Effect of tris-based extenders with sunflower lecithin or egg yolk on the post-thaw qualities of Kalahari Red buck semen

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Submission: 5 November 2023 Revised: 17 April 2024 Accepted: 28 April 2024

ABSTRACT

Background and Objective: This study aimed to determine the effect of sunflower lecithin (SL) concentrations, egg yolk, and sugar types in tris-based extenders on post-thaw qualities of Kalahari Red (KR) buck semen. SL was used as an alternative to egg yolk in the semen extender during cryopreservation due to its characteristic phospholipid content which played a major cryoprotective role and also to prevent the deleterious effect of egg yolk on semen when included in the extender.

Methodology: Semen was collected from four KR bucks between 2 and 2 ½ years of age using an artificial vagina, evaluated for sperm motility, pooled, and then diluted in tris-sucrose and tris-fructose extenders, each containing 1.5, 3.0, 4.5% SL and 20% egg yolk (0% SL). Diluted semen was loaded in 0.25 mL plastic straws, cooled to 4 °C, and suspended in liquid nitrogen (LN) for 10 min. where the temperature reached -120 °C before plunging directly into the LN for 7 days. Sperm qualities, including motility, acrosome integrity, membrane integrity, malondialdehyde (MDA) concentration, seminal arginase activity, and cholesterol level, were evaluated after thawing. Data were analyzed using a 2 × 4 factorial arrangement.

Main Results: Results showed that generally, tris-fructose extender had significantly better results as regards motility, cholesterol level, and seminal arginase activity (23.33 ± 3.25%, 78.62 ± 12.83 mg/dL, and 0.41 ± 0.06 units/mg protein respectively; $P < 0.05$) while there was no significant difference in acrosome integrity, membrane integrity, and malondialdehyde concentration when compared with tris-sucrose extender. However, interactive results showed that the tris-fructose extender had better ($P < 0.05$) post-thaw semen qualities with egg yolk. In contrast, the tris-sucrose extender had better ($P < 0.05$) results with 4.5% (SL) in the cryopreservation of Kalahari buck semen.

Conclusions: Tris-fructose extender with egg yolk had better post-thaw semen qualities than other extenders. However, with a tris-fructose extender where egg yolk is to be replaced with sunflower lecithin, this should be done at 3.0% inclusion, while 4.5% sunflower lecithin inclusion in sucrose extender will also give the desired result.

Keywords: Artificial vagina, tris-extenders, egg yolk, sunflower lecithin, Kalahari Red goat

Thai J. Agric. Sci. (2024) Vol. 57(1): 45−57

INTRODUCTION

One of the most important elements in the handling and storage of semen was the preparation of the eligible semen extender, ensuring high survivability and fertility of the spermatozoa for a long period (Maxwell and Watson, 1996; Purdy, 2006). In most cases, the extender used for chilling or freezing semen includes egg yolk, skimmed milk, glycerol, or their combination (Leboeuf *et al*., 2000; Medeiros *et al*., 2002; Sharafi *et al*., 2009). The most commonly used extenders for freezing goat semen are tris-egg yolk (TS) and skimmed milk (SM). Regardless of the good fertility rate after adding egg yolk, milk, or glycerol, some limitations to the use of these protectors have been detected in goats (Lowry *et al*., 1951).

Those extenders that contained additives of animal origin (egg yolk and/or milk) posed a potential risk of microbial contamination (Bousseau *et al*., 1998; Thun *et al*., 2002; Bittencourt *et al*., 2008) on the semen, thereby deteriorating it's quality (Aleem *et al*., 1990; Dela Pena *et al*., 1995; Akhter *et al*., 2008), causing reproductive problems in the female which could result in low fertility rate (Andrabi *et al*., 2001; Akhter *et al*., 2007). Extenders containing egg yolk and/or milk represent a risk of contamination if microorganisms, such as bacteria and fungi, are present in the fresh product. Such contamination can release endotoxins that reduce the fertilization capacity of sperm (Bousseau *et al*., 1998; Bittencourt *et al*., 2008). Therefore, a well-defined and pathogen-free substitute for egg yolk (preferably of non-animal origin) would be more suitable for extenders used for semen (Stradaioli *et al*., 2007).

