396	0.438^{NS}	0.231	0.521^{NS}	0.643	0.242^{NS}	-0.061	0.794^{NS}
Acrosome integrity (%)	-0.701	0.121^{NS}	-0.011	0.976^{NS}	0.166	0.790^{NS}	-0.608	0.003**
DNA integrity (%)	-0.244	0.642^{NS}	-0.058	0.873^{NS}	0.321	0.598^{NS}	-0.486	0.026*
Mitochondrial membrane potential (%)	-0.486	0.329^{NS}	0.381	0.277^{NS}	-0.555	0.332^{NS}	-0.339	0.133^{NS}
MDA (ng/mL)	0.198	0.707^{NS}	-0.256	0.475^{NS}	-0.496	0.396^{NS}	-0.070	0.765^{NS}
CAT (ng/mL)	-0.526	0.283^{NS}	0.470	0.170^{NS}	-0.202	0.745^{NS}	-0.133	0.564^{NS}
GPx (ng/mL)	0.194	0.712^{NS}	-0.058	0.873^{NS}	-0.206	0.740^{NS}	-0.731	0.000**
SOD (ng/mL)	0.223	0.671^{NS}	-0.398	0.255^{NS}	-0.402	0.503^{NS}	-0.424	0.055^{NS}

*- Significant ($p<0.05$)
**- Highly Significant ($p<0.01$)
NS- Not Significant

Furthermore, current study also revealed a highly significant ($p<0.01$) negative correlation of age with acrosome integrity ($r = -0.608$, $p = 0.003$) and GPx concentration ($r = -0.731$, $p = 0.000$).

CONCLUSION

From the present study it can be concluded that increase in lipid peroxidation and defective antioxidant defense system (excluding GPx) in the seminal plasma

may not be a universal feature of aging. Furthermore, sperm characteristics can get affected at any age since different compounding factors associated with semen and testicular pathologies are impossible to differentiate.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Veterinary Physiology, Madras Veterinary College, TANUVAS, Chennai-600007 for giving necessary permission and facility to carry out the research work.

CONFLICT OF INTEREST

There is no conflict between the authors regarding the preparation of the manuscript. The research was carried out as a part of fulfillment of M.V.Sc. Degree Programme. All authors read and approved the final manuscript.

REFERANCES

Abedin, S.N., Leela, V., Suganya, G., Loganathasamy, K. and Roy, N.K. (2020). Correlation and association of hypo osmotic swelling test (HOST) on conventional semen parameters in dogs. *J. Ent. Zool. Stud.* **8(4)**: 1704-1707.

Ahmad, E., Ahmad, N., Naseer, Z., Aleem, M., Khan, M.S., Ashiq, M. and Younis, M. (2011). Relationship of age to body weight, scrotal circumference, testicular ultrasonograms and semen quality in Sahiwal bulls. *Trop. Anim. Health Prod.* **43(1)**: 159-164.

Ahmed, S., Khan, M.I.U.R., Ahmad, M. and Iqbal, S. (2018). Effect of age on lipid peroxidation of fresh and frozen-thawed semen of Nili-Ravi buffalo bulls. *Ital. J. Anim. Sci.* **17(3)**: 730-735.

Amann, R.P. and Graham, J.K. (1993). Spermatozoal function. In: Equine Reproduction, Mcinnon, A.O., Voss, J.L. (Edts.), Lea and Febiger, London, UK, pp. 715-745.

Chohan, K.R., Griffin, J.T. and Carrell, D.T. (2004). Evaluation of chromatin integrity in human sperm using acridine orange staining with different fixatives and after cryopreservation. *Andrologia.* **36(5)**: 321-326.

Dowsett, K.F. and Knott, L.M. (1996). The influence of age and breed on stallion semen. *Theriogenology.* **46(3)**: 397-412.

Haidl, G., Jung, A. and Schill, W.B. (1996). Ageing and sperm function. *Hum. Reprod.* **11**: 558–60.

Kadirvel, G., Kumar, S. and Kumaresan, A. (2009). Lipid peroxidation, mitochondrial membrane potential and DNA integrity of spermatozoa in relation to intracellular reactive oxygen species in liquid and frozen-thawed buffalo semen. *Anim. Reprod. Sci.* **114(1-3)**: 125–134.

Kelso, K.A., Redpath, A., Noble, R.C. and Speake, B.K. (1997). Lipid and antioxidant changes in spermatozoa and seminal plasma throughout the reproductive period of bulls. *Reproduction.* **109(1)**: 1-6.

Kidd, S.A., Eskenazi, B. and Wyrobek, A.J. (2001). Effects of male age on semen quality and fertility: a review of the literature. *Fertil. Steril.* **75(2)**: 237-248.

Oettle, E.E. (1993). Sperm morphology and fertility in the dog. *J. Reprod. Fertil. Suppl.* **47**: 257–260.

Selvaraju, S., Ravindra, J.P., Ghosh, J., Gupta, P.S.P. and Suresh, K.P. (2008). 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. *Anim. Reprod. Sci.* **106(3-4)**: 311-321.

Waheed, M.M., El-Bahr, S.M. and Al-Haider, A.K. (2013). Influence of seminal plasma antioxidants and osteopontin on fertility of the Arabian horse. *J. Eq. Vet. Sci.* **33(9)**: 705-709.

Winkle, T., Rosenbusch, B., Gagsteiger, F., Paiss, T. and Zoller, N. (2009). The correlation between male age, sperm quality and sperm DNA fragmentation in 320 men attending a fertility center. *J. Assist. Reprod. Genet.* **26(1)**: 41-46.