EFFECTS OF AVOCADO SEED EXTRACT IN DIFFERENT TRIS-EXTENDERS ON SPERM AND OXIDATIVE STRESS INDICES OF VITRIFIED GOAT SPERMATOZOA

**Daramola James Olamitibo[1*], Onanuga Oluwakemi Dayo[1],
Abioja Monsuru Oladimeji[1], Adeleke Mathew[2], Olowofeso Olajide[2],
Oke Oyegunle Emmanuel[1], Adekunle Ezekiel Oluwafemi[1],
Sorongbe Taiwo Amidu[1] and Iyanda Olayinka Ayobami[1]**

[1]Department of Animal Physiology, Federal University of Agriculture,
Abeokuta, Nigeria
[2]Animal Breeding and Genetics Department, Federal University of Agriculture,
Abeokuta, Nigeria

Abstract: Effects of avocado seed extract (ASE) in different tris-extenders on sperm and seminal oxidative stress indices of vitrified spermatozoa of West African Dwarf (WAD) goats during vitrification were assessed. Semen samples were diluted with varying levels (0, 5, 10, 15 and 20 mL/100 mL of diluent) of ASE in tris citric acid (TCA), tris sodium phosphate (TSP) and tris sodium citrate (TSC) extenders while a tris-extender without citric acid, sodium phosphate and sodium citrate served as control. Diluted semen samples were vitrified and preserved. The results revealed higher ($P<0.05$) progressive sperm motility in TCA extenders at 10%, 15% and 20%, and TSP extender at 20% ASE. Acrosome integrities were higher ($P<0.05$) in the different tris-extenders at 15% and 20% ASE. TCA, TSP and TSC extenders had higher ($P<0.05$) membrane integrities at 10%, 15% and 20% ASE. TSP extenders had higher ($P<0.05$) live sperm at 20% ASE. TCA extenders had higher acrosome reaction while TSP at 20% ASE and TSC at 15% and 20% of ASE had higher sperm capacitation. TCA extender at 20% ASE had reduced malondialdehyde (MDA) concentrations while higher acrosin activities were observed at 20% ASE in TCA extender ($P<0.05$). The findings indicated that 20% ASE in the tris-extenders improved sperm and oxidative stress indices and the tris-extenders were ranked best in this order: TCA > TSP > TSC.

Key words: antioxidant, freezing, bucks, oxidative stress, semen extenders, sperm quality.

[*]Corresponding author: e-mail: daramolajames2003@yahoo.com

Introduction

Sperm cryopreservation is a viable method for preserving economically genetic traits of male gametes (Blash et al., 2005). Application of this technique to conserve gametes of West African Dwarf (WAD) goats (*Capra hircus*) is necessary as a means of improving production of this breed. Cryopreservation however reduces sperm quality (Pegg, 2007; Bagchi et al., 2008). Milk and egg yolk are animal products commonly used as constituents of semen diluents in most species to preserve sperm viability (Correa et al., 2006; Purdy, 2006). They, however, pose the risk of contamination if microorganisms are present in the fresh product. Moreover, the major limitation with respect to egg yolk or milk diluents is linked to the bulbourethral gland secretion in seminal plasma that produces egg yolk coagulating enzyme (Iritani and Nishikawa, 1961) or due to a protein fraction called SBUIII that interacts with milk components in diluents and inhibits motility of buck spermatozoa (Leboeuf et al., 2000). Accordingly, extenders free of animal products could be a possible alternative option to solve these problems. The seed of avocado (*Persea americana*) is rich in some important compounds such as minerals, lutein and antioxidants that are essential components of semen extenders necessary for cryosurvival of spermatozoa (USDA, 2011; Gómez et al., 2014; Vedamurthy et al., 2015). The beneficial effects of ASE in semen extenders have not been reported. The aim of this experiment was therefore to evaluate the effects of ASE in different tris-extenders on sperm and oxidative stress indices of WAD goat bucks during vitrification.

Material and Methods

Location and animal management

The experiment was conducted at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria. The area is located in the south-western part of Nigeria and is characterized by average rainfall and temperature of 1,037 mm and 34.7ºC respectively, and between 7^0 10'N and 3^0 2'E and altitude 76 m above sea level. The animals used for this study consisted of five WAD goat bucks aged 3–5 years. The bucks were managed intensively and fed with concentrate feed and guinea grass (*Panicum maximum*) as supplements.

Preparation of avocado seed extract

ASE was prepared using the method of soymilk preparation (Odu et al., 2012) and further modified as follows. Avocado fruit was washed with distilled water and split open with a knife to retrieve the seed. The outer coat was removed and then transferred into boiling water for 10 min. The boiled seed was chopped and

blended. After blending, 500 mL of distilled water was added to the slurry, mixed and sieved with a clean white cloth. The extract was then transferred into a beaker and used fresh with a tris-based extender.

Semen collection and dilution

Semen samples were obtained with the aid of an artificial vagina from the bucks consisting of five semen samples (each semen sample emanated from five individual bucks). The samples that showed >80% motility and >90% live sperm, acrosome integrity, and membrane integrity were pooled (Bucak and Tekin, 2007). The pooled semen samples were diluted at room temperature with a tris-based extender. The sample was divided into 15 equal parts, diluted with ASE (0, 5, 10, 15 and 20 mL/100 mL of diluent) each in tris citric acid (TCA), tris sodium phosphate (TSP) and tris sodium citrate (TSC) extenders. The tris-extender (control) consisting of 2 M tris-hydroxymethyl-aminomethane (2.42 g), 0.5 M glucose (1 g), 0.0005 M penicillin (0.028 g), and distilled water made up 100 ml as control plus either TCA (0.6 M citric acid [1.34 g]) or TSP (0.2 M sodium phosphate [0.8g]) or TSC (1 M sodium citrate [2.9 g]).

Vitrification

The diluted semen samples (270×10^6 sperm/mL) at pH of 7.02 were vitrified at room temperature using varying cryoprotectants in line with the procedure described by Srirattana et al. (2013) with some modifications.