Lecithin from soybean has been reported to offer protection to animal spermatozoa from cold shock during semen cryopreservation (Thibier and Guerin, 2000; Aires *et al*., 2003). Many researchers (Phutikanit *et al*., 2011; Salmani *et al*., 2013; Vidal *et al*., 2013) have reported the replacement of the animal origin components with soybean lecithin. This phospholipid represents a mixture of phosphatidylcholine and different fatty acids and protects the sperm cell membrane by restoring the phospholipids lost during heat shock (Oldenhof *et al*., 2013; El-Sisy *et al*., 2016). In industrial volumes, lecithins are also produced from sunflower and rapeseed oils (Van Nieuwenhuyzen, 2014; Lehri *et al*., 2019)

Sunflower lecithin was used as an alternative to egg yolk in semen extender, where it maintained sperm motility and other viability parameters and also protected the sperm membrane against lipid peroxidation (Adeyanju *et al*., 2018). The main phospholipids in lecithin from soya and sunflower are phosphatidylcholine, phosphatidyl inositol, phosphatidyl ethanolamine, and phosphatidic acid. An ideal extender should have nutrients as an energy source, buffer against harmful changes in pH, provide a physiological osmotic pressure and concentration of electrolytes, prevent bacterial growth, and protect the cells from cold shock during the freezing and thawing processes. Extenders used for semen cryopreservation preserve sperm motility and fertility by promoting the stabilization of the plasma membrane and providing an energy substrate.

The cryoprotective properties of various sugars against freezing-thawing-induced damage have been documented in various cell types (Yodmingkwan *et al*., 2016). Sugars are divided into monosaccharides and disaccharides, and each of them has cryoprotective properties to protect spermatozoa during freezing and thawing (Moussa *et al*., 2002; Yodmingkwan *et al*., 2016). Disaccharides are effective in stabilizing biomembrane bilayers, and sperm metabolism can be better sustained in diluents containing degradable sugar (Aisen *et al*., 2005). Lactose, sucrose, raffinose, trehalose, and dextran are not able to diffuse across the plasma membrane, creating an osmotic pressure that induces cell dehydration and a lower incidence of intracellular ice formation. These sugars interact with phospholipids in the plasma membrane, increasing sperm survival to cryopreservation (Aisen *et al*., 2002). They increase membrane fluidity before freezing, leading to greater resistance of spermatozoa against freeze-thawing damage (Aboagla and Terada, 2003). The addition of sucrose and trehalose for freezing bull semen resulted in an improvement

in sperm survival (Yildiz *et al*., 2000). Therefore, the aim of this study was to evaluate the effect of sunflower lecithin concentrations and the sugar types in tris-based extenders on the post-thaw sperm qualities of Kalahari Red buck.

MATERIALS AND METHODS

Animals and Semen Collection

Four Kalahari Red bucks between 2 and 2 ½ years old weighing between 80 and 90 kg were used for semen collection in the presence of a female induced into oestrus as a stimulus, using an artificial vagina. Semen collection was done early in the morning and evaluated for progressive motility. The animals were on an intensive feeding management system with the provision of guinea grass (*Panicum maximum*) supplemented with concentrate.

Sunflower Lecithin

Sunflower lecithin (SL) is a type of phospholipid found in sunflower seeds. It is a fatty substance obtained by dehydrating a sunflower seed and separating it into three parts, namely the oil, the gum, and other solids. Lecithin is the gum by-product. Sunflower lecithin is organic and natural with no genetic engineering. It is a good source of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. The fatty acid composition includes stearic acid and linoleic acid, which help lower cholesterol levels (American Chemical Society, 2005).

Semen Analyses and Freezing

Sperm viability and abnormality were assessed by determining the rate of live sperm using a modification of the eosin-nigrosin stain procedure as described by Agarwal *et al*. (2016). A mixture of 10 µL of diluted spermatozoa and 10 µL eosin-nigrosin stains was smeared on a slide and allowed to air dry in a dust-free environment. Two hundred spermatozoa from different microscopic fields were examined under a bright-field microscope (400x magnifications), and the number of non-stained (viable) spermatozoa was counted. The sperm cell concentration was also determined by using a hemocytometer. The sperm cell concentration was recorded in millions per mL.

