CRYOSURVIVAL OF GOAT SPERMATOZOA IN TWO TRIS BASED EXTENDERS SUPPLEMENTED WITH VITAMIN E

Adekunle, E.O., Majekodunmi, B.C., Logunleko, M.O. & Daramola, J.O.
Department of Animal Physiology, Federal University of Agriculture Abeokuta, Nigeria

ABSTRACT
Tris Coconut Water (TCW) and Tris Skim Milk (TSM) extenders supplemented with different levels of vitamin E were studied for their ability to support *in vitro* cryosurvival of spermatozoa obtained from West African Dwarf (WAD) goat bucks. Semen samples collected with the aid of artificial vagina were diluted with two tris extenders supplemented with 2, 4, 6 and 8mM of vitamin E for 30 days in liquid nitrogen. Spermatozoa cryopreserved with 6mM and 8mM inclusion levels of vitamin E for TCW extenders and 8mM inclusion levels of vitamin E for TSM based extenders had highest ($P < 0.05$) percentage motility. However, the results showed highest ($P < 0.05$) percentage acrosome integrity and membrane integrity at 4mM, 6mM, and 8mM inclusion levels of vitamin E in TCW extender. Highest ($P < 0.05$) percentage live spermatozoa were observed at TSM extenders supplemented with 4mM, 6mM and 8mM inclusion levels. Spermatozoa cryopreserved with 6mM and 8mM TCW extenders had the lowest ($P < 0.05$) percentage abnormality. Moreover, TCW and TSM extenders supplemented with 6mM and 8mM of vitamin E had highest ($P < 0.05$) spermatozoa that underwent acrosome reaction and capacitation. The findings indicated that spermatozoa obtained from WAD goat bucks were better preserved in TCW extender.
Keywords: cryopreservation, goat, oxidative stress, sperm viability, Trisbased extenders

INTRODUCTION
Goat is the most numerous domestic livestock species in Nigeria and presents a great potential to alleviate the problem of protein malnutrition (FAO, 2006). As the demand for these animals is constantly increasing, key steps in rapid multiplication of these animals will be important tools to reduce malnutrition. Frozen semen is normally used for artificial insemination (AI) in goat, sheep and cattle and semen extenders are the main constituents to accomplish freezing; semen extenders provide energy, maintain osmolarity, reduce bacterial contamination and protect sperms at low temperature. Quality of frozen-thawed semen is the main factor for successful application of AI. However, it is proven that the fertility of frozen semen is lower than fresh semen in all mammals including caprine (Lessard *et al.,* 2000; Watson, 2000).
The mechanism by which cryopreservation causes sperm damage is still to be explored, however, it is proven that reduction in viability and fertility occurs due to the damage on sperm plasma membrane (Abavisani *et al.,*2013). Sperm membrane is a highly dynamic structure that controls extra and intra cellular processes and plays an important role during fertilization. Formation of ice crystals reorders lipid, reduces membrane fluidity and permeability during freezing. Choice of semen extender is an important aspect of semen processing for AI and many extenders have been proposed for goat semen (Iaffaldano *et al.,* 2005). The adoption of AI technique in animal production in Nigeria has been generally low and this has accounted for the little or no documented information on storage and AI technique for goat reproduction in Nigeria. Vitamin E (α-tocopherol) functions as a peroxyl radical scavenger which helps to maintain the integrity of long chain polyunsaturated fatty acids in the membranes of cells and thus maintain their bioactivity (Traber and Atkinson, 2007).

MATERIALS AND METHODS
Experimental Location and animal
The study was carried out at the Teaching and Research Farm of Federal University of Agriculture, Abeokuta. Eight intact WAD bucks aged 2.5-3 years were used for this study.

