A MODIFIED GIEMSA STAINING TECHNIQUE FOR EVALUATION OF ACROSOME INTEGRITY OF FROZEN SEMEN

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ABSTRACT

A simplified technique to evaluate the acrosome integrity using Giemsa stain in frozen semen doses at room temperature was discussed.

Key words: Acrosome integrity, Giemsa stain, Frozen semen

Acrosomal evaluation of sperm is one of the important laboratory tests conducted in frozen semen to assess the potential fertility of a breeding bull. Acrosome, a cap like structure on the head of the spermatozoa covers 60 per cent of the anterior portion of the nucleus. Acrosome integrity is essential for the sperm to undergo capacitation and acrosome reaction in the female reproductive tract. Acrosome intergrity is affected during both freezing and thawing processes thereby affecting the capacitation of the sperm and fertilization. The acrosome morphology therefore is a significant criterion in the evaluation of frozen semen. The stock solution of Giemsa is prepared by dissolving one gram of Giemsa powder in 66 ml of methanol and 60 ml glycerol. After complete dissolution of the Giemsa powder, the stock solution is filtered and preserved in airtight containers.

Thin smear of frozen semen is made on a clean glass slide and air dried. The slide is then immersed in a jar consisting of 2 ml stock Giemsa stain, 20 ml absolute methanol and 23 ml single distilled water of pH 7.4 and left at room temperature for 5-6 hours. The slide from the jar was removed, washed in tap water, air dried and were examined microscopically under oil immersion of a differential interference contrast (DIC) microscope. This simplified technique has yielded best results with improved clarity and contrast with respect to acrosomal evaluation.

Giemsa staining technique (Hancock, 1952 and Pant, 2000) is routinely followed in frozen semen laboratories for staining to evaluate acrosomal integrity. Addition of egg yolk beyond certain level (Benjamin *et al.*, 1990) and increase in glycerol in extender (Mixner and Saroff, 1954) were found to interfere with the stain permeability and results in poor staining quality. The modified technique differs from that developed by Hancock (1952) in avoiding pretreatment of the semen smear with 5 per cent formaldehyde for 30 minutes at 37°C and washing of the slide after formaldehyde treatment. The simplified staining technique gives better staining at room temperature with improved clarity and contrast with respect to acrosome morphology. Hence this technique can be adopted in semen laboratories for the routine evaluation of frozen semen samples to assess their *in vitro* fertility status.

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