Preparation of cryoprotectants

Phosphate-buffered saline (PBS) was supplemented with 20% bovine serum albumin (BSA) (v/v) as a holding medium (HM). Vitrification solution I (VS-I) and vitrification solution II (VS-II) consisting of ethylene glycol (EG), dimethylsulfoxide (DMSO) and HM were prepared prior to use. VS-I was made by mixing 0.2 M EG (12.5%,), 0.15 M DMSO (12.5%) and the HM in the ratio of 1:1:6. VS-II on the other hand was made by mixing 0.4 M EG (25%), 0.3 M DMSO (25%) and the HM in the ratio of 1:1:2.

Vitrification procedures

The semen sample previously diluted was added to the different cryoprotectants consecutively for each treatment. The sample (1 mL) was first added to 1 mL of HM for 10 min. The mixture (diluted semen sample + HM) was then placed in 1 mL of VS-I for 4 min, and the mixture (diluted semen sample +

HM + VS-I) was finally put into 1 mL of VS-II for 1 min. During equilibration in VS-II, the sample was loaded into 0.2 mL straws and treatment was replicated twice. Immediately after loading, the straws were put vertically into liquid nitrogen tank and preserved for thirty days. Thereafter, the straws were thawed in Clifton water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 38°C.

Microscopic evaluation of sperm motility

Assessment of progressive sperm motility was carried out (Bearden and Fuquay, 1997). The thawed semen sample was assessed for progressive sperm motility using Celestron PentaView microscope (LCD-44348 by RoHS, China) at 400x magnification. The sample (5 µL) of semen was placed on a warmed microscope slide and overlaid with a cover slip (22 x 22 mm). Ten microscopic fields were examined using different slides for each semen sample to observe progressively motile sperm cells that moved forward in a straight line by three observers. The average of the ten consecutive observations was noted as the final motility result.

Acrosome Integrity

Assessment of sperm cells with intact acrosome was carried out using the method described by Ahmad et al. (2003). Formalin citrate solution consisting of 96 mL 2.9% sodium citrate and 4 mL 37% formaldehyde was prepared. Subsequently, 50 mL of the thawed semen sample was mixed with 500 µL formalin citrate solution. A minute drop of the mixture was placed on a microscope slide. Two hundred sperm cells were observed in different microscopic fields for each sample using Celestron PentaView LCD microscope (400x magnification). The acrosome that showed a normal apical ridge of sperm cells was recorded.

Sperm Membrane Integrity

Membrane integrity of the sperm cells was evaluated using the hypo-osmotic swelling test as described by Zubair et al. (2013). In this test, a hypo-osmotic solution consisting of 9 g fructose plus 4.9 g sodium citrate mixed with 1000 mL of distilled water was prepared prior to use. Subsequently 10 µL of semen was incubated in 100 µL hypo-osmotic solution at 37°C for 30 min. Thereafter, 0.1 mL of the mixture was placed on a warmed slide and overlaid with a cover slip. Two hundred spermatozoa (200) were observed under Celestron PentaView LCD digital microscope (400 x magnification). Swelled spermatozoa with a characteristic coiled tail were recorded as the intact plasma membrane.

Live sperm

Eosin-nigrosin smears were used to evaluate live sperm as described by Bearden and Fuquay (1997). A slight smear of combination of semen and eosin-nigrosin solution was placed on a microscopic slide and dried. A total of 240 sperm cells were observed using Celestron PentaView LCD digital microscope (400x magnification). Spermatozoa that appeared white were recorded as live spermatozoa and those that picked up the stain were recorded as dead spermatozoa.

Malondialdehyde concentrations

The concentration of malondialdehyde (MDA) in the preserved semen was evaluated using a thiobarbituric acid reactive substances as described by Pipan et al. (2014). Following incubation of a sample of the stored semen (0.1 mL) in 0.1 mL of 150 mM tris-HCl (pH 7.1) for 20 min at 37°C, 1 mL of 10% trichloroacetic acid (TCA) and 2 mL of 0.375% thiobarbituric acid were added and further incubated at a boiling point for 30 min. Subsequently, the incubated sample was centrifuged (3000 xg) inside the blank tube for 15 min. UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) was used to read the absorbance at 532 nm. MDA was subsequently estimated using the formula shown below: MDA (nmol/mL) = $AT - AB/1.56 \times 10^5$; where AT = the absorbance of the sample, AB = the absorbance of the blank, 1.56×10^5 molar absorptivity of MDA.

Acrosin activity

Evaluation of acrosin activity was carried out as described by Rosatti et al. (2004). Specific substrate for acrosin consisting of N-a-benzoyl-DL-arginine p-nitroanilide (BAPNA) was used. HCl solution (10 M) was used to inhibit the activation of proacrosin to acrosin. A solution of 10 M HCl was mixed with a control and incubated at 38°C. Equal parts were taken from each sample at a time and incubated. Furthermore, a solution of 10 M HCl was added to all the samples with the exception of the control and the total acrosin ones. Samples were made uniformed and centrifuged (14,000 xg) for 10 min. A solution of 0.2 M buffer tris and 100 µL of BAPNA (100 mM) was added to each supernatant, which were then incubated for 3 min at room temperature. UV spectrophotometer (SW7504 model by Surgifriend Medicals, England) was used to read the sample absorbance (Ab) at 410 nm. Acrosin activity (mIU/10^6 sperm) was calculated as follows: Acrosin activity = [Ab sample – Ab control] x 10^6/9.9 mM^{-1}x cm^{-1} x 3 min x 10^6 sperm x vol. of cuvette.

In vitro acrosome reaction

The proportion of acrosome reaction was evaluated in line with the procedure described by Somanath and Gandhi (2002) and further modified as follows: vitrified samples of semen were centrifuged in phosphate-buffered saline (PBS), and re-suspended in culture medium consisting of calcium chloride dihydrate 265 mg/L, magnesium chloride anhydrous 46 mg/L, potassium chloride 200 mg/L, sodium chloride 8000 mg/L, sodium dihydrogen phosphate anhydrous 50 mg/L and D-Glucose 1000 mg/L. Following inclusion of 0.9% wt/vol PBS, induction of acrosome reaction was carried out by incubating sperm cells for 20 min with progesterone (2.5 mg/mL) at 38.5°C (5% CO_2 in air; 100% humidity). Subsequently, an equal volume of PBS was added without progesterone to estimate the proportion of spontaneous acrosome reaction. One hundred sperm cells were observed per slide using an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics. Intact acrosome was characterized by intense fluorescence over the acrosome while spermatozoa that underwent acrosome reaction showed no fluorescence or a dull fluorescence along the equatorial segment.

