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The Effect of Trehalose Level in Tris-Based Medium on Sperm Membrane Integrity of Boer Goat Semen after Cooling

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ABSTRACT: This study was conducted to evaluate the effect of trehalose level in tris-based medium on sperm membrane integrity of Boer goat semen after cooling. Semen was collected from 6 bucks using artificial vagina. Fresh semen evaluated for colour, pH, volume, concentration, mass motility, individual motility, life sperm, sperm abnormality and sperm membrane integrity. Semen was diluted with tris-based medium suplemented with different level of trehalose (1.5; 2.5 and 3.5%) with the ratio of 1 semen : 9 diluter. Sampling was conducted as purposive sampling, i.e. semen used should has mass motility of 2+ and individual motility of 70%. Immediately after dilution semen was stored in 3-5ºC and sperm membrane integrity percentage was observed at 0, 24 and 48 h. The obtained data were analyze with Analysis of Variance (ANOVA) and continued by Least Significant Different if there was significant or very significant difference between groups. The experiment was designed using completely random design (3 treatments and 10 replications). The results showed that the level of trehalose (1.5, 2.5 and 3.5% had very significant effect (P<0,01) on sperm membrane integrity percentage in 0 h of cooling (65.99; 64.67 and 57.46% respectively), 24 h of cooling (56.58; 55.89 and 48.80% respectively); 48 h of cooling (47.49; 48.75 and 39.38% respectively). It was concluded, that the trehalose levels for resulting in optimal sperm membrane integrity were 1.5%. It was suggested, that for resulting in optimal sperm membrane integrity after cooling of Boer goat semen in tris-based medium should be supplemented with 1.5% trehalose.

Key words: Boer goat semen, cooling, trehalose, sperm membrane integrity

INTRODUCTION

Sperm membrane integrity is an important indicator for characterization of sperm quality after processing. During dilution and cooling, sperm membrane integrity reduce correponding to appropiate processing technique and cryoprotectant used. Disaccharides have a stabilizing efect on biological membrane. Trehalose, a disaccharide of glucose can be viewed as naturally occuring stabilizing agents. Trehalose is found in animals capable of enduring cold temperatures (Sum *et al.*, 2003). This study was conducted to evaluate the effect of trehalose level in tris-based medium on sperm membrane integrity of Boer goat semen after cooling.

MATERIALS AND METHODS

Semen was collected from 6 male mature (2.0 - 2.5 y) with about 100 kg in weight, using artificial vagina (Evans and Maxwell, 1987). The bucks were maintained at Field Laboratory of the Faculty of Animal Husbandry Sumber Sekar, Brawijaya University Malang. After collection, fresh semen was evaluated macroscopically (colour, pH, volume) and microscopically (concentration, mass motility, individual motility, life sperm, abnormal sperm and membrane integrity of sperm). Sampling was conducted as purposive sampling. Only semen with mass motility minimal 2+ and individual motility of sperm more than 70% was used for research material. Semen collection was regularly conducted twice a week per individu of animal.

The selected semen was diluted with tris-based medium suplemented with different level of trehalose (1.5; 2.5 and 3.5%) with the ratio of 1 semen : 9 diluter. Immidiately after

dilution semen was stored in the refrigerator (3-5ºC) and sperm membrane integrity was observed at 0, 24 and 48 h after cooling (Neild, *et al.*, 1999). The obtained data were analyze with Analysis of Variance (ANOVA) and continued by Least Significant Different if there was significant or very significant difference between groups. The experiment was designed using completely random design (3 treatments and 10 replications).

RESULTS AND DISCUSSION

Characteristics of fresh Boer goat semen

Table 1. shows that the characteristics of Boer goat semen used in this study was normal.

Table 1. Characteristics of fresh semen of Boer goat semen used in the experiment (n=10)

Parameter	Value
Color	Creamy
Ph	7.00 ± 0.0
Volume (ml)	0.81± 0.33
Concentration (106/ml)	3387 ± 230.32
Mass motility	2+ - 3+
Sperm Individual motility (%)	74.50 ± 3.69
Life sperm (%)	88.03 ± 3.07
Abnormal sperm (%)	6.87 ± 1.98
Sperm membrane integrity (%)	72.67 ± 3.66

Sperm membrane integrity after cooling

Percentage of sperm membrane integrity of Boer goat semen after cooling diluted with tris-based diluent containing different levels of trehalose is shown in Table 2.

Table 2. Percentage of sperm membrane integrity following treatment with different trehalose level after cooling

Time of cooling (h)	Trehalose levels (%)	Mean ± SD
0	1.5	65.99 ± 6.94[b]
	2.5	64.67 ± 4.41[b]
	3.5	57.46 ± 5.50[a]
24	1.5	56.58 ± 4,50[b]
	2.5	55.89 ± 4.95[b]
	3.5	48.80 ± 6.74[a]
48	1.5	47.49 ± 7.05[b]
	2.5	48.75 ± 6.57[b]
	3.5	39.38 ± 9.31[a]

[a, b] within collumn highly significant different (P<0.01)

Table 2. shows that the membrane integrity of sperm was highly significant different (P<0.01) after cooling between trehalose treatment groups. In general, it was shown that both 1.5 and 2.5% trehalose in tris-based diluent showed an optimal level for maintaining the sperm membrane integrity after cooling compared to the level of 3.5% trehalose, but 1.5% trehalose is more economic. Trehalose 3.5% meybe too high level for maintaining sperm membrane integrity. The role of trehalose in the protection of sperm during cooling acted by maintaining the membrane integrity of sperm (lipid bilayer) by formation of hydrogen bounds at O2, O3 and O4 of trehalose structure with phosphate and carbonyl groups of lipid the sperm membrane stability could maintained and the sperm damage originated from diluent and temperature shock could be maintained (Sum *et.al,* 2003).

CONCLUSION

Based on the study, it was concluded that the addition 1.5% trehalose in tris-based medium resulting optimal sperm membrane integrity Boer goat semen after cooling. It was suggested, that for resulting optimal sperm membrane integrity after cooling of Boer goat semen in tris-based medium should be supplemented with 1.5% trehalose.

REFERENCES

Evans, G. and W.M.C. Maxwell. 1987. Salamon's Artificial Insemination of Sheep and Goat. Butterworths, Sidney.

Nield, D. G. Chaves, M Flores, N. Mora, M. Beconi and A. Aguero. 1999. Hypoosmotic Test in Equine Spermatozoa. Thereogenology 51 (4): 721-728.

Sum, A.K., R. Faller and J.J. de Pablo. 2003. Molecular Simulation Study of Phospholipid Bilayers and Insight of The Interactions with Disaccharides. J. Biophys. 85: 2830-2844