After evaluation, semen showing ≥ 70% motility were pooled. The pooled semen was diluted in an egg yolk extender as the control and a lecithin-based extender as the experimental group. The extenders contained tris-hydroxyl methyl-aminomethane (2.42 g), citric acid (1.36 g), fructose/sucrose (1 g), glycerol (7%), penicillin (0.028 g). They made up to 100 mL with 20 mL egg yolk in the control and sunflower lecithin at 1.5%, 3.0%, and 4.5%, respectively, in the experimental group (Table 1) in three replicates for each treatment. Aliquots of pooled semen were diluted with the extenders, and the diluted semen was then loaded into a 0.25 mL plastic straw (Purdy, 2006) and sealed with polyvinyl, equilibrated by cooling it to 4 °C at a rate of 0.25 °C/min in TYFSF refrigerated incubator (Model: SPX-70B 111, Hebei, China). Subsequently, the straws were then placed at 4 cm above liquid nitrogen in the vaporous phase for 10 min, where it reached a temperature of -120 °C before plunging directly and quickly into liquid nitrogen for 7 days, after which it was evaluated for sperm viability parameters in a 2 × 4 factorial design.

Thawing and Sperm Evaluation

After 7 days of storage, frozen semen was thawed in a Clifton water bath (Model: 74178, Nickel Electro Ltd., Weston-S-Mare Somerset, England) at 37 °C for 30 s. The thawed semen was then analyzed for sperm motility, acrosome integrity, plasma membrane integrity, seminal arginase activity, cholesterol content, and malondialdehyde (MDA) concentration.

Sperm Motility

In determining sperm motility, a five µL sample of semen was placed directly on a warmed microscope slide and overlaid with a 22 × 22 mm coverslip, as described by Bearden and Fuquay (1997). Each sample was measured on different slides, and different microscopic fields were examined to observe progressively motile spermatozoa that move forward in a straight line.

Table 1 Composition of tris fructose/sucrose extender

Extender composition	T_0	T_1	T_2	T_3
Tris (g)	2.42	2.42	2.42	2.42
Citric acid (g)	1.36	1.36	1.36	1.36
Fructose/sucrose (g)	1.00	1.00	1.00	1.00
Sunflower lecithin (%)	-	1.5	3.0	4.5
Egg yolk (%)	20	-	-	-
Glycerol (%)	7	7	7	7
Penicillin (g)	0.0028	0.0028	0.0028	0.0028
Distilled water made up to (mL)	100	100	100	100

Note: T_0 = 20% egg yolk or 0% sunflower lecithin, T_1 = 1.5% sunflower lecithin, T_2 = 3.0% sunflower lecithin, T_3 = 4.5% sunflower lecithin.

Acrosome Integrity

Sperm acrosome integrity was determined according to the method described by Ahmad *et al*. (2003). 50 µL of each semen sample was added to a 500 µL formalin citrate solution (96 mL 2.9% sodium citrate, with 4 mL 37% formaldehyde) and mixed. A small drop of the mixture was placed on a microscope slide, and a total of 200 spermatozoa were counted in at least three different microscopic fields for each sample, using (400x magnifications). The intactness of acrosome characterized by a normal apical ridge of 200 spermatozoa was assessed using a microscope.

Sperm Plasma Membrane Integrity

Sperm plasma membrane integrity was determined using the Hypo-osmotic swelling test (HOST) assay as described by Correa *et al*. (1997), where 10 µL semen was incubated in Hypo - osmotic solution (9 g fructose and 4.9 g sodium citrate/100 mL distilled water) at 37 °C for 30 min. 0.1 mL of the mixture was spread over a warm slide covered with a cover slip and observed under a microscope (400x magnifications). Two hundred spermatozoa were counted for their swelling characterized by the coiled tail, indicating an intact plasma membrane.

Arginase Activity

Arginase activity was determined following the procedure of Malo *et al*. (2010), in which 0.1 g bovine serum albumin (BSA) as standard in 10 mL water was used. 1 mL alkaline copper reagent (a mixture of copper sulphate reagent, sodium dodecyl sulphate solution, and sodium hydroxide solution in a ratio of 1:2:1) and 0.1 mL semen sample were mixed and incubated for 10 min at room temperature. 4 mL folin Ciocalteu's phenol reagent was then added to the sample, mixed, and incubated for 5 min at 55 °C. The absorbance of the samples was recorded at 650 nm in a spectrophotometer (UV spectrophotometer, SW7504 model, Surgifriend Medicals, England). The results were expressed as units/mg protein (specific activity).

