

EFFECTS OF APPLE AND ORANGE JUICES ON QUALITY OF REFRIGERATED GOAT SEMEN

Ezekiel O. Adekunle¹, James O. Daramola¹, Olusiji S. Sowande²,
John A. Abiona¹ and Monsuru O. Abioja^{1*}

¹Department of Animal Physiology,
Federal University of Agriculture, Abeokuta, Nigeria

²Department of Animal Production and Health,
Federal University of Agriculture, Abeokuta, Nigeria

Abstract: This study investigated the effects of apple and orange juices on quality of refrigerated spermatozoa of goat bucks. Semen samples from WAD goat bucks were diluted with Tris-egg yolk extenders each supplemented with apple and orange juices at 0, 2.5, 5, 7.5 and 10/100 ml of diluents. The diluted semen samples were assessed for sperm viability and malondialdehyde (MDA) concentration after *in vitro* storage for 240 hours at 5°C. The ability to maintain sperm motility was higher in the extenders with 7.5% orange juice followed by 10% apple juice compared to other treatments ($P<0.05$). The extenders supplemented with 2.5%, 5% and 7.5% apple juice, and 5% orange juice had higher intact acrosome compared to other treatments and the control ($P<0.05$). The 10% orange juice had higher percentage membrane integrity compared to other treatments. Consistent and reduced ($P<0.05$) MDA levels were observed in the extenders supplemented with fruit juices and lower MDA was observed in the extenders supplemented with 10% apple juice compared to other treatments and the control ($P<0.05$). The findings reveal that additions of the fruit juices to semen extenders to maintain the viability of refrigerated spermatozoa were best at concentrations of 10 ml/100 ml of apple juice and 7.5 ml/100 ml of orange juice.

Key words: antioxidants, bucks, fruit juice, liquid storage, sperm viability.

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. The genetic improvement of goats requires the selection of superior breeding stock and

*Corresponding author: e-mail: dimejiabioja@yahoo.com

the application of an artificial insemination technique. The prospect of artificial insemination in goats generally varies with methods of semen preservation and insemination (Sugulle et al., 2006). In Nigeria, a lack of a reliable method for short-term storage of semen is a limiting factor. Short-term storage with diluents adequate in nutrients for the spermatozoa and a buffer against changes in pH as well as reduced oxygen environment provide a simple means for storage of buck semen. However, during storage, a decline in some semen quality indices such as motility, functional integrity and fertilizing capability of the sperm is a major problem encountered (Vishwanath and Shannon, 2000). One major factor contributing to poor quality semen is seminal oxidative stress caused by lipid peroxidation (Henkel, 2011). Small ruminants usually have a higher concentration of polyunsaturated fatty acids in the sperm membrane compared to other species rendering the sperm susceptible to oxidative stress damage (Henkel, 2011). Sperm cells generally have antioxidants as a defense mechanism against the attack of reactive oxygen species, however, this defense system is inadequate (Saleh et al., 2002). The semen extender supplemented with antioxidants may reduce this effect of reactive oxygen species caused by lipid peroxidation. The addition of antioxidants to the extender has been shown to improve sperm viability of bovine semen (Krzyzosiak et al., 2000; Bilodeau et al., 2002). Vitamins A, C and E have been shown to be potent antioxidants (Breininger et al., 2005; Ondeï et al., 2009). The inclusion of fruit juices from orange (*Citrus sinensis*), cucumber (*Cucumis sativus*) and pineapple (*Ananas comosus*) as constituents of semen extenders has recently been proved to improve the quality of stored spermatozoa (Daramola et al., 2016). Apple (*Malus domestica*) and orange (*Citrus sinensis*) are antioxidant-rich fruits with high levels of these vitamins (Cutler et al., 2008) that probably outweigh other food nutrients in reducing the effect damage by reactive oxygen species. Information on the effects of apple and orange juices in extenders on sperm preservation during refrigeration has not been obtained. The aim of this experiment therefore was to assess the effects of juices of these antioxidant-rich fruits on the quality of refrigerated semen of WAD goat bucks.

Materials and Methods

Location of the experiment and management of animals

The Goat Unit of University Farm, Federal University of Agriculture, Abeokuta, located in the south-west of Nigeria was used for the experiment. Six intact West African Dwarf goat bucks and one matured teaser doe were used for the experiment. The bucks ranged between 4 and 5 years with an average weight of 18kg. The animals were managed intensively and fed with concentrate feed and guinea grass (*Panicum maximum*).

Juice preparation

The procedure of Adeyemo et al. (2007) with some modifications was used to prepare the fruit juice. Washed fresh fruits of apple and orange were first peeled (orange), cut into pieces, seeds removed and thereafter blended for five minutes. The blended fruits were sieved and pressure was applied manually to squeeze the juice from the blended fruits. The juices collected were centrifuged (3000 rpm) for 20 minutes and the supernatant fluid obtained was decanted into a clean beaker and used fresh with a tris-based extender.

