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Comparison between tris-citric acid yolk, yolk albumin citrate and skimmed milk extenders on sperm motility, livability and mass movement in frozen-thawed goat sperm.

Nor-Ashikin MNK[1] and RB Abdullah[2]

[1]Institute of Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia,

[2]Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya 50603, Kuala Lumpur, Malaysia.

Abstract

This experiment was carried out with the aim to compare the effect of three extenders on frozen-thawed sperm characteristics in goat. Sperm of Katjang and their crossbreds; Jermasia and Jermana were cryopreserved in three extenders namely tris-citric acid yolk (TCAYE), yolk albumin citrate (YACE) and skimmed milk (SME) and the frozen-thawed sperm parameter were examined. The finding of this study showed that the Jermasia and Jermana buck sperm cryopreserved in TCAYE provided the best results in terms of motility (52.64 ± 0.93% and 53.20 ± 1.13%) and livability (56.47± 0.96% and 57.72 ± 1.11%), respectively. In the crossbreds, sperm motility and livability in TCAYE were found to be significantly higher compared to the sperm cryopreserved in YACE and SME. Therefore, TCAYE extender apparently is better than YACE followed by SME for the cryopreservation of goat sperm.

Key words: Cryopreservation, Extender, Buck sperm

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Introduction

Variation in the results of buck semen cryopreservation is attributed to many confounding factors which include processing techniques, choice of cryoprotectants, extenders dilution, cooling and thawing rates [1, 2]. According to Purdy [1], an extender for cryopreservation serves as an energy source and protects sperm cells from cryogenically-induced damage, thus maintaining sperm survivability. The choice of extender plays an important role in determining the viability of deep frozen semen. Different species have dissimilar nutritional requirements, and thus the type of extender used must cater for its specific needs. For example, the egg yolk-coagulating enzyme (EYCE) secreted by the bulbourethral gland of the goat has been shown to cause egg yolk coagulation and sperm death [3]. This phospholipase-A enzyme hydrolyses egg yolk lecithin to fatty acids and lysolecithin [4, 5]. Lysolecithin is toxic to goat sperm. A protein identified as SBUIII from the same gland, was also found to decrease survival of cooled or frozen goat sperm diluted in milk-based extenders [6]. The component that causes this effect was later identified as tricylglycerol lipase [7]. The component caused a decrease in sperm motility and the quality of movement in skim milk extender.

Materials and Methods

Experimental animals

Three local Katjang bucks and nine crossbred Jermasia and Jermana bucks ageing nine months to three years were used in this study. Animals for semen collection were selected based on the criteria suggested by Mukherjee [8]. The crossbreds in the study were the products of a crossbreeding project jointly carried out between the Institute of Advanced Studies, University of Malaya, Malaysia and the Technical University of Berlin, Germany.

Local Katjang goats were inseminated with imported frozen semen from improved German Fawn bucks to obtain the F_1 generation. The following generations, F_2 to F5 goats were produced by inter-insemination procedure named Jermasia. Goats having 75% improved German Fawn genome and 25% Katjang goat genome were mated to improved German Fawn to upgrade them. The upgraded animals were then inter-inseminated to produce the Jermana genotype.

Collection of semen

Semen collection was done between 0800 to 0900 hr by using an artificial vagina (AV). The semen collection tube was wrapped by aluminum foil to minimise exposure to light. AV method required the use of restrained female goats in oestrus as teasers.

Preparation of extenders

Tris-citric acid yolk extender (TCAYE)

The TCAYE consisted of 3.8 g tris (hydroxymethyl) aminomethan, 2.1 g citric acid to make up to 100 mL with milli-Q water. After which, 0.05 g of streptomycin sulfate and 0.03 g penicillin-G (sodium salt) were added. Ratio of buffer to egg yolk was 4:1. After centrifugation at 650 x G for 15 min, 6.8% glycerol and 1.0% fructose were added to the supernatant.

Yolk albumin citrate extender (YACE).

The YACE extender consisted of 2.9 g sodium citrate, 1.3 g D-glucose, 0.05 g streptomycin sulfate and 0.03 g penicillin-G (sodium salt) in 70 ml milli-Q water. Ratio of buffer to egg yolk was 4:1. After centrifugation at 650 x G for 15 min, 15 ml egg albumin and 6 ml glucose were added to 15 ml of supernatant.

Skimmed milk extender (SME)

A mixture containing 10.0 g skim milk (less than 1% fat content), 0.2 g D-glucose in 100 ml milli-Q water was heated at 91 ± 1ºC for 10 min, cooled to room temperature and added with 0.03 g streptomycin sulfate and 0.02 g penicillin-G (sodium salt). Then 14% glycerol was added to 86% of this mixture.

Cryopreservation

French-type 0.25 ml polyvinylchloride (PVC) straws were used in the packaging of semen for freezing. All semen-filled straws were left with an air space at its distal end and sealed with polyvinyl alcohol (PVA) powder. Straws were lined on a grooved perspex frame and kept in nitrogen vapour (-150°C) for 9 min before plunging into liquid nitrogen.