SEMEN COLLECTION
Semen samples were collected from eight WAD bucks with the aid of artificial vagina.
Semen dilution and storage
The effect of adding different levels of vitamin E in two tris extenders on viability of spermatozoa obtained from WAD goat bucks was carried out in a study repeated twice. Only ejaculates showing >80% motility were pooled to minimize individual differences (Bucak and Tekin, 2007). The pooled semen samples were diluted at 32°C in a two-step process with a Tris-based extender composed of 2

fractions. The Fraction 1 solution contained Tris-hydroxymethyl-aminomethane (2.42g), citric acid (1.36g), glucose (1g), penicillin (0.028g), Sodium citrate (2.9g) and distils water made up 100 mL as control followed by addition of Tris-egg yolk, skimmed milk and coconut water based extender each at (20mL), consisted of Tris-hydroxymethyl-aminomethane (2.42g), citric acid (1.36g), glucose (1g), penicillin (0.028g), and distils water make up 100mL. Fraction 2 solution had the same composition as the Fraction 1 solution with the addition of 14.0% glycerol (v/v). The pooled semen samples were splited into 9 equal aliquots, diluted with the Fraction 1 solution and supplemented each with 0mM, 2mM, 4mM, 6mM and 8mM of vitamin E respectively with pH of 7.05 at a final concentration of 789 $\times 10^6$ spermatozoa per millilitre. Fraction 2 solution was subsequently added to fraction 1. Diluted semen samples were then loaded into 2 mL plastic straws, sealed with polyvinyl, cooled to 4°C at a rate of 0.25°C/min and equilibrated at 4°C for 10 min in TYFSF Refrigerated Incubator. Subsequently, the straws were then placed in a canister at 4cm above liquid nitrogen in the vaporous phase for 10min before plunging them directly and quickly into liquid nitrogen for 30 days and thereafter evaluated for sperm quality characteristics.

SEMEN EVALUATION

Subjective microscopic evaluation of sperm motility, acrosome Integrity, sperm membrane integrity, sperm abnormality, live sperm, *In vitro* acrosome reaction and *In vitro* capacitation were carried out.

RESULTS

The results in Table 1 showed that spermatozoa cryopreserved with 6mM and 8mM inclusion levels of vitamin E for TCW based extenders had highest ($P < 0.05$) percentage motility. Similarly, spermatozoa cryopreserved with 8mM inclusion levels of vitamin E for TSM extenders had highest ($P < 0.05$) percentage motility. Highest ($P < 0.05$) percentage acrosome integrity and membrane integrity in 4mM, 6mM, and 8mM inclusion levels of vitamin E were observed among TCW extender and highest ($P < 0.05$) membrane integrity was also realized when 6mM and 8mM inclusion levels of vitamin E in TSM extender was used. Highest ($P < 0.05$) percentage live spermatozoa was observed among both extenders supplemented with 4mM, 6mM and 8mM inclusion levels of vitamin E and TSM supplemented with 6mM and 8mM inclusion levels of vitamin E. However, Spermatozoa cryopreserved with TCW extender supplemented with 6mM and 8mM inclusion levels of vitamin E had the lowest ($P < 0.05$) percentage abnormality.

The results in Table 2 showed that more spermatozoa ($P < 0.05$) cryopreserved with vitamin E underwent acrosome reaction and capacitation compared to the control. However, TCW and TSM based extenders supplemented with 6mM and 8mM of vitamin E had highest ($P < 0.05$) spermatozoa that underwent induced acrosome reaction.

Table I: Effects of spermatozoa viability of buck semen using three different Tris-based extenders supplemented with vitamin E

		Parameters				
		MOT (%)	ACI (%)	MI (%)	LIVE (%)	ABN (%)
	Control	29.20± 3.97^{e}	46.50 ± 2.22^{e}	48.50 ± 1.50^{e}	52.50 ± 5.00^{e}	3.75 ± 0.63^{a}
	2 mM	50.60 ± 2.69^{c}	69.50 ± 0.96^{b}	66.50 ± 0.96^{b}	67.00 ± 4.08^{b}	3.00 ± 0.41^{b}
Tris-	4 mM	62.40 ± 3.11^{b}	74.50 ± 1.26^{a}	77.50 ± 0.96^{a}	67.50 ± 2.50^{b}	2.50 ± 0.50^{c}
coconut	6 mM	70.00 ± 4.38^{a}	77.00 ± 1.73^{a}	75.50 ± 0.50^{a}	72.50 ± 2.50^{a}	1.40 ± 0.41^{d}
water	8 mM	71.20 ± 4.50^{a}	78.50 ± 0.96^{a}	74.50 ± 0.96^{a}	70.59 ± 4.79^{a}	1.00 ± 0.41^{d}
	2 mM	56.00 ± 5.29^{c}	55.50 ± 2.63^{d}	61.00 ± 0.58^{c}	65.00 ± 6.45^{b}	3.00 ± 0.00^{b}
Tris-skim	4 mM	55.00 ± 7.34^{c}	58.50 ± 2.21^{c}	67.00 ± 1.00^{b}	70.00 ± 0.02^{a}	2.75 ± 0.63^{b}
milk	6 mM	62.60 ± 5.68^{b}	63.50 ± 1.50^{c}	72.50 ± 0.50^{a}	70.00 ± 5.77^{a}	2.50 ± 0.29^{c}
	8 mM	71.20 ± 2.69^{a}	66.50 ± 2.22^{b}	75.00 ± 1.29^{a}	75.00 ± 2.89^{a}	2.00 ± 0.41^{c}

