

## The Effect of Straw Position in Nitrogen Vapour during Equilibration on Post-Thawing Motility and Membrane Integrity Following Quick Freezing in Madura Cattle Sperm

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### ABSTRACT

Processing semen needs appropriate technology to produce high quality of sperm for successful artificial insemination (AI) program. Quick freezing is developed freezing technique from conventional freezing in sperm cryopreservation. In this technique, the straw containing sperm was equilibrated in liquid nitrogen vapour before direct plunged into liquid nitrogen. The present study was conducted to evaluate the effect of height position of straw in liquid nitrogen vapour on the quality of Maduran cattle sperm after thawing. Fresh semen was diluted with tris-aminomethane base extender, then loaded into 0.25 ml straw and refrigerated for 30 min. Before plunging into liquid nitrogen, the straws were placed at 5 cm, 10 cm or 15 cm height upper the surface of liquid nitrogen in its vapour for 5 min. Immediately after thawing, the sperm was evaluated for motility and membrane integrity by hypo-osmotic swelling test (HOST). The results showed that sperm motility and HOST significantly improved at 5 cm height upper the liquid nitrogen surface ( $p < 0.05$ ), T5 ( $31.00 \pm 6.15$ ), T10 ( $26.00 \pm 9.37$ ) and T15 ( $10.00 \pm 0.00$ ) and HOST T5 ( $31.90 \pm 4.73$ ), T10 ( $29.20 \pm 6.39$ ) and T15 ( $19.20 \pm 2.70$ ). Conclusion, the effect of straw position in nitrogen vapour during equilibration at 5 cm height showed higher post thawing motility and membrane integrity than height 10 cm and 15 cm following quick freezing in Maduran cattle sperm. There was significant correlation between sperm motility and membran integrity.

**Key Words:** Quick freezing, Motility, Sperm membrane integrity, Artificial insemination, Maduran cattle sperm

### INTRODUCTION

The development of Madurans bull cattle as one of local cattle required in order to beef self-sufficiency for fulfill public nutrition still faced many problem of reproduction especially about poor semen quality that required handling to increase fertility rate use appropriate technology semen processing. Previous study reported, that the percentage of post thawing sperm motility in Maduracattle still achieve about 37%- 44 % (Salim et al, 2012). Processing of frozen semen requires appropriate technology beginning step of cooling, nitrogen vapour equilibration, freezing in liquid nitrogen and semen storage. Whereas, for that processing frequently occurs decreasing in motility, viability, membran integrity and increasing number of sperm abnormality. It was indicate that each step processing semen causing physiology damaged especially at level sperm membrane that affected in poor motility.

Slower freezing method using a programmable freezer was required long processing time and produced low rate of motility (Thachil and Jewett, 1981) or (Serafini and Marrs, 1986). Another research have done about ultra-rapid freezing of very low numbers of sperm placed in liquid nitrogen vapour 3 cm upper the surface of the liquid nitrogen for 5 min prior to submersion (ultra-rapid freezing). Vials were left submerged in the liquid nitrogen for a minimum of 10 min prior to thawing. The result of ultra-rapid freezing is feasible using suspended in liquid nitrogen vapour for 5 min (Timothy et al., 2003). The hypothesis of the study was improving that height of equilibration nitrogen vapour for 5 min influence on

post-thawing semen quality that could be predicted from semen analyses included motility and Hypoosmotic Swelling Test (HOST) of membran integrity at Madurans bull cattle and there was significant correlation between sperm motility and membrane integrity.

## MATERIALS AND METHODS

**Semen samples.** Semen sample were used from Madurans cattle with sperm motility  $\geq 70\%$ , at Singosari National Artificial Insemination Centre Malang, Indonesia. Semen collection was carried out two times per week using an artificial vagina. One aliquot of 1 ml of fresh semen was separated and diluted by Egg-yolk Tris Aminomethane, glycerol 7%, penicilline 1000 IU/ml, streptomycin 100 mg. Sample were cooling at  $5^{\circ}\text{C}$  for 30 min, sealing/chilling into 0,25 ml straw.

**Quick Freezing.** Straw were placed horizontal upper tray and equilibrated with nitrogen vapour at various height ( 5cm, 10cm and 15cm) upper liquid nitrogen surface for 5 min (quick freezing) then plunged into liquid nitrogen ( $-196^{\circ}\text{C}$ ) and finally storage in the container that contain liquid nitrogen

**Semen thawing.** Thawing process was carried out after  $>10$  min after storage into container that contain liquid nitrogen by placed straw into a water bath at  $37^{\circ}\text{C}$  for 30 sec.