Semen collection, dilution and refrigeration

Semen samples collected using an artificial vagina from six WAD goat bucks were pooled and diluted with a Tris-egg yolk based extender consisting of tris-hydroxymethyl-aminomethane (2.42g), citric acid (1.36g), glucose (1g), penicillin (0.028g), egg yolk (20 ml) and distilled water made up 100 ml as the control. Prior to dilution with the semen sample, the extender was supplemented each with apple and orange juice at 2.5, 5.0, 7.5 and 10/100 ml of the diluents respectively. Following dilution, the semen was dispensed into 5 ml tubes, sealed and chilled at 5°C for 240 hours in a refrigerator. The pH of the fruit-juice extenders with the aid of a digital pH meter was determined as follows: control: 7.03, apple juice extender: 7.14 and orange juice extender: 6.98.

Semen evaluation

Sperm motility

Following the procedure described by Bearden and Fuquay (1997), semen samples were kept warmed at 37°C for 3 minutes and accessed for sperm progressive motility by three observers using a Celestron Penta View LCD digital microscope (400x magnification) with a warmed slide (37°C). Motility assessment was carried out at an interval of 24 hours for 240 hours. Estimations were performed for the pooled semen samples in repeated observations using five different slides for each treatment. Sperm abnormality was evaluated with the use of eosin-nigrosin smears under a Celestron Penta View LCD digital microscope (400x magnification) for percentage of defects in the head, mid-piece and tail as described by Bearden and Fuquay (1997).

Acrosome and membrane integrities

Assessment of acrosome integrity was carried out in a formalin citrate solution (96 ml 2.9% sodium citrate and 4 ml 37% formaldehyde) as earlier described

(Ahmad et al., 2003) to identify the acrosome that showed a normal apical ridge of sperm cells using a Celestron Penta View LCD digital microscope (400x magnification). Fructose (9 g) plus sodium citrate (4.9 g) mixed with 1000 ml of distilled water was used as a hypo-osmotic swelling test to assess the membrane integrity of the sperm cells as described by Zubair et al. (2013). This is used to observe swelled spermatozoa that showed a coiled tail as against those with the intact plasma membrane under a Celestron Penta View LCD digital microscope (400x magnification).

Malondialdehyde

At the end of every 24 hours, a thiobarbituric acid reactive substance was used to assess the concentration of malondialdehyde (MDA) in the preserved semen as described by Pipan et al. (2014).

Statistical analysis

Data obtained were subjected to a 2 x 5 x 11 factorial arrangement, using SPSS version 16 and significantly different means were separated by Duncan's multiple range test (Duncan, 1955). The model used is provided below:

$$Y_{ijkl} = \mu + A_i + L_j + T_k + (AL)_{ij} + (AT)_{ik} + (LT)_{jk} + (ALT)_{ijk} + \sum_{ijkl} \quad (1)$$

where,

Y_{ijkl} = Dependent variables,

μ = Population mean,

A_i = Effect due to the i^{th} fruit juices, $i = 1, 2$,

L_j = Effect due to the j^{th} level of inclusion, $j = 0, 2.5, 5, 7.5, 10$,

T_k = Effect due to the k^{th} duration of storage, $k = 0, 24, 48, 72, 96, \dots, 240$,

$(AL)_{ij}$ = Effect due to the ij^{th} interaction between fruit juices and levels of inclusion,

$(AT)_{ik}$ = Effect due to the ik^{th} interaction between fruit juices and refrigeration period,

$(LT)_{jk}$ = Effect due to the jk^{th} interaction between levels of inclusion and refrigeration period,

$(ALT)_{ijk}$ = Effects due to the ijk^{th} interaction between fruit juices, levels of inclusion and refrigeration period, and

\sum_{ijkl} = Experimental error.

Results and Discussion

The effects of apple and orange juices on progressive spermatozoa motility of chilled spermatozoa of WAD goat bucks are presented in Table 1. The results indicate consistently higher ($P < 0.05$) sperm motility in 7.5% and 10% apple juice and 7.5% orange extenders compared to the control group, except 7.5% apple juice

at 216 h of storage. The ability to maintain higher sperm motility was higher in the extenders supplemented with 7.5% orange juice compared to other extenders and the control group ($P < 0.05$).

Table 1. Progressive motility (%) of refrigerated spermatozoa in Tris-egg yolk extenders supplemented with juices.