$^{a, b, c,d,e}$Values within the same column with different superscripts differ ($P < 0.05$), MOT: Motility, ACI: Acrosome Integrity, MI: Membrane Integrity,ABN: Abnormality,SE:standard error.

Table 2: Effect of two Tris-based extenders on *in vitro* acrosome reaction (%) and capacitation (%) of spermatozoa cryopreserved with vitamin E

	Extenders	Parameters	
		*In-vitro*acrosome reaction (%)	*In-vitro*sperm capacitation (%)
Tris coconut water	Control	43.00 ± 1.91[e]	40.00 ± 1.63[e]
	2 mM	61.00 ± 6.61[c]	58.00 ± 1.15[c]
	4 mM	73.00 ± 5.00[b]	71.50 ± 8.09[b]
	6 mM	84.50 + 2.06[a]	78.00 + 5.29[a]
	8 mM	81.50 ± 1.50[a]	80.00 ± 4.90[a]
Tris skim milk	2 mM	72.00 ± 10.67[b]	69.00 ± 5.74[b]
	4 mM	76.50 ± 2.36[b]	71.00 ± 4.43[b]
	6 mM	78.00 ± 0.81[a]	72.00 ± 5.89[b]
	8 mM	78.50 ± 2.99[a]	81.00 ± 2.52[a]

[a, b, c, d, e] Values within the same column with different superscripts differ significantly, ($P < 0.05$), SE: standard error.

DISCUSSION

The addition of different levels of vitamin E in this present study to TCW and TSM based extenders improved the percentage of sperm motility and viability, The improvement in motility in this tris based extenders supplemented with vitamin E suggests the beneficial effect of vitamin E on sperm motility and viability which could be due to its potential antioxidant capacity. The highest sperm motility and percentage live sperm observed from TCW following supplementation of vitamin E could be linked to the fact that vitamin E suppressed deleterious effect of ROS on WAD buck and the protective effect of coconut water which result to high content of lipids which is a major component of sperm membrane that is involved in a series of biochemical and functional change ultimately required for fertilization (Brque *et al*., 2003). The combination of vitamin E and TCW is a more complete nutrient composition that provide important source of energy for sperm to maintain their lifespan during storage and also coconut water is poor in phospholipids and rich in complex organic molecules, such as proline, glycine, glutamic acid and indole-acetic acid (IAA) which protect and extend the spermatozoa life span.

The higher acrosome and membrane integrity during cryopreservation with the tris based extenders supplemented with vitamin E indicated that vitamin E as antioxidant has capacity to increase the onset of the physiological acrosome reaction and the Indole Acetic Acid present in coconut water had a beneficial effect on goat sperm acrosomal and membrane integrity.

Evaluation of sperm abnormality is one of the commonest methods of investigating survival of frozen thawed semen and the relationship between sperm abnormality and fertility has been evaluated (Raina, 2002). Vitamin E functions as antioxidants which in turn may provide direct protection of sperm cells from morphological damage by preventing free radical oxygen from damaging sperms (Luvoni, 2006).

The physiological acrosome reaction involves activation of several signal transduction pathways, the induced acrosome reaction mainly involves a chemical effect on calcium influx that partially reflects the physiological acrosome reaction process (Breitbart and Spungin, 1997) and only capacitated spermatozoa have ability to undergo acrosome reaction (Arnoult *et al.,*1996).The present *in vitro* study indicated that vitamin E was able to maintain fertilizing capacity of spermatozoa in, TCW and TSM extenders as evidenced by better percentage of capacitation and acrosome reaction of cryopreserved spermatozoa. In ram spermatozoa, vitamin E administration increases plasminogen activator activity, known to be involved in sperm capacitation and acrosome reaction (Tsantarliotou *et al.,* 2008). This result was in agreement with Arabi and Seidaie (2008) who demonstrated that vitamin E determined capacitation of buffalo spermatozoa *in vitro*.