**Semen evaluation.** Semen evaluation at first for volume and mass motility, sperm concentration was count using spectrofotometer, mass motility rates from +3 to +4, and less than 20% morphological abnormalities. Motility was expressed qualitatively on a motility scale (0-4) as described by Matharoo et al. (1985) using a 10X objective lens on a phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan). HOST for sperm membrane integrity was assessed using the hypo-osmotic swelling test according to the methods described by Correa and Zavos (1994). Sperm tail coiled was recorded of 200 spermatozoa using a 40 x objective lens on a phase contrast microscope (Nikon Eclipse E600, Tokyo, Japan).

**Statistical analysis.** We used SPSS program to perform the statistical analyses. Mean percentages of motility and membrane integrity spermatozoa measure by one-way analysis of variance (Snedecor and Cochran, 1967) and the relationship between sperm motility and membran integrity with Pearson correlation at significant correlation  $p > 0.05$  (Hauke And Kossowski. 2011). Prior to the analysis, proportionality data (motility and HOST) were transformed using percent (Snedecor and Cochran, 1994). Comparison between different treatment groups was done by Fisher's Least Significant Difference (LSD) test, the differences at  $p < 0.05$  were considered to be statistically significant.

## RESULTS AND DISCUSSION

The mean percentages of sperm motility and membran integrity at various height are presented in Table 1.

**Tabel 1.** The percentage of sperm motility and membran integrity with various height nitrogen vapour equilibration following freezing at Madurans cattle

Height (T) cm	Motility $\bar{x} \pm sd$	HOST $\bar{x} \pm sd$
T5	$31.00 \pm 6.15^a$	$31.90 \pm 4.73^a$
T10	$26.00 \pm 9.37^b$	$29.20 \pm 6.39^b$
T15	$10.00 \pm 0.00^c$	$19.20 \pm 2.70^c$

The difference superscript at the same collum, indicate significant difference ( $p < 0,05$ );  $\bar{x} \pm sd$  (mean  $\pm$  standart deviation); T5 ( height 5 cm), T10 ( height 10 cm) and T15 ( height 15 cm).

The result showed significant difference ( $p < 0.05$ ) percentage of sperm motility between three height equilibration, T5 more higher than T10 and T15. The correlation between sperm motility and membran integrity was 0.689 showed significant correlation ( $p > 0.05$ ).

In this study the effect nitrogen vapour equilibration upper liquid nitrogen surface on post thawing semen at height 5 cm has more beneficial effect in motility and membran integrity than height 10 cm and 15 cm. Gradually decrease of sperm motility from 5 cm, 10 cm and 15 cm ( $< 40\%$  standart motility for AI), caused by the faster and the slower freezing rate, the temperature changes that spermatozoa are subjected to during the process nitrogen vapour equilibration was the physical effects of cooling and then freezing result in number of changes in the environment of water and solute movement affected cell injurious, whereas the rate water move out of the cell during this process plays an important role in determining cooling rates, which have to be optimized for cell survival after thawing, similar as reported by Mazur (1977); Watson (1979, 1995) and Woelders (1997). The results of the present investigation showed highly significant correlation between HOST score and progressive motility in Sahiwal cow bull semen. In this study, reported that percentage membran integrity at equilibration height respectively decrease 5 cm, 10 cm and 15 cm, it was caused injurious plasma membran sperm affected by ice nucleation formed at highest or lowest rate freezing (Vishwanath and Shannon, 2000), where as HOST more easy obserable in the tail than in the head of sperm because the plasma membrane surrounding the tail appears to be more loosely attached than the membrane surrounding the head, look as coiled tail.

The lower sperm survival caused by the translocation of water and the injurious that affected by ice nucleation during the freezing and thawing process, as reported by Vishwanath and Shannon (2000), rapid leakage of intracellular ATP through the damaged sperm plasma membrane due to death or anisotomic condition is certain to affect sperm motility (Bohlooliet al., 2012; Christensen et al., 1999). The results of the present study were in agreement with some previous work on cattle (Correa and Zavos, 1994; Kumar, 2004; Lodhiet al., 2008). The correlation that was recorded between motility, viability, acrosomal integrity and HOST was expected since they are all related to plasma membrane integrity (Brito et al., 2003) and also between percentages of live sperms and acrosome intact sperms with percentage of motile sperms (Kirk et al., 2005). In this study the higher sperm membran integrity, the higher sperm motility there was showed significant correlation between Motility and membran integrity.

## **CONCLUSION**

The effect of straw position in nitrogen vapour during equilibration at 5 cm height showed higher post thawing motility and membrane integrity than height 10 cm and 15 cm following quick freezing in Maduran cattle sperm. The higher sperm membran integrity, the higher sperm motility.

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