In vitro capacitation

The proportion of capacitated sperm cells was determined as described by Collin et al. (2000) using the chlortetracycline (CTC) fluorescence method. CTC (750 µM) was prepared in tris buffer (20 mM) containing 130 mM NaCl and 5 mM DL-cysteine (final pH 7.8). A vitrified semen sample (5 µL) was added to CTC solution (5 µL) on a slide (37°C). Following expiration of 30 s, 5 µL of 0.2% glutaraldehyde in 0.5 M tris (pH 7.4) was added. Finally, 5 µL of 90% glycerol and 10% PBS (pH adjusted to 8.6) were added to retard fluorescence fading. A drop of the sample was placed on a slide and overlaid with a coverslip and observed using an upright Carl Zeiss Fluorescent Microscope (Primo Star, Germany) equipped with phase contrast and epifluorescence optics. One hundred sperm cells were observed per slide for sperm cells that showed the bright anterior head and faint fluorescence in the post-acrosomal region.

Statistical analysis

The results analyzed for the vitrified semen samples in each treatment consisted of two straws in repeated measurements using a 3 x 5 factorial arrangement in SAS 2000 package. Significantly different means (P<0.05) were separated by the Duncan multiple range test (Duncan, 1955). The model used is presented below:

$$Y_{ijk} = \mu + E_i + M_j + (EM)_{ij} + \sum_{ijk} \qquad (1)$$

where, Y_{ijkl} = Dependent variables, μ = Population mean, E_i = Effect due to the ith different tris based extender, i = (1, 2, 3), M_j = Effect due to the jth level of ASE inclusion, j = (0, 5, 10, 15, 20), EM_{ij} = Effect of the ij^{th} interaction between different tris based extenders and levels of ASE inclusion, $\sum_{ijkl}$ = Experimental error.

Results and Discussion

Sperm quality indices

The motilities, acrosome integrities, membrane integrities and live sperm of WAD goat semen vitrified with ASE in different tris-extenders are presented in Figures 1, 2, 3 and 4 respectively. The results showed higher (P<0.05) sperm motility in TCA extenders at 10%, 15% and 20%, and in TSP extender at 20% ASE compared to other extenders and the control. Semen vitrified with ASE in TSC extender had no motile spermatozoa compared to the control. The results (Figure 2) showed higher (P<0.05) acrosome integrities in the different tris-extenders at 15% and 20% ASE. TCA, TSP and TSC extenders had higher (P<0.05) membrane integrities at 10%, 15% and 20% ASE (Figure 3). The results (Figure 4) showed that semen vitrified with TSP extenders had higher (P<0.05) live sperm at 20% ASE compared to other extenders and the control.
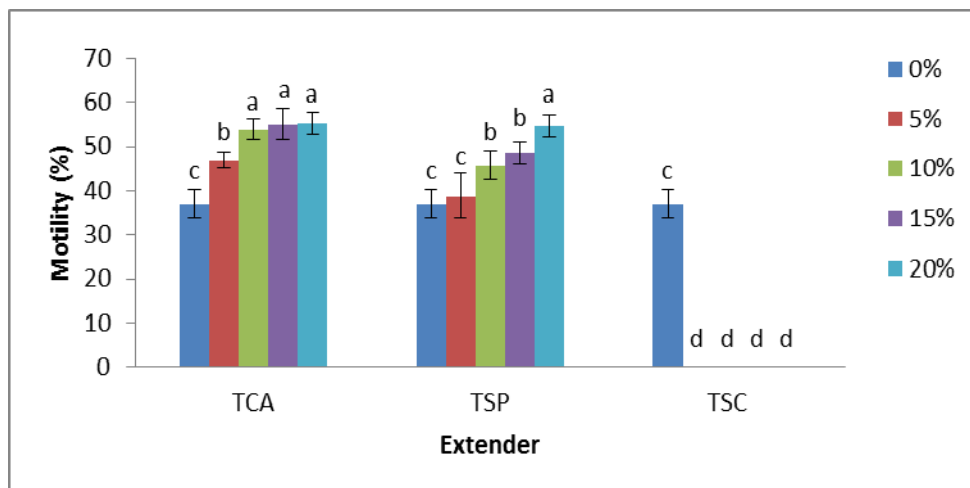

