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Morphometric evaluation of caprine spermatozoa by HT-IVOS System at pre-freeze and post-thaw stages of cryopreservation*

M.N.SUNDARARAMAN[1†] AND M.JOHN EDWIN[2]

Semen Bank, Department of Animal Genetics & Breeding, Madras Veterinary College
Tamilnadu Veterinary and Animal Sciences University, Chennai - 600 007 (TN)

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ABSTRACT

Semen samples were collected from two adult Boer Grade (Boer x Local) goats by artificial vagina method, evaluated, extended in Tris-Egg yolk-Glycerol based diluent and frozen by French straw technique. After dilution, a portion of extended semen was further diluted to prepare thin sperm smears. The smears were stained with Stat III, Andrology stain for morphometric evaluation by HT-IVOS system. The frozen samples were thawed and samples showing acceptable sperm motility were further diluted for preparation of semen smears for staining and evaluation by HT-IVOS system (CASA). A total of 1000 spermatozoa each at pre-freeze and post-thaw stages of cryopreservation, involving 6 different samples each from two bucks were analysed for morphometric traits *viz.*, major axis, minor axis, elongation, head area, perimeter and tail length. Highly significant ($P \leq 0.01$) differences were noticed for most of the morphometric traits except for minor axis of the sperm head and tail length between different stages of semen processing for cryopreservation. The variations obtained in major axis, minor axis, elongation, head area and perimeter of the sperm head between bucks were also highly significant ($P \leq 0.01$). Most of the morphometric traits of the sperm heads of frozen-thawed spermatozoa also exhibited highly significant ($P \leq 0.01$) variation between bucks. Cryopreservation resulted in irreversible changes in structural integrity of caprine spermatozoa.

Key words: Goat spermatozoa, cryopreservation, morphometry, CASA

Distinct variations in morphometric features of spermatozoa of different species establish the fact that they are genetically controlled. The head of spermatozoa, which carries the genetic material, largely determines the fertilizing capacity of spermatozoa (Mann, 1964). It is well known that dimensions of spermatozoa vary significantly between breeds and between individuals within breeds in several species. Therefore morphometric characteristics of spermatozoa become relevant in evaluation of sperm for fertility. Most of the sperm evaluation protocols include morphological estimation of spermatozoa based on visual approximation of shape and size of sperm but not by morphometric characteristics. Conventional methods of measurement of morphometry of spermatozoa is cumbersome, time consuming and subjective. In a study conducted by Jequier and Ukombe (1983), 26 professionals were employed to evaluate morphology of single semen sample. They gave the abnormal sperm per cent between 5 and 85. Concurrently, three skilled technicians estimated the percentage of abnormalities for the same sample as 25 to 40%. Therefore, a definite need for improved methodology for accurate and repeatable morphology assessment was felt. To get over these difficulties, computer assisted semen analyser (CASA) is the equipment of choice which facilitate accurate and reliable morphometric analysis of spermatozoa. Spermatozoa are subjected to rigorous stress during the process of cryopreservation which may possibly alter the morphology of spermatozoa thereby reduces the fertilizing ability of sperm. These morphometric changes may not be appreciable by conventional microscopic evaluation of spermatozoa. Therefore, a study was undertaken to measure the morphometric features of goat spermatozoa and to investigate the influence of cryopreservation on morphology of spermatozoa.

MATERIALS AND METHODS

Semen samples were collected by Artificial Vagina method from 2 Boer grade (Boer x Local) half-bred adult bucks, maintained under stall-fed conditions. The samples were evaluated for semen characteristics by conventional methods. Samples suited for cryopreservation were frozen

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[1]Professor

[2]Professor and Head (Retd.)

†Corresponding author

by French straw technique and stored in liquid nitrogen at -196°C until evaluation.

The extended semen sample preserved for evaluation by CASA was further diluted to reduce the sperm concentration for fair analysis by CASA. Thin semen smears were prepared and the slides were stained using STAT III Andrology stain. Dried smears were first immersed in Solution I (fixative), methanol for 30 seconds. After drying, the slides were immersed in Solution II (Xanthene Dye based stain), for 60 seconds. Again after drying in air, the smears were counter stained by dipping in Solution III (Thiazine Dye) for 60 seconds. The stained smears were again dried in air. The excess stain was washed with distilled water and the smears were dried again in air for evaluation by CASA.

The frozen semen samples were thawed in a water bath at 37°C for 30 seconds and further extended to minimise the sperm number for accurate analysis by CASA. The procedure followed for pre-freeze samples was repeated for preparation of stained slides from frozen-thawed semen. Sperm slides were prepared only from frozen thawed samples having at least 40 per cent of progressively motile spermatozoa. The morphometric evaluation of pre-freeze and post-thaw spermatozoa was done by HT-IVOS system. Major axis, minor axis, elongation, head area, perimeter and tail length were the morphometric traits studied in the present investigation. The data were analysed statistically for interpretation.