Thawing

Frozen straw was taken out from liquid nitrogen and immersed in a water bath at 37°C for 2 min. All straws were wiped-dry before cutting off the tips and releasing cryopreserved sperm into microfuge tubes in a floating water-bath rack at 37°C.

Evaluation of semen quality

The quality of neat and frozen-thawed semen were evaluated based on composite characteristics. Percentage of sperm motility, livability and mass movement ratings (Nelson and Lin, 1983) [9] as shown in Table 1 were recorded. Semen was diluted with basic tris-citric acid buffer (pH 6.75) prior to assess sperm motility.

Table 1*:Rating system for mass movement (Nelson and Lin, 1983) [9]*

Score	Appearance
5	Vigorous, churning, swirling movement
4	Active, massive movement without the vigorous activity shown in 5
3	Slow massive movement, very little massive activity
2	Individual movement, very little massive activity
1	Large quantities of sperm present, essentially no movement
0	No sperm present

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) programme. Data were analysed through analysis of variance and Duncan's Multiple Range Test. A *P* value of < 0.05 was considered to be statistically significant.

Results

Table 2 shows results of post-thaw sperm characteristics of different buck genotypes in different extenders.

The finding of this study showed that the Jermasia and Jermana buck sperm cryopreserved in TCAYE provided the best results in terms of motility (52.64 ± 0.93% and 53.20 ± 1.13%) and livability (56.47± 0.96% and 57.72 ± 1.11%), respectively. In the crossbreds, sperm motility and livability in TCAYE were found to be significantly higher compared to the sperm cryopreserved in YACE and SME.

***Table 2**: Genotype X extender interaction means ± S.E.M. of frozen-thawed semen*

	Characteristics		
	Motility (%)	**Live (%)**	**Mass movement**
Interactions			
Katjang x TCAYE	49.67 ± 0.66^{de}	55.37 ± 0.61^{ef}	3.98 ± 0.02^{e}
Katjang x YACE	29.21 ± 1.10^{a}	37.38 ± 1.41^{b}	2.73 ± 0.09^{a}
Katjang x SME	47.09 ± 1.71^{cd}	52.52 ± 1.96^{de}	3.54 ± 0.07^{c}
Jermasia x TCAYE	52.64 ± 0.93^{e}	56.47 ± 0.96^{ef}	3.96 ± 0.02^{e}
Jermasia x YACE	26.50 ± 1.15^{a}	33.17 ± 1.36^{a}	2.55 ± 0.10^{a}
Jermasia x SME	44.25 ± 1.61^{c}	48.89 ± 1.75^{cd}	3.53 ± 0.06^{c}
Jermana x TCAYE	53.20 ± 1.13^{e}	57.72 ± 1.11^{f}	3.85 ± 0.04^{de}
Jermana x YACE	38.21 ± 1.40^{b}	46.62 ± 1.54^{c}	3.21 ± 0.09^{b}
Jermana x SME	48.48 ± 1.02^{d}	54.10 ± 0.92^{ef}	3.74 ± 0.05^{d}

Values indicate mean ± S.E.M. Means within a column with different superscripts are significantly different ($P < 0.05$)
Note: Mass movement score was based on Nelson and Lin, 1983 [9]

Discussion

Interactions between Jermasia and Jermana bucks with TCAYE gave the best results in terms of motility (52.64 ± 0.93% and 53.20 ± 1.13%) and live sperm percentage (56.47± 0.96% and 57.72 ± 1.11%), respectively. In the crossbreds, sperm motility and livability in TCAYE were significantly higher compared to sperm cryopreserved in YACE and SME. The mean values for motility and livability sperm in all extenders were higher in Jermana bucks compared to Katjang and Jermasia bucks which is also reported by Noran [10]. The sperm motility and mass movement were found to be higher for crossbred goat. For all genotypes, TCAYE was found to be the best extender in terms of frozen-thawed sperm motility, livability and mass movement. This finding is consistent with the finding of Dorado *et al*. [11], who found that tris extender produced better frozen-thawed sperm motility and velocity parameters compared to skimmed milk extender, as measured by sperm class analyser (CASA). Hidalgo *et al*. [12] in their comparative study by using commercial tris- and skimmed milk-based extenders and found that milk extender caused less reduction of sperm head size in Florida bucks. However, cryopreservation of Florida goat semen using TCAYE extender results in slightly improved rate of motility (56.07 ± 1.32) [13]. Studies have shown that the removal of seminal plasma by washing immediately after collection increases motility and livability of sperm during storage in egg yolk or milk extenders [14, 15].

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Correspondence:

Nor Ashikin Mohamed Noor Khan
Institute of Medical Molecular Biotechnology
Faculty of Medicine, Universiti Teknologi MARA
Sungai Buloh Campus, Jalan Hospital
47000 Sungai Buloh, Selangor
Malaysia