Cholesterol Content

This was determined as follows: 10 µL each of the sample, standard, and distilled water (blank) was mixed with 1,000 µL of the reagent inside a cuvette and incubated for 5 min at +37°C the absorbance of the sample (A_{sample} against the reagent blank was read within 60 min in a spectrophotometer at 500 nm (Table 3). The calculation is shown below:

$$\text{Concentration of cholesterol in sample} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Concentration of standard}$$

Malondialdehyde (MDA) Concentration

Malondialdehyde (MDA) concentration was measured in a thiobarbituric acid reactive substances (TBARS), which constitutes 0.37% TBA, 15% TCA, and 0.25M HCl 0.1 mL of sperm suspension was incubated with 2 mL TBA/TCA/HCl (1:1:1) in a water bath for 15 min at 95 °C, after which it was placed in ice and centrifuged for 10 min at 3,000 rpm. The absorbance of the clear supernatant was measured with a UV spectrophotometer (SW7504 model, Surgifriend Medicals, England) at 552 nm. The concentration of MDA was calculated as follows: MDA (μmol/L) = $A/1.55 \times 10^6 b$, where A = the absorbance of the sample, b = size of the cuvette to the nearest cm, 1.55×10^6 molar absorptivity of MDA.

Statistical Analysis

Data obtained were subjected to a 2 × 4 factorial arrangement, and means were separated by the Duncan multiple range test (Duncan, 1955) using the model (Sharafi *et al*., 2009) below:

$$y_{ij} = \mu + E_i + L_j + (EL)_{ij} + \sum_{ijk}$$

where y_{ijk} = dependent variables, μ = population mean, E_i = effect due to i^{th} different tris-based extender, i = (1, 2,) L_j = effect due j^{th} level of SL inclusion, j = (0, 1.5, 3.0, and 4.5%), $(EL)_{ij}$ = effect of ij^{th} interaction between different tris-based extender and levels of SL inclusion, $\sum_{ijk}$ = experimental error.

RESULTS AND DISCUSSION

The main effect of tris-sucrose and tris-fructose extenders on the viability parameters of the frozen-thawed Kalahari Red buck semen Table 2 shows. There were significant differences in post-thaw quality parameters of Kalahari Red spermatozoa in response to the sugar types added to the extender; as a result, showed that tris-fructose had a significantly higher value (23.33 ± 3.25%) for motility than tris-sucrose (16.25 ± 3.66%). This result agrees with some studies that show that the cryoprotective effect of the sugars on sperm cells may differ according to chemical functionality and molecular weight (Devireddy *et al*., 2002).

There was no significant difference in the values obtained for acrosome integrity (86.92 ± 3.12% and 81.50 ± 4.35%) and membrane integrity (78.41 ± 4.34% and 76.00 ± 3.52%), respectively. There was no significant difference in the values of MDA obtained for both tris-fructose (0.62 ± 0.03 nmol/mL) and tris-sucrose (0.63 ± 0.09 nmol/mL). Tris-fructose had a significantly ($P < 0.05$) higher cholesterol (78.62 ± 12.83 mg/dL) and seminal arginase activity (0.41 ± 0.06 units/mg protein) than tris-sucrose (76.18 ± 14.75 mg/dL and 0.27 ± 0.01 units/mg protein, respectively).

The use of either fructose or sucrose as part of the composition of diluents to serve as energy substrate has been confirmed in previous studies (Farshad and Akhondzadeh, 2008; Qureshi *et al*., 2013; Panyaboriban *et al*., 2015; Yodmingkwan *et al*., 2016). Sucrose tended to be used as a non-permeating cryoprotective agent (Hoffmann *et al*., 2011; Oldenhof *et al*., 2013). Aboagla and Terada (2003) reported the use of trehalose and sucrose in semen cryopreservation as they play a key role in preventing deleterious alteration to the membrane during the reduced water phase. It has also been reported that disaccharides are effective in stabilizing membrane bilayer (De Leeuw *et al*., 1993), they have a protective role against osmotic effect and form specific interaction between membrane phospholipids (Ahmad *et al*., 2013; Singh *et al*., 2016). Monosaccharides (glucose or fructose) can be permeable to the membrane because of their small molecular weight (Akourki *et al*., 2004; Anakkul *et al*., 2013). According to Salamon and Maxwell (2000), the ram contains monosaccharides in its seminal plasma. Hence, it is very necessary to include monosaccharides in the semen-freezing extender as an energy source and a protective agent for the spermatozoa. The results obtained in this experiment agreed with Yodmingkwan *et al*. (2016), where tris-fructose egg yolk extender had significantly ($P < 0.05$) higher percentages for motility and other viability parameters than tris-sucrose soybean lecithin which was contrary to the report by Yotov (2015).