Figure 1. Motility of semen vitrified with ASE in different tris-extenders (n=20). [a,b,c,d] Means with different superscripts differ significantly (P<0.05). ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium phosphate; TSC = Tris sodium citrate.
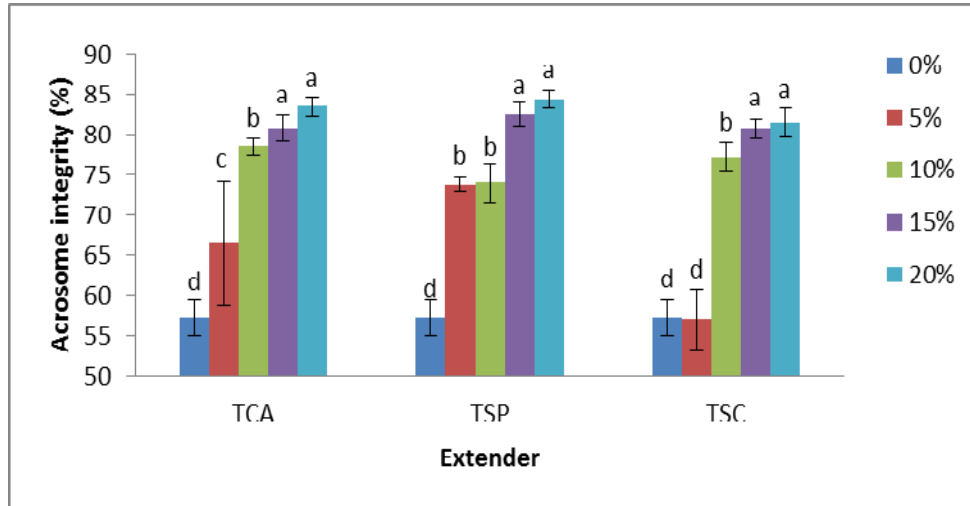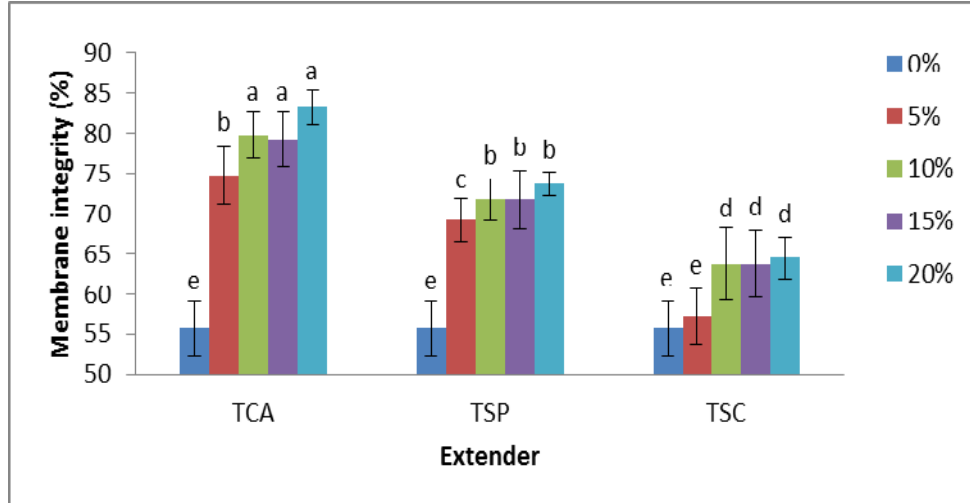
Figure 2. Acrosome integrity of semen vitrified with ASE in different tris-extenders (n=8). [a,b,c,d] Means with different superscripts differ significantly (P<0.05). ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium phosphate; TSC = Tris sodium citrate.

Figure 3. Membrane integrity of semen vitrified with ASE in different tris-extenders (n=8). [a,b,c,d] Means with different superscripts differ significantly (P<0.05). ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium phosphate; TSC = Tris sodium citrate.
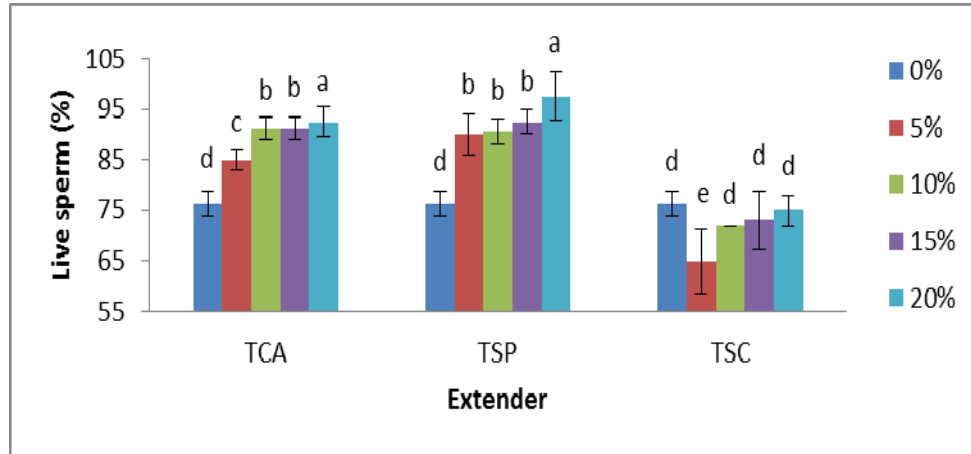
Figure 4. Live sperm of semen vitrified with ASE in different tris-extenders (n=8).
[a,b,c,d] Means with different superscripts differ significantly (P<0.05).
ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium
phosphate; TSC = Tris sodium citrate.

Seminal oxidative stress indices

The oxidative stress indices of WAD goat semen vitrified with ASE in different tris-extenders are presented in Figures 5 and 6.
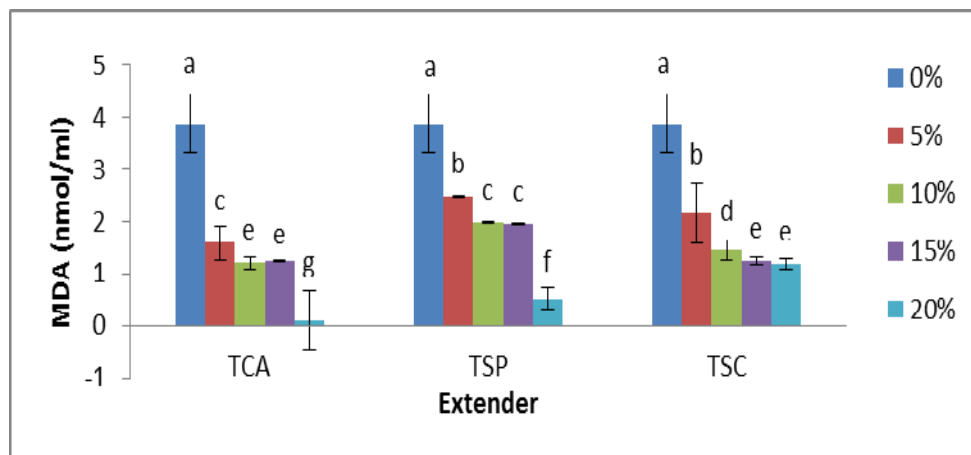