CONCLUSION

The findings of the present study indicated that the use of coconut extenders supplemented with vitamin E for goat semen cryopreservation could, in the future, improve the effectiveness of goat AI programs and it is concluded that there were no apparent disadvantages to switching from egg yolk extenders to coconut extenders for goat semen cryopreservation.

RECOMMENDATION

Coconut extender supplemented with 6mM and 8mM vitamin E could be used to preserve goat semen.

REFERENCES

Abavisani, A., Arshami, J., Naserian, A.A., Kandelousi, M.A.S. and Azizzadeh, M., 2013.Quality of bovine chilled or frozen-thawed semen after addition of omega-3 fatty acids supplementation to extender.*Int. J. Fertil. Steril*. 7: 161-168.

Arabi, M., and Seidaie, S.R.,2008. Assessment of motility and membrane peroxidation of bull spermatozoa in the presence of different concentration of vitamin C. *Vet. Med. J.* 2: 39-46.

Arnoult, C., Zeng, Y., and Florman, H.M., 1996. ZP3-dependent activation of sperm cation channels regulates acrosomal secretion during mammalian fertilization. *J. Cell Biol.* 134, 637-645.

Breitbart, H., Spungin, B. 1997. The biochemistry of the acrosome reaction. Mol.Hum.*Reprod.* 3:195-202.

Brèque, C., Surai, P., and Brillard, J. P. 2003. Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro.*Mol. Reprod. Dev*. 66: 314–323

Bucak, M.N. and Tekin, N. 2007. Protective effect of taurine, glutathione and trehalose on the liquid storage of ram semen. *Small Ruminant Res*. 73, 103-108

Etches, R.J., 1996.Reproduction in Poultry. CABInternational, Wallingford, UK, pp: 241-250.

FAO (Food and Agriculture Organization) 2006. World agriculture towards 2030/2050. Interim report, Global Perspective Studies United Nation. Rome, Italy

Iaffaldano, N., Rosato, M.P., Manchisi, A.,Centoducati, G. and Meluzzi, A., 2005. Comparison of different extenders on the quality characteristicsof turkey semen during storage.Ital.*J.Anim. Sci*.4: 513-515.

Leeuw, F.E.; Leeuw, A.M.; Den Daas, J.H.G.; Colenbrander, B. and Verkleij, A.J. 1993. Effects of various cryoprotective agents and membrane-stabilizing compounds on bull sperm membrane integrity after cooling and freezing. *Cryobiolo.* 30: 32-44.

Lessard, C., Parent, S., Leclerc, P., Baileys, J.L. and Sullivan, R., 2000. Cryopreservation alters the levels of the bull sperm surface protein P25b. *J. Androl.,* 21: 700-707.

Luvoni G.C. 2006.Gamete cryopreservation in the domestic cat. *Theriogenol* 66: 101-111.

Moussa, M., Martinet, V.,Trimeche, A.,Tainturier, D., and Anton, M., 2002. Low density lipoproteins extracted from hen egg yolk by an easy method: cryoprotective effect on frozen-thawed bull semen. *Theriogenol*57:1695-1706.

Raina, V. S., A. K. Gupta and K. Singh, 2002.Effect of antioxidant fortification on preservability ofbuffalo semen.Asian-Austr.*J. Anim. Sci*. 15: 16-18.

SAS. 1999. SAS User's Guide: Statistics Inc., Cary, N.C., Pp. 923.

Toniolli, R.; Bussière, J.; Courot, M.; Magistrini, M. and Combarnous, Y. 1996.Effect of indole-3-acetic acid (plant auxin) on the preservation at 15°C of boar semen for artificial insemination.*ReprodNutr Dev*. 36: 503-511.

Traber, M.G. and Atkinson, J. 2007.Vitamin E antioxidant and nothing more. Free radic. Bio. Med.43: 4-15

Tsantarliotou, M.P., Kokolis, N.A., and Smokovitis, A., 2008. Melatonin administration increased plasminogen activator activity in ram spermatozoa. *Theriogenol.* 69, 458-465.

Watson, P., 2000. The causes of reduced fertility with cryopreserved semen.*Anim. Reprod. Sci.* 60: 481-492.