Table 2 Main effect of tris-sucrose and tris-fructose extenders on the sperm qualities after cryopreservation

Parameters	Tris-sucrose	Tris-fructose	P-value
Motility (%)	16.25 ± 3.66[b]	23.33 ± 3.25[a]	0.014
Acrosome integrity (%)	81.50 ± 4.35	86.92 ± 3.12	0.789
Membrane integrity (%)	76.00 ± 3.52	78.41 ± 4.34	0.815
Malondialdehyde (nmol/mL)	0.63 ± 0.09	0.62 ± 0.03	0.986
Cholesterol (mg/dL)	76.18 ± 14.75[b]	78.62 ± 12.83[a]	0.050
Seminal arginase activity (units/mg protein)	0.27 ± 0.01[b]	0.41 ± 0.06[a]	0.049

Note: [a,b] [a,b] Means within the same row with differemt superscript differ (P < 0.05)

Table 3 shows the interaction between tris-base extenders and sunflower lecithin and egg yolk. The table shows that there were significant differences (P < 0.05) in the values obtained for all the semen parameters post-thawed in both tris-sucrose and tris-fructose extenders across all the levels of sunflower lecithin used. Tris-sucrose extender with 4.5% sunflower lecithin had the highest (P < 0.05) motility (30.00%) and acrosome integrity (94.00%) compared to egg yolk (17.67% and 78.33%, respectively). Tris-fructose extender, on the other hand, had the highest (P < 0.05) motility and acrosome integrity with egg yolk (38.67% and 93.33%, respectively) compared with 4.5% sunflower lecithin (12.67% and 80.33% motility and acrosome integrity, respectively). Tris-fructose extender had the highest (P < 0.05) malondialdehyde (MDA) concentration with 4.5% sunflower lecithin (0.72 nmol/mL) compared with egg yolk (0.48 nmol/mL). In contrast, the highest level (P < 0.05) of cholesterol (133.38 mg/dL) and seminal arginase (0.68 units/mg protein) were produced with egg yolk. However, the tris-sucrose extender had the highest MDA concentration, cholesterol, and seminal arginase with egg yolk (1.11 nmol/mL, 128.10 mg/dL, and 0.31 units/mg protein, respectively).

Cholesterol is known to be one of the major components of seminal plasma (Ozkavukcu *et al*., 2008; Perumal, 2008). Several studies have reported that cholesterol efflux reduces spontaneous acrosome reactions (Wabersk, 1995; Ozkavukcu *et al*., 2008; Perumal, 2008). Arginase is an enzyme that synthesizes nitric oxide (NO), the principal mediator of penile erection (Bivalacqua *et al*., 2001). The effect of nitric oxide on spermatogenesis and sperm function has been studied by several research groups and nitric oxide was found to reduce or inhibit sperm motility (Weinberg *et al*., 1995). A report of the study carried out by Gur and Kandemir (2012) showed that seminal plasma arginase activity may be a biological criterion for determining sperm quality besides classical semen analysis parameters in ram.

Higher motility observed with tris-fructose was supported by previous researchers, except that higher motility and viability were observed in 3% soybean lecithin (Phutikanit *et al*., 2011) and 1.5% soybean lecithin (Hoffmann *et al*., 2011; Yotov, 2015) unlike with egg yolk in this present study. However, 3.0% sunflower lecithin had higher motility when compared with 1.5 or 4.5% sunflower lecithin. This was confirmed by Purdy (2006). Contrary to the results obtained in this present study with sucrose (disaccharide) when compared with fructose (monosaccharide) in egg yolk extender, a study with common carp spermatozoa revealed that disaccharide had a greater cryoprotective effect on it than monosaccharide due to the higher molecular weight of the disaccharide. A study by Thananurak *et al*. (2019) showed that sucrose (disaccharide) gave a better cryoprotectant result when compared with raffinose (trisaccharide) on chicken sperm. In mammalian sperm, different studies reported that disaccharides provide high protection of sperm by increasing motility, plasma membrane integrity, and fertilizing capacity of post-thawed sperm in mouse and deer (Tada *et al*., 1990; Yokoyama *et al*., 1990; Garde *et al*., 2008).