Figure 5. Malondialdehyde concentrations of semen vitrified with ASE in different tris-extenders (n=8). [a,b,c,d] Means with different superscripts differ significantly (P<0.05). ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium phosphate; TSC = Tris sodium citrate; MDA = Malondialdehyde.
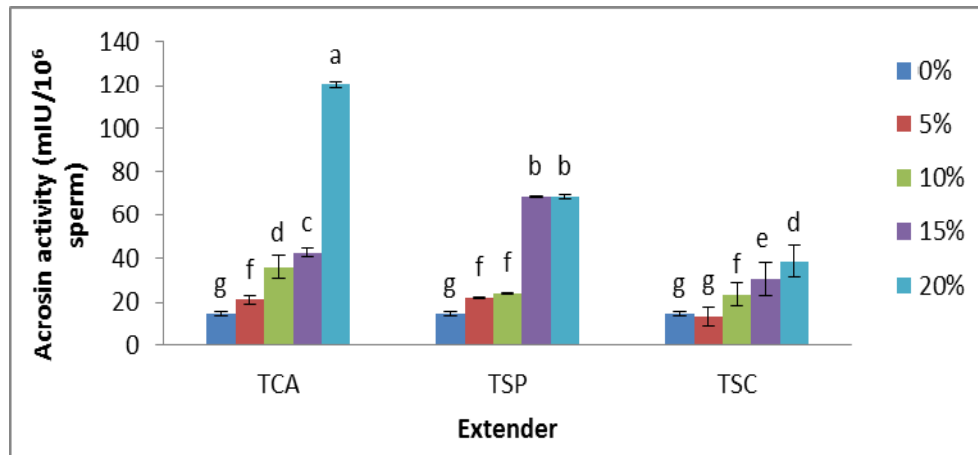
Figure 6. Acrosin activity of semen vitrified with ASE in different tris-extenders
(n=8). [a,b,c,d] Means with different superscripts differ significantly (P<0.05).
ASE = Avocado seed extract; TCA = Tris citric acid;
TSP = Tris sodium phosphate; TSC = Tris sodium citrate.

The results showed that semen vitrified with ASE in the different tris-extenders had reduced MDA concentrations compared to the control. Semen vitrified with TCA extender at 20% ASE had reduced MDA concentrations compared to other tris-extenders and the control. Similarly, higher acrosin activities were observed in semen vitrified with ASE in the different tris-extenders compared to the control and optimal values were recorded at 20% ASE in TCA extender.

Fertilizing ability indices

The fertilizing ability indices (acrosome reaction and capacitation) of WAD goat semen vitrified with ASE in different tris-extenders are presented in Figures 7 and 8. The results showed higher acrosome reaction in semen vitrified with all levels of ASE in TCA followed by 15% and 20% of ASE in TSP and TSC extenders. However, semen vitrified with TSP at 20% ASE and TSC at 15% and 20% of ASE had higher sperm capacitation compared to other tris-extenders and the control.

The improvement in sperm quality and fertilizing ability indices in this study revealed the preservative effect of these extenders and could be attributed to the components of ASE that are essential for optimal survival of spermatozoa during vitrification. Avocados contain some minerals, lutein and antioxidants that are essential in cell functions (USDA, 2011; Dreher and Davenport, 2013; Gómez et al., 2014; Vedamurthy et al., 2015).
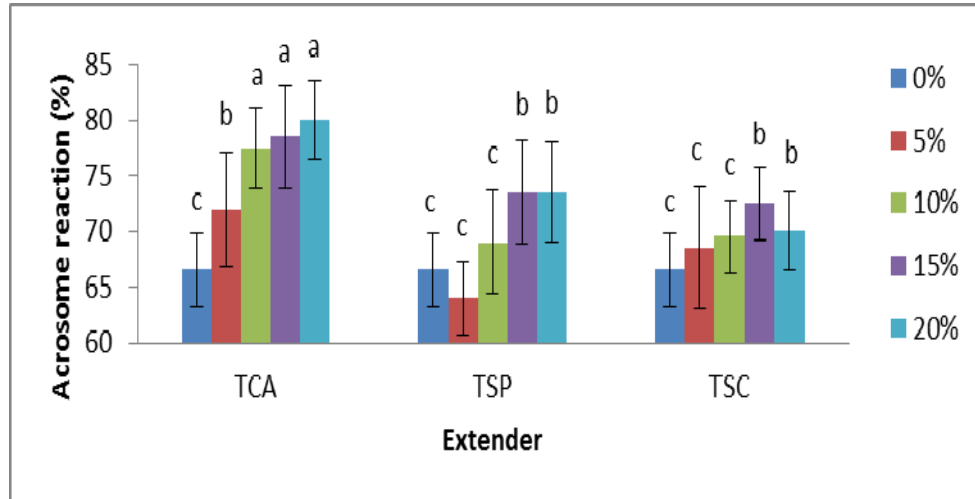
Figure 7. Acrosome reaction of semen vitrified with ASE in different tris-extenders (n=8). [a,b,c] Means with different superscripts differ significantly (P<0.05). ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium phosphate; TSC = Tris sodium citrate.

