# ASSESSMENT OF ACROSOMAL INTEGRITY OF DOG SPERMATOZOA USING SPERMAC STAINING TECHNIQUE.

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## ABSTRACT

Semen was collected by digital manipulation from eighteen matured dogs of three different breeds. The sperm rich second fraction of semen samples were diluted, cooled and frozen in programmable freezer at three different cooling rates. In an attempt to monitor any changes occurring to the acrosome during freezing, smears made with fresh semen and with frozen semen at various cooling rates were stained with spermac stain and examined under 400x magnifications. Most acrosomal damage appeared to occur during fast and medium cooling rates than in slow cooling rate of programmable freezing. Further, the use of Spermac stain with slight modification in staining technique in the present study provides simple and accurate assessment of the acrosomal membrane changes in frozen thawed dog spermatozoa.

**Key words:** Acrosome, Spermac stain, Programmable freezing.

Cryopreservation of canine sperm is a process that provokes changes in the sperm membrane structure and function, particularly during the extension, cooling, freezing and thawing process. However, even the best cryopreservation techniques were found to cause lethal and sub lethal injury and hence standard diagnostic evaluation based on physical parameters such as sperm motility, sperm morphology and number of spermatozoa in an ejaculate were not sufficient to account for the fertility status of an ejaculate at any time (Keel and Webster,1990).

Although many tests that have been developed to predict the fertility of semen, the most reliable of these seems to be the morphological assessment of acrosomal integrity (Foote, 1975). Since, various stains as well as phase contrast microscopy have been used to examine acrosomal morphology it becomes more difficult to stain extended semen than fresh semen, as factors like egg yolk /milk present in the diluents interfered with most stains. Thus, the present study was undertaken to assess acrosomal integrity of fresh and frozen thawed dog spermatozoa using Spermac staining.

Semen from eighteen healthy and sexually matured dogs of three breeds aged between 2 to 6 years owned by private dog owners was collected by digital manipulation. The sperm rich second fraction was evaluated immediately after collection for membrane changes using Spermac stain (Fertipro, Belgium). The Semen samples were diluted with Tris-Fructose Citric acid extender, cooled, packed in French Mini Straws and frozen in programmable freezer at three different cooling rates viz. slow, medium and fast with -1p C/min

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for 30min, -5p C/min for 10min and -10p C/min for 7min respectively. After 24 hrs of storage at -196°C, the semen straws were thawed in water bath at 37°C for about 1min and the post thaw acrosomal Integrity was assessed using modified spermac staining technique where in the prepared semen smears were fixed, air dried and stained with Spermac stain as per the method prescribed by the manufacturer with slight modification in the staining procedure.(i.e.) the slides were kept 5 to 6 min in solution A instead of 1 to 2 min. and then placed in into solution B and C for 1 to 2 min. respectively. The stained smears (Fig. 2) were examined under 400X magnification and a minimum of 200 spermatozoa were evaluated per slide.Sperms were accessed and scored as follows,

Normal sperm possess a deep green acrosome, mid piece and tail, distinct pale green equatorial zone and a red post acrosomal region of the head (Fig. 2a).

Sperm with altered acrosome seen as swollen, fuzzy acrosome, with decreased green staining affinity (Fig. 2b).

Sperm which did not possess an acrosome, the head stains red (Fig. 2c).

The intact acrosome percentage for fresh semen in the present study ranged from 76 to 82 with an overall mean (± SE) of 76.83 ± 2.63 which was lower than the values reported by Farstad and Andersen Berg, 1989 (96 per cent) and Kurien, 2000 (92 per cent).

The mean percentage of Acrosomal Integrity for slow, medium and fast cooling rates under programmable freezing was 46.38±1.46, 31.50±1.98 and 28.38±1.20, respectively. The post thaw values of acrosomal integrity were found to be significantly higher ($P \leq 0.05$) in slow than with medium and fast cooling rate of programmable freezing. This result concurred with the finding of Strom *et al.* (1997) but lower than the value reported by Nair (1996), Kurien (2000) and Ponglowhapen *et al.* (2004).

The lower value obtained in the present study could be due to the highly precise Spermac staining technique used to evaluate the acrosomal integrity of frozen thawed spermatozoa. Oettle (1988) and Hay *et al.* (1997) reported that Spermac stain provides a better resolution and accurate assessment of the membrane changes and the type of extender used did not appear to influence the staining affinity of spermac. The present study shows that the Spermac staining technique posses the advantage of being rapid, reliable and can practically be used in the examination of acrosomal defects in pre and post freezing dog semen as with that of other species.

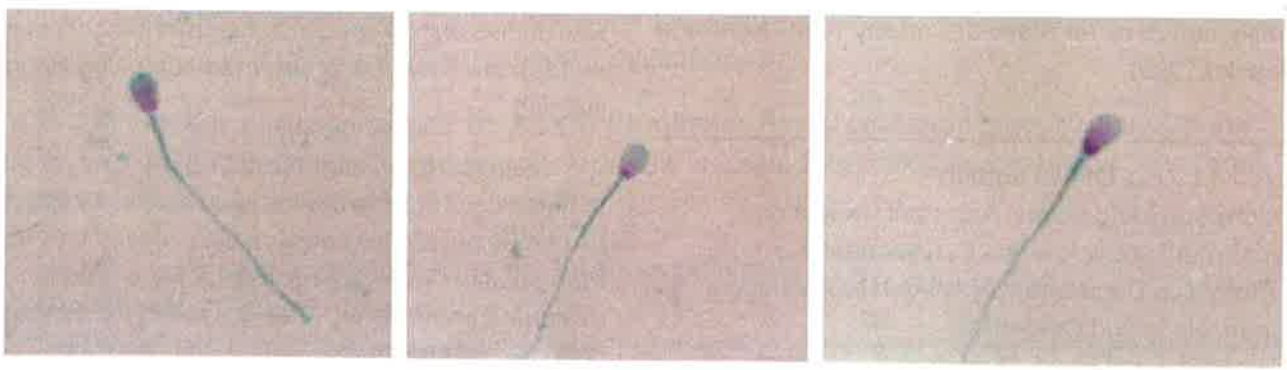

*Fig.2 : Acrosomal integrity of canine spermatozoa(400x magnification) – Spermac staining. (a) Sperm with intact acrosome possess deep green acrosome, mid piece and tail and a red post acrosomal region of the head. (b) Sperm with altered acrosome seen as swollen, fuzzy acrosome with decreased green staining affinity. (c) Sperm which did not possess an acrosome, the head stains red.*

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