EVALUATION OF EXTENDERS FOR CRYOPRESERVATION OF MALABARI BUCK SEMEN

M. PAWSHE[1]*, H.M. HARSHAN[2], A. JOHN[3], M.O. KUREIN[4] AND SUNANDA C.[5]

Department of Animal Reproduction, Gynaecology and Obstetrics,
College of Veterinary and Animal Sciences,
Kerala Veterinary and Animal Science University, Mannuthy - 680 651

Received: 09.06.2017 Accepted: 15.08.2017

ABSTRACT

The efficacy of commercially available Soybean lecithin (Bioxcell) and egg yolk (Triladyl) based extender and lab made Skim milk and Tris based extender was assessed for the cryopreservation of Malabari buck semen. Semen was collected from two bucks and equally divided twenty pooled semen samples were extended using four extenders followed by cryopreservation and storage in liquid nitrogen. Spermatozoa parameters evaluated after initial extension, and at pre-freeze and post-thaw stage revealed better ($p<0.05$) characteristics at all stages with commercial egg yolk extender in comparison to other extenders. Thus, the commercial egg yolk (Triladyl) based extender is better for cryopreservation of Malabari buck semen.

Keywords: Cryopreservation, Extender, Malabari buck, Semen, TRIS

A major bottleneck in goat semen cryopreservation is the failure to replicate the success rate achieved with cryopreserved cattle semen. The composition of semen extender plays a vital role in the success of cryopreservation. Tris egg yolk based extender have been widely used for the freezing of buck semen. However, a wide variability of yolk constituents and the chance of microbial contamination in the yolk necessitates yolk replacers. Soybean lecithin, a plant derived lecithin is a potential candidate. The present study was planned to compare the efficacy of commercially available soybean lecithin (Bioxcell) and Tris (Triladyl) based extender and lab made Skim milk and Tris based extender for the cryopreservation of Malabari buck semen.

Four adult healthy Malabari bucks (age, 2-7 yr) were subjected to semen collection using a Danish type of artificial vagina maintained at 41-43ºC under standard pressure. Immediately after collection, semen ejaculates were transferred to a water bath maintained at 37ºC. Semen ejaculates collected from two randomly selected bucks were pooled to contain equal number of progressively motile spermatozoa from each buck in the pooled semen sample. Twenty pooled semen samples were used for the cryopreservation of semen. The pooled samples were equally divided in four aliquots for the further extension of semen using extenders maintained at 37ºC. These extenders were, a) commercial soybean lecithin based extender (Bioxcell, IMV International, USA), b) lab made goat skim milk extender (Goat skim milk 93 ml, Glycerol 7 ml, Penicillin G sodium 1000 IU/ml and Streptomycin sulfate 1000 µg/ml), c) commercial egg yolk based extender (Triladyl; 3.8% Tris, 2.2% citric acid, 0.6% glucose, double distilled water, Gentamycin, Tylosin, Spectinomycin and Lincomycin; Apu *et al*., 2012; Minitube, Germany), and d) lab madel Tris based extender (Tris 3.028 g, Citric acid 1.675 g, Fructose 1.25 g, Egg yolk 5 ml, Benzyl penicillin 1000 IU/ ml, Streptomycin 1000µg/ ml, Glycerol 12 ml and triple distilled water up to 100 ml).

[1,3]Ph.D. Scholar, [4]Associate Professor; [2, 5]Assistant Professor, College of Veterinary and Animal Sciences, Pookot – 673 576; *drmayurpawshe@gmail.com

Table 1: Impact of semen extender on progressive motility, viability, sperm abnormality and acrosome integrity of Malabari buck semen (n=20 pooled semen samples) at different stages of cryopreservation