Figure 8. Sperm capacitation of semen vitrified with ASE in different tris-extenders (n=8). [a,b,c,d] Means with different superscripts differ significantly (P<0.05). ASE = Avocado seed extract; TCA = Tris citric acid; TSP = Tris sodium phosphate; TSC = Tris sodium citrate.
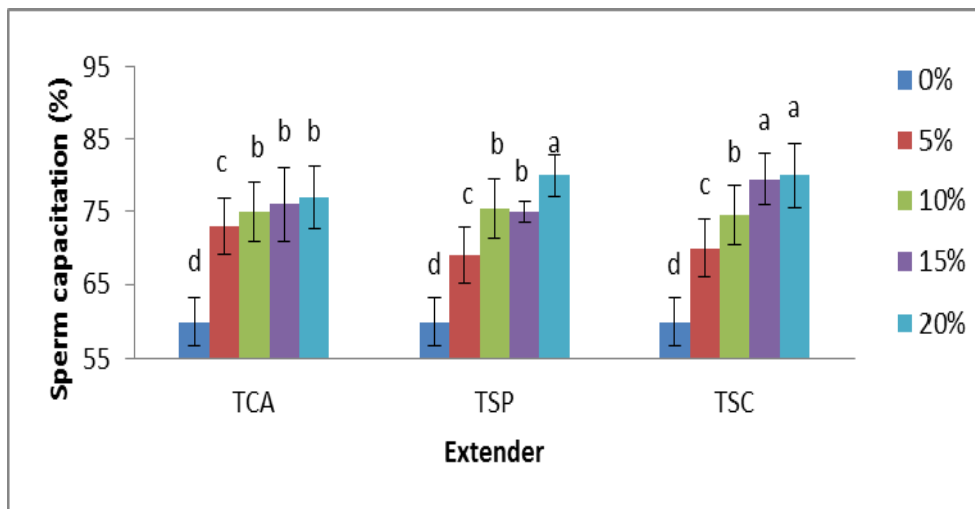
Adverse effects of seminal oxidative stress on sperm quality have been evaluated using MDA concentration (Arabi and Seidaie, 2008; Piyali et al., 2009; Pasqualotto et al., 2000). The reduction in levels of MDA coupled with elevated acrosin activities observed in different tris-extenders compared to the control and the optimal benefit at 20% ASE suggested the protective effect of these treatments against oxidative stress damage associated with cryopreservation. A substantial level of polyunsaturated fatty acids (PUFA) is found in the plasma membrane of mammalian sperm which makes them prone to lipid peroxidation (Mandal et al., 2014; Niki et al., 2005). Consequently, effective antioxidant components are essential to preserve sperm cells against damage due to lipid peroxidation (Breque et al., 2003). The findings to greater extent supported the preservative effect of ASE due to antioxidant capacity of avocado (Wu et al., 2004). The antioxidant property of avocado seed extract has recently been reported to remove 1,1-diphenyl,2-picrylhydrazyl (Vedamurthy et al., 2015). A considerable percentage of polyphenols are also present in avocado (Gómez et al., 2014). Polyphenols are able to prevent cell damage caused by lipid peroxidation (Perumalla and Hettiarachchy, 2011).

Furthermore, considerable levels of vitamins such as C, E, folate and pyridoxine are found in avocados (USDA, 2011). The antioxidative properties of these vitamins as scavengers for free radicals have been observed (Kannan and Jain, 2004). Vitamin C in particular is involved in recycling vitamin E for maintaining circulatory antioxidant protection (USDA, 2011; Dreher and Davenport, 2013). Vera-Munoz et al. (2009) observed that motility and integrity of sperm plasma membrane were efficiently preserved in the low-density lipoprotein (LDL) extender. In addition, Mendez and Hernandez (2007) found that antioxidative activity of avocados could modify the high-density lipoproteins-cholesterol structure by improving lipophilic antioxidant capacity.

Carotenoids are natural antioxidants and perform various biological functions in animals (Surai, 2002). Xanthophylls and fat-soluble antioxidants are the primary carotenoids found in avocado (Voutilainen et al., 2006; USDA, 2011). Lutein, a product of xanthophyll, has ability to inhibit lipid peroxidation of liposomes (Stahl et al., 1998). Xanthophylls as fat-soluble compounds in membranes could scavenge free radicals (Woodall et al., 1996). Avocados have considerable lipophilic antioxidant capacity in different fruits and vegetables (Wu et al., 2004). Studies have revealed protective effects of xanthophylls on DNA due to their antioxidant capacity (Thomson et al., 2007; Hughes et al., 2009).

The variations in sperm functional indices during vitrification with ASE in tris-extenders suggested differences in the beneficial effects of these tris-extenders. The optimal beneficial effects observed in TCA on the sperm quality indices compared to TSC agreed with Rakha et al. (2013) who observed higher sperm motility, viability, acrosome and plasma membrane integrity in tris-citric acid extender compared to sodium citrate extender. Sodium, in spite of its inability to cross cell membranes, has

protective properties against the harmful effect of cold (Toner et al., 1993). The improved sperm quality indices at 20% ASE indicated the ability of the extenders at this level of ASE to effectively use the potassium contained in avocados and sperm cells for the survival of the vitrified spermatozoa. Avocado has a considerable concentration of potassium (Vinha et al., 2013). Earlier studies have revealed the important role of potassium on the quality of diluted sperm cells (Dott and White, 1964). Quinn et al. (1966) reported higher potassium concentration in sperm cells compared to seminal plasma. Loss of potassium in ram sperm cells diluted with sodium phosphate buffer was also observed (Dott and White, 1964). The present study confirms the observation of Dott and White (1964) that semen diluted with sodium phosphate buffer resulted in reduced potassium concentration within the spermatozoa.

Conclusion

The findings indicated that 20% ASE in the tris-extenders effectively improved quality indices of vitrified spermatozoa of the WAD goat and the best results in these indices were observed in TCA and TSP extenders. The study therefore revealed the beneficial effect of ASE due to the presence of essential components of semen extenders necessary for cryosurvival of spermatozoa. Vitrified semen samples obtained from WAD goat bucks would be satisfactory for an artificial insemination programme at 20% ASM inclusion in TCA and TSP extenders.

Acknowledgements

References

Ahmad, Z., Anzar, M., Shahab, N., Ahmad, S. & Andrabi, M.H. (2003). Sephadex and sephadex ionexchange filtration improves the quality and freezability of low-grade buffalo semen ejaculates. *Theriogenology,* 59, 1189-1202.

Arabi, M., & Seidaie, S.R. (2008). Assessment of motility and membrane peroxidation of Bull spermatozoa in the presence of different concentration of vitamin C. *Veterinary Medicine Shahrekord*, 2, 39-46.

Bagchi, A., Woods, E.J. & Critser, J.K. (2008) Cryopreservation and vitrification: Recent Advances in fertility preservation technologies. Expert *Review Medical Devices*, 5, 359-370.

Bearden, H.J. & Fuquay, J.W. (1997). *Applied Animal Reproduction*. 4thed. New Jersey: Prentice Hall; pp.158-169.

Blash, S., Chen, L., Harvey, M. & Gavin, W.G. (2005). Reestablishment of a transgenic rabbit line by artificial insemination using cryopreserved semen. *Laboratory Animals*, 34, 61-63.

Breque, C., Surai, P. & Brillard, J.P. (2003). Roles of antioxidants on prolonged storage of avian spermatozoa in vivo and in vitro. *Molecular Reproduction and Development*, 66, 314-323.