RESULTS AND DISCUSSION

The major axis (μm) of head of Boer Grade buck spermatozoa obtained in the present study was higher than those reported by conventional methods of measurements for several exotic, indigenous and local goats (Bardoloi and Sharma, 1983; Misra and Mukherjee, 1984; Deka and Rao, 1987). The major axis as determined by Gravance *et al.* (1995) for Alpine goats by computer automated analysis was lower than those obtained for Boer Grade bucks in this study. The mean values for minor axis and head area recorded in the present study for Boer grade bucks were lower than those measured by conventional methods for different breeds by other workers (Bardoloi and Sharma, 1983; Misra and Mukherjee, 1984; and Deka and Rao, 1987) but higher than the mean values obtained by Gravance *et al.* (1995) on computer automated sperm head morphometry analysis in Alpine goats. Information on morphometric trait of elongation of goat spermatozoa head was not reported in the works by conventional methods. Nevertheless, from the studies of Gravance *et al.* (1995) the estimated value for elongation of spermatozoa was higher than those recorded in the present study for Boer Grade goats.

The mean value for Perimeter of Boer Grade spermatozoa head recorded in this study was similar to those observed for Alpine bucks by Gravance *et al.* (1995) by computer automated method. Barodoloi and Sharma (1983) and Deka and Rao (1987) reported the mid piece and tail length separately in different breeds by conventional methods. However, in the present analysis the tail length was referred as the total length of the tail including both mid piece and tailpiece length. The estimated tail length (μm) of spermatozoa in different breeds from the reports of Barodoloi and Sharma (1983) and Deka and Rao (1987) were almost similar to the mean value for tail length obtained by present CASA analysis for Boer Grade bucks. Highly significant ($P \leq 0.01$) differences were found between Boer Grade bucks in this study for major axis, minor axis, elongation, head area and perimeter of the pre-freeze sperm heads. Similar results were published by Misra and Mukherjee (1984) and Deka and Rao (1987) for major and minor axis of spermatozoa of certain other breeds of goats.

In automated sperm morphometry analysis, specimen preparation, image acquisition, image processing and analysis method were reported to influence the accuracy of the measurement (Verstegin *et al.*, 2002). Considering the above factors, the variations observed in this study for morphometric traits of Boer Grade goats from those of Alpine bucks as reported by Gravance *et al.* (1995) might be because of different computer automated equipment employed for morphometric analysis and various methods employed for staining of spermatozoa besides however the basic genotypic difference. The variations observed for most of the morphometic traits in the present analysis from those of reports of other workers, who had determined the dimensions of spermatozoa by manual methods, might be because of different breeds of goats involved in the studies as it has been well-established that dimensional characteristics of spermatozoa are controlled by genes (Koley *et al.*, 1985) besides obviously, the different methods adopted for morphometric analysis.

Most of the morphometric traits of frozen-thawed sperm heads also showed highly significant ($P \leq 0.01$) variation between bucks. These findings confirmed the existence of individual genetic variation among bucks for morphometry of spermatozoa. Except for Minor Axis of sperm

Table 1. Mean (±S.E.) values of morphometric traits of goat spermatozoa at different stages of cryopreservation

Buck No.	Stage of Processing	Mean (±S.E)					
		Major Axis (mm)	Minor Axis (mm)	Elongation (%)	Head Area (μm^2)	Perimeter (mm)	Tail Length (mm)
BL 5	Pre-freeze	8.22 ± 0.01 (500)	3.93 ± 0.01 (500)	47.86 ± 0.10 (500)	26.24 ± 0.07 (500)	20.46 ± 0.03 (500)	50.84 ± 0.15 (136)
	Post-thaw	8.16 ± 0.01 (500)	3.96 ± 0.01 (500)	48.41 ± 0.10 (500)	26.10 ± 0.09 (500)	20.35 ± 0.03 (500)	50.85 ± 0.16 (136)
BL 6	Pre-freeze	8.47 ± 0.01 (500)	3.97 ± 0.01 (500)	46.85 ± 0.11 (500)	27.28 ± 0.08 (500)	20.96 ± 0.03 (500)	50.93 ± 0.11 (136)
	Post-thaw	8.33 ± 0.02 (500)	3.92 ± 0.01 (500)	47.01 ± 0.10 (500)	26.32 ± 0.07 (500)	20.62 ± 0.03 (500)	50.69 ± 0.09 (136)
Overall	Pre-freeze	8.34 ± 0.01 (1000)	3.95 ± 0.01 (1000)	47.35 ± 0.01 (1000)	26.76 ± 0.05 (1000)	20.71 ± 0.02 (1000)	50.88 ± 0.09 (272)
	Post-thaw	8.24 ± 0.01 (1000)	3.94 ± 0.01 (1000)	47.71 ± 0.01 (1000)	26.21 ± 0.06 (1000)	20.48 ± 0.02 (1000)	50.77 ± 0.07 (272)

Figures in parenthesis indicate number of observations.

head and Tail Length all the other morphometric traits of Boer Grade buck spermatozoa exhibited highly significant ($P \leq 0.01$) differences between pre-freeze and post-thaw stages of cryopreservation indicating that freezing and thawing distinctly alter the morphology of spermatozoa. Since the head of the spermatozoa, which carries the genetic material largely determines the fertilizing ability of spermatozoa, the variation in morphometeric traits of spermatozoa between individual males and between stages of processing during cryopreservation possibly explains the variation in fertility although the samples showed acceptable quality for sperm motility. From this study, it was inferred that cryopreservation resulted in irreversible changes in the structural integrity of caprine spermatozoa.

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