Table 3 Effect of interaction between tris-based extenders and levels of sunflower lecithin on the viability parameters of Kalahari Red goat during cryopreservation

Parameters	Tris-sucrose				P-value	Tris-fructose				P-value
	0%	1.5%	3.0%	4.5%		0%	1.5%	3.0%	4.5%	
Motility (%)	17.67 ± 2.33^{b}	4.33 ± 1.20^{c}	13.00 ± 5.86^{b}	30.00 ± 9.02^{a}	0.032	38.67 ± 4.81^{a}	18.33 ± 1.45^{c}	23.67 ± 3.48^{b}	12.67 ± 2.91^{d}	0.032
Acrosome integrity (%)	78.33 ± 6.36^{bc}	65.33 ± 6.39^{c}	82.33 ± 5.36^{b}	94.00 ± 3.58^{a}	0.041	93.33 ± 4.41^{a}	85.00 ± 8.50^{bc}	89.00 ± 6.08^{ab}	80.33 ± 6.33^{c}	0.043
Membrane integrity (%)	70.67 ± 9.68^{ab}	81.67 ± 5.46^{a}	68.00 ± 1.53^{b}	83.67 ± 7.42^{a}	0.049	82.33 ± 12.99^{a}	62.00 ± 2.89^{b}	81.67 ± 6.49^{a}	87.67 ± 2.03^{a}	0.050
MDA (nmol/mL)	1.11 ± 0.01^{a}	0.53 ± 0.01^{c}	0.61 ± 0.01^{b}	0.30 ± 0.00^{d}	0.042	0.48 ± 0.01^{d}	0.57 ± 0.00^{c}	0.69 ± 0.01^{b}	0.72 ± 0.00^{a}	0.047
Cholesterol (mg/dL)	128.10 ± 0.19^{a}	34.70 ± 0.15^{b}	121.49 ± 0.27^{a}	20.44 ± 0.29^{c}	0.004	133.38 ± 0.20^{a}	39.06 ± 0.11^{c}	106.85 ± 0.25^{b}	35.14 ± 0.15^{d}	0.005
Seminal arginase activity (units/mg protein)	0.31 ± 0.00^{a}	0.28 ± 0.00^{b}	0.26 ± 0.00^{b}	0.22 ± 0.00^{c}	0.050	0.68 ± 0.01^{a}	0.18 ± 0.00^{b}	0.25 ± 0.00^{b}	0.55 ± 0.00^{a}	0.050

Note: ^{a,b,c,d} Means within the same row, by tris-based extenders, with different superscripts differ ($P < 0.05$) MDA = Malondialdehyde.

According to Mara *et al*. (2007), disaccharides can form hydrogen bonds with polar head groups of phospholipids, binding to the membrane interface and thereby replacing water molecules more efficiently than monosaccharides. According to the results of some studies, the presence of disaccharides in the extender after freezing of fish spermatozoa was effective in prolonging post-thaw survival of semen (Woelders *et al*., 1997; Dziewulska *et al*., 2011). It was noted that the substitution of sucrose with trehalose results in slightly better post-thaw motility figures in common carp spermatozoa (Woelders *et al*., 1997). Using sucrose as a component of the extender improved post-thaw motility and motility durations in carp sperm (Iwahashi *et al*., 1995). A sucrose extender with 4.5% sunflower lecithin had improved post-thaw qualities of the spermatozoa. This could be due to the reduction of the injury caused by ice crystallization as sucrose is a non-permeable sugar that renders hypertonic media decreasing intracellular freezable water (Bakás and Disalvo, 1991). Ohkawa *et al*. (1979) also stated the protective layer of lecithin, which is a phospholipid at the surface of spermatozoa membrane against ice crystal formation. Interaction of this sugar with phospholipids in the plasma membrane resulted in a reduction in cryodamage of spermatozoa and increased membrane fluidity, leading to the greater resistance of spermatozoa against freeze-thawing damage (Salamon and Maxwell, 2000; Aboagla and Terada, 2003; Kulaksiz *et al*., 2013). This extender also recorded lower MDA concentration, which is an indication of a reduced level of lipid peroxide due to the antioxidant capacity of sunflower lecithin, thereby reducing the oxidative stress that would have been caused by cryopreservation.

CONCLUSIONS

The tris-fructose extender with egg yolk had higher post-thaw semen qualities than the tris-sucrose extender. This means that semen extender with egg yolk and fructose as a source of sugar should be considered for semen cryopreservation. However, with a tris-fructose extender where egg yolk is to be replaced with sunflower lecithin, this should be done at 3.0% inclusion. Sucrose extender with sunflower lecithin had higher post-thaw sperm qualities than egg yolk. Replace egg yolk with 4.5% sunflower lecithin and use sucrose as a source of sugar in tris-based extender inclusion will give the desired result.

ACKNOWLEDGEMENT

The authors acknowledge the Centre of Excellence in Agricultural Development and Sustainable Environment (CEADESE), World Bank Africa Centre of Excellence, Federal University of Agriculture, Abeokuta, Nigeria, for funding the research.

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