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

Collin, S., Sirard, M.A., Dufour, M. & Bailey, J.L. (2000). Sperm calcium levels and chlortetracycline fluorescence patterns are related to the in vivo fertility of cryopreserved bovine semen. *Journal of Andrology*, 21, 938-943.

Correa, M.N., Lucia, T., Bianchi, I., Schmitt, E., Bordignon, J., Rech, D.C., Peruzzo, I.A. & Deschamps, J.C. (2006). Swine semen cooled at 5ºC with PIGPEL-5 extender: effects on semen quality in vitro and fertility estimators in vivo. *Animal Reproduction*, 3, 41-48.

Dott, H.M. & White, I.G. (1964). Effect of potassium on ram spermatozoa studied by a flow dialysis technique. *Journal of Reproduction and Fertility*, 7, 127-134.

Dreher, M.L. & Davenport, A.J. (2013). Hass Avocado composition and potential health effects. *Critical Reviews in Food Science and Nutrition*, 53 (7), 738-750.

Duncan, B.D. (1955). Multiple range test and multiple F-test. *Biometrics*, 11, 1-42.

Gómez, F.S., Sánchez, P.S., Gallego, Iradi, M.G.G., Azman, N.A.M. & Almajano, M.P. (2014). Avocado seeds: Extraction optimization and possible use as antioxidant in food. *Antioxidants*, 3 (2), 439-454.

Hughes, K.J., Mayne, S.T., Blumberg, J.B., Ribaya-Mercardo, J.D., Johnson, E.J. & Cartmel, B. (2009). Plasma carotenoids and biomarkers of oxidative stress in patients with prior head and neck cancer. *Biomarker Insights*, 4, 17-26.

Iritani, A. & Nishikawa, Y. (1961). Studies on the egg-yolk coagulating factors in goat semen: II Properties of the coagulating factor and influential conditions for coagulation. In: Proceedings of Silver Jubilee Lab. Anim. Husbandry, (pp. 97-104) Kyoto University.

Kannan, K. & Jain, S.K. (2004). Effect of vitamin B6 on oxygen radicals, mitochondrial membrane potential, and lipid peroxidation in H2O2-treated U937 monocytes. *Free Radical Biology and Medicine*, 36, 423-8.

Leboeuf, B., Restall, B. & Salomon, S. (2000). Production and storage of goat semen for Artificial insemination. *Animal Reproduction Science*, 62, 113-141.

Mandal, R., Badyakar, D. & Chakrabarty, J. (2014). Role of membrane lipid fatty acids in sperm cryopreservation. *Advances in Andrology*, Article ID 190542, 9 pages, from http://dx.doi.org/10.1155/2014/190542

Mendez, P.O. & Hernandez, G.L. (2007). HDL-C size and composition are modified in the rat by a diet supplementation with "Hass" avocado. *Archivos de cardiología de México*, 77 (1), 17-24.

Niki, E., Yoshida, Y., Saito, Y., & Noguchi, N. (2005). Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochemical and Biophysical Research Communications*, 338, 668-676.

Odu, N.N., Egbo, N.N. & Okonko, I.O. (2012). Assessment of the effect of different preservatives on the shell-life of soymilk stored at different temperatures. *Researcher*, 4 (6). from http://www.sciencepub.net/researcher

Pasqualotto, F.F., Sharma, R.K., Potts, J.M., Nelson, D.R., Thomas, A.J. & Agarwal, A. (2000). Seminal oxidative stress in patients with chronic prostatitis. *Urology*, 55, 881-885.

Pegg, D.E. (2007). Principles of cryopreservation. *Methods in Molecular Biology*, 368, 39-57.

Perumalla AVS, Hettiarachchy NS, 2011: Green tea and grape seed extracts: Potential applications in food safety and quality. *Food Research International*, 44, 827-839.

Pipan, M.Z., Mrkun, J., Kosec, M., Svete, A.N. & Zrimšek, P. (2014). Superoxide dismutase: A predicting factor for boar semen characteristics for short-term preservation. *Biomedical Research International*, 2014, 105280.

Piyali, D., Choudhari, A.R., Singh, A.K. & Ramji, S. (2009). Correlation among routine semen parameters, sperm viability and malondialdehyde levels in human subjects with different fertility potential. *Indian Journal of Physiology and Pharmacology*, 53 (3), 253-258.

Purdy, P.H. (2006). A review on goat sperm cryopreservation. *Small Ruminant Research*, 63, 215-225.

Quinn, P.J., White, I.G. & Wirrick, B.R. (1966). The effect of dilution on the concentration of sodium, potassium, calcium and magnesium in ram and bull spermatozoa. *Journal of Reproduction and Fertility*, 12, 133-138.

Rakha, B.A., Hussain, I., Akhter, S., Ullah, N., Andrabi, S.M. & Ansari, M.S. (2013). Evaluation of tris-citric acid, skim milk and sodium citrate extenders for liquid storage of Punjab Urial (Ovis vignei punjabiensis) spermatozoa. *Reproductive Biology*, 13 (3), 238-42.

Rosatti, M.I., Beconi, M.T. & Córdoba, M. (2004). Proacrosin-acrosin activity in capacitated and acrosome reacted sperm from cryopreserved bovine semen. *Biocell*, 28 (3) Mendoza ago. /dic.

Somanath, P.R. & Gandhi, K.K. (2002). Role of Calcium and Calcium Channels in Progesterone Induced Acrosome Reaction in Caprine Spermatozoa. Asian-Australian Journal *Animal Science*, 15 (7), 949-956.

Srirattana, K., Sripunya, N., Sangmalee, A., Imsoonthornruksa, S., Liang, Y., Ketudat-Cairns, M. & Parnpai, R. (2013). Developmental potential of vitrified goat oocytes following somatic cell nuclear transfer and parthenogenetic activation. *Small Ruminant Research*, 112 (1-3), 141-146.

Stahl, W., Junghans, A., De Boer, B., Driomina, E.S., Briviba, K. & Sies, H. (1998). Carotenoid mixtures protect multilamellar liposomes against oxidative damage: synergistic effects of lycopene and lutein. *FEBS Letters*, 427, 305-308.

Surai, P.F. (2002). *Natural antioxidants in avian nutrition and reproduction.* Nottingham University Press, Nottingham 3, 167.