Test	Stage	Extender base			
		Soybean lecithin	Skim milk	Egg yolk	TRIS
Progressive motility, %	**Post-initial extension**	85.2±0.8^{a}	80.7±0.7^{b}	87.0±1.0^{a}	86.0±0.6^{a}
	Pre-freeze	71.7±1.4^{b}	62.5±2.2^{c}	77.0±1.4^{a}	71.2±0.9^{b}
	Post-thaw	41.0±1.3^{b}	24.7±1.1^{c}	45.2±2.1^{a}	40.5±1.2^{b}
Viability, %	**Post-initial extension**	91.1±0.4^{b}	86.8±0.8^{c}	92.8±0.5^{a}	90.6±0.7^{b}
	Pre-freeze	79.0±1.0^{b}	68.8±2.1^{c}	84.2±1.0^{a}	78.5±0.8^{b}
	Post-thaw	56.0±2.0^{b}	32.5±1.1^{c}	61.2±2.5^{a}	51.3±1.3^{b}
Sperm abnormality, %	**Post-initial extension**	2.8±0.2	3.4±0.2	2.6±0.2	2.8±0.3
	Pre-freeze	5.0±0.2^{b}	5.8±0.3^{a}	4.4±0.2^{b}	5.0±0.3^{b}
	Post-thaw	7.3±0.2^{b}	9.2±0.3^{a}	6.4±0.2^{c}	7.7±0.2^{b}
Acrosome integrity, %	**Post-initial extension**	91.0±0.5	91.3±0.4	92.3±0.5	91.2±0.5
	Pre-freeze	80.1±1.1^{b}	79.9±0.8^{b}	86.2±1.0^{a}	80.4±1.0^{b}
	Post-thaw	59.8±1.9^{b}	57.6±1.7^{b}	66.8±2.3^{a}	59.0±1.6^{b}

$p<0.05$, Mean values with different superscripts within a row differ significantly

Semen was extended with each extender to contain 400 million progressive motile spermatozoa per ml of extended semen. The manual filling of extended sample was carried out in 0.5 ml French medium straws at room temperature. An equilibration period of 4 h was provided and manual freezing of straws was carried out by allowing the straws to freeze under liquid nitrogen vapour in a styrofoam box for a period of 8 min. The frozen straws were transferred to a pre-cooled goblet and plunged into liquid nitrogen using a canister in a liquid nitrogen refrigerator for storage. Thawing of semen was carried out by immersing the straws in a water bath, maintained at 37°C, for a period of 60 sec. Progressive sperm motility, sperm viability, sperm abnormality and acrosome integrity of the samples were assessed at post-initial extension, pre-freeze and post-thaw stage. Between groups variation was measured using repeated measures ANOVA and analysis was done with the help of statistical software SPSS, version 21.

The Malabari buck semen extended in commercial egg yolk (Triladyl) extender had better progressive motility at all the stages of storage in comparison to other three extenders ($p<0.05$, Table 1). Furthermore, at pre-freeze stage and post-thaw stages, semen extended in Triladyl had higher ($p<0.05$) viability and acrosome integrity than the other three groups (Table 1). The spermatozoa abnormalities were also less in the Triladyl extended group ($p<0.05$, Table 1). Of the three extenders, the semen extended with skim milk had least ($p<0.05$) progressive motility, viability and acrosome integrity during pre-freeze and post-thaw stage of cryopreservation (Table 1). The observations on semen extended with commercial egg yolk extender were similar to a previous study in crossbred Malabari buck semen (Shiny, 2011).

In African buffalo and North American buffalo, Triladyl had better post-thaw motilities and acrosomal integrity of spermatozoa in comparison to Andromed, a soybean lecithin based extender (Herold *et al.*, 2004 and Lessard *et al*., 2009). It was suggested that soybean lecithin based extender may lack mechanism of sequestering binder of sperm proteins associated with sperm cryodamage during cryopreservation

due to induction of cholesterol efflux (Nauc and Manjunathm, 2000). The amount of glycerol used in Triladyl was comparable with glycerol concentration used in the other extenders. Hence, the advantages of the extender might be due to the other non-disclosed factors present in the extender or their interaction with one another.

REFERENCES

Apu, A.S., Khandoker, M.A.M., Husain, S.S., Fakruzzaman, M. and Notter, D.R. (2012). A comparative study of fresh and frozen-thawed semen quality in relation to fertility of Black Bengal Goats. *Iranian J. Application Anim. Sci.,* **2**(2): 157-161.

Herold, F.C., Aurich, J.E. and Gerber, D. (2004). Epididymal sperm from the African buffalo (Syncerus ®caffer) can be frozen successfully with Andromed but the addition of bovine seminal™ and Triladyl plasma is detrimental. *Theriogenology,* **61**: 715-724.

Lessard, C., Danielson, J., Rajapaksha, K., Adams, G.P. and McCorkell, R. (2009). Banking North American buffalo semen. *Theriogenology,* **71**: 1112-1119.

Nauc, V. and Manjunath, P. (2000). Radio immunoassays for bull seminal plasma proteins (BSP-A1/A2, BSP-A3 and BSP-30-kDa) and their quantification in seminal plasma and sperm. *Biol. Reprod*., **63**: 1058-1066.

Shiny, M. (2011). Freezability of buck spermatozoa treated with chelating agent for fibronectin type II proteins M.V.Sc. Thesis. *Kerala Veterinary and Animal Sciences University, Thrissur, Kerala*, 75p.