Thomson, C.A., Stendell-Hollis, N.R., Rock, C.L., Cussier, E.C., Flatt, S.W. & Pierce, J.P. (2007). Plasma and dietary carotenoids are associated with reduced oxidative stress in women previously treated for breast cancer. *Cancer Epidemiology and Biomarkers Preview*, 16 (10), 2008-2015.

Toner, M., Cravalho, E.G., Stachecki, J., Fitzgerald, T., Tompkins, R.G., Yarmush, M.L. & Armant, D.R. (1993). Non-equilibrium freezing of one-cell mouse embryos. Membrane integrity and developmental potential. *Biophysics Journal*, 64, 1908-1921.

USDA. (2011). *Avocado, almond, pistachio and walnut Composition.* Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 24. Washington, DC: U.S. Department of Agriculture.

Vedamurthy, A.B., Padmaa, M.P. & Jogaiah, S. (2015). Antibacterial and antioxidant activities of fractionated persea gratissim seed extracts. *World Journal of Pharmaceutical Sciences*, 4 (06), 480-486.

Vera-Munoz, O., Amirat-Briand, L., Diaz, T., Vasquez, L., Schmidt, E., Desherces, S., Anton, M., Bencharif, D. & Tainturier, D. (2009). Effect of semen dilution to low-sperm number per dose on motility and functionality of cryopreserved bovine spermatozoa using low-density lipoproteins (LDL) extender: Comparison to Triladyl[®] and Bioxcell[®]. *Theriogenology*, 71 (6), 895-900.

Vinha, A.F., Moreira, J. & Barreira, S.V.P. (2013). Physicochemical parameters, phytochemical composition and antioxidant activity of the ALgarvian avocado (Persea Americana Mill.). *Journal of Agricultural Science*, 5(12), 100-109.

Voutilainen, S., Nurmi, T., Mursu, J. & Rissanen, T.H. (2006). Carotenoids and cardiovascular health. *American Journal of Clinical Nutrition*, 83, 1265-1271.

Woodall, A.A., Britton, G. & Jackson, M. (1996). Dietary supplementation with carotenoids: effects on a-tocopherol levels and susceptibility of tissues to oxidative stress. *British Journal of Nutrition*, 76, 307-317.

Wu, X., Beecher, G.R., Holden, J.M., Haytowitz, D.B. & Prior, R.L. (2004). Lipophilic and hydrophilic antioxidant capacity of common foods in the U.S. *Journal of Agriculture and Food Chemistry*, 52, 4026-4037.

Zubair, M., Akbar Lodhi, L., Ahmad, E. & Muhammad, G. (2013). Osmotic swelling test as screening for evaluation of semen of bull. *Journal of Entomology and Zoology Studies*, 1 (6), 124-128. from http://www.entomoljournal.com.

UTICAJI EKSTRAKTA SEMENA AVOKADA U RAZLIČITIM TRIS-RAZREĐIVAČIMA NA INDEKSE SPERME I OKSIDATIVNOG STRESA VITRIFIKOVANIH SPERMATOZOIDA JARCA

Daramola James Olamitibo[1*], Onanuga Oluwakemi Dayo[1], Abioja Monsuru Oladimeji[1], Adeleke Mathew[2], Olowofeso Olajide[2], Oke Oyegunle Emmanuel[1], Adekunle Ezekiel Oluwafemi[1], Sorongbe Taiwo Amidu[1] i Iyanda Olayinka Ayobami[1]

[1]Odsek za fiziologiju životinja, Federalni poljoprivredni univerzitet, Abeokuta, Nigerija
[2]Odsek za oplemenjivanje životinja i genetiku, Federalni poljoprivredni univerzitet, Abeokuta, Nigerija

R e z i m e

Ocenjeni su uticaji ekstrakta semena avokada (engl. *avocado seed extract* – ASE) u različitim tris-razređivačima na indekse sperme i oksidativnog stresa sperme vitrifikovanih spermatozoida zapadno-afričkih patuljastih (engl. *West African Dwarf* – WAD) koza tokom vitrifikacije. Uzorci sperme su razblaženi uz pomoć različitih nivoa (0, 5, 10, 15 i 20 mL/100 mL razređivača) ASE-a u razređivačima tris limunske kiseline (engl. *tris citric acid* – TCA), tris natrijuma fosfata (engl. *tris sodium phosphate* – TSP) i tris natrijuma citrata (engl. *tris sodium citrate* – TSC), dok je tris-razređivač bez limunske kiseline, natrijum fosfata i natrijum citrata služio kao kontrola. Razblaženi uzorci sperme su vitrifikovani i sačuvani. Rezultati su pokazali višu ($P<0,05$) progresivnu pokretljivost sperme u razređivaču TCA pri nivou od 10%, 15% i 20%, i razređivaču TSP pri nivou od 20% ASE-a. Integriteti akrozoma su bili viši ($P<0,05$) u različitim tris-razređivačima pri nivou od 15% i 20% ASE-a. Razređivači TCA, TSP i TSC imali su više ($P<0,05$) integritete membrana pri nivoima od 10%, 15% i 20% ASE-a. Razređivači TSP su imali više ($P<0,05$) živih spermatozoida pri nivou od 20% ASE-a. Razređivači TCA su imali višu reakciju akrozoma, dok su TSP pri nivou od 20% ASE-a i TSC pri nivoima od 15% i 20% ASE-a imali višu kapacitaciju spermatozoida. Razređivač TCA pri nivou od 20% ASE-a imao je smanjene koncentracije malondialdehida (engl. *malondialdehyde* – MDA), dok su više aktivnosti akrozina zapažene pri nivou od 20% ASE-a u razređivaču TCA ($P<0,05$). Ovi rezultati su ukazali da 20% ASE u tris-razređivačima poboljšava indekse sperme i oksidativnog stresa i da su tris-razređivači najbolje rangirani prema sledećem redosledu: TCA > TSP > TSC.

Ključne reči: antioksidant, zamrzavanje, jarčevi, oksidativni stres, razređivač sperme, kvalitet sperme.

---

[*]Autor za kontakt: e-mail: daramolajames2003@yahoo.com