Duration (h)	Control	Apple (%)				Orange (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
n	5	5	5	5	5	5	5	5	5	
0	94.00 ^a	82.00 ^{bc}	98.00 ^a	86.00 ^b	88.00 ^b	76.00 ^d	98.00 ^a	88.00 ^b	96.00 ^a	4.081
24	74.00 ^{bc}	80.00 ^a	64.00 ^d	82.00 ^a	72.00 ^c	70.00 ^c	78.00 ^b	82.00 ^a	84.00 ^a	4.727
48	62.00 ^d	66.00 ^{bc}	58.00 ^e	70.00 ^b	68.00 ^b	66.00 ^{bc}	58.00 ^f	78.00 ^a	58.00 ^e	4.402
72	54.00 ^d	58.00 ^c	58.00 ^c	66.00 ^b	66.00 ^b	64.00 ^b	54.00 ^d	74.00 ^a	58.00 ^c	4.271
96	54.00 ^d	56.00 ^{bc}	50.00 ^{ed}	58.00 ^b	62.00 ^{ab}	52.00 ^d	50.00 ^{ed}	64.00 ^a	52.00 ^d	4.943
120	54.00 ^b	50.00 ^b	48.00 ^c	62.00 ^a	62.00 ^a	46.00 ^c	42.00 ^d	62.00 ^a	46.00 ^c	5.250
144	52.00 ^c	48.00 ^c	44.00 ^d	58.00 ^b	62.00 ^a	26.00 ^f	30.00 ^f	62.00 ^a	40.00 ^e	5.015
168	42.00 ^b	42.00 ^b	20.00 ^e	40.00 ^{bc}	60.00 ^a	28.00 ^e	24.00 ^e	60.00 ^a	32.00 ^{de}	5.505
192	40.00 ^{cd}	42.00 ^c	16.00 ^f	40.00 ^{cd}	54.00 ^b	26.00 ^e	22.00 ^e	58.00 ^a	26.00 ^e	5.271
216	40.00 ^c	12.00 ^e	6.00 ^f	30.00 ^d	44.00 ^b	10.00 ^e	14.00 ^e	52.00 ^a	10.00 ^e	5.690
240	16.00 ^{de}	10.00 ^e	8.00 ^e	20.00 ^{bc}	26.00 ^b	0.00 ^f	8.00 ^e	34.00 ^a	10.00 ^e	4.872

a, b, c, d, e, f Values within a row with different superscripts differ significantly ($P < 0.05$).

Table 2. Acrosome integrity (%) of refrigerated spermatozoa in Tris-egg yolk extenders supplemented with juices.

Duration (h)	Control	Apple (%)				Orange (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
n	5	5	5	5	5	5	5	5	5	
0	94.50 ^b	97.00 ^a	98.00 ^a	97.50 ^a	98.50 ^a	95.50 ^{ab}	94.00 ^b	98.00 ^a	96.50 ^{ab}	1.390
24	92.50 ^a	90.50 ^{ab}	90.00 ^{ab}	92.00 ^a	89.50 ^b	90.00 ^{ab}	90.50 ^{ab}	93.50 ^a	91.00 ^{ab}	1.095
48	86.00 ^a	77.50 ^c	84.00 ^a	83.50 ^a	85.00 ^a	85.00 ^a	82.00 ^{ab}	86.50 ^a	80.50 ^b	2.525
72	76.00 ^b	72.00 ^c	78.50 ^a	76.00 ^b	76.50 ^b	76.00 ^b	76.00 ^b	80.50 ^a	74.50 ^{bc}	1.878
96	73.00 ^{bc}	69.50 ^{cd}	66.50 ^d	70.00 ^c	86.50 ^a	66.00 ^d	68.00 ^d	74.00 ^b	71.00 ^c	2.628
120	64.00 ^b	61.50 ^c	60.50 ^c	66.00 ^a	66.50 ^a	61.50 ^c	62.00 ^c	62.50 ^c	62.50 ^c	2.466
144	57.50 ^b	53.00 ^d	54.50 ^c	55.5 ^{bc}	59.50 ^a	55.00 ^{bc}	52.00 ^d	55.00 ^{bc}	56.00 ^b	2.568
168	52.00 ^a	47.00 ^c	46.00 ^d	49.00 ^b	49.50 ^b	47.50 ^c	46.50 ^{cd}	46.00 ^d	44.50 ^d	2.666
192	40.00 ^c	44.50 ^a	41.00 ^b	41.00 ^b	38.50 ^c	39.50 ^c	41.50 ^b	36.25 ^d	34.00 ^c	2.856
216	32.00 ^d	39.00 ^a	35.00 ^b	36.50 ^b	33.50 ^{bc}	33.50 ^{bc}	34.00 ^{bc}	32.50 ^d	30.00 ^c	2.090
240	28.00 ^c	35.00 ^a	30.25 ^b	32.00 ^b	27.00 ^c	28.50 ^c	30.00 ^b	27.50 ^c	25.50 ^d	2.150

a, b, c, d, e Values within rows with different superscripts differ significantly ($P < 0.05$).

The effects of apple and orange juices on the intact acrosome of chilled spermatozoa of WAD goat bucks are presented in Table 2. The values obtained for the intact acrosome did not follow a particular pattern. However, higher values of the intact acrosome were obtained when the extender was supplemented with 7.5% orange juice at 24, 48, and 72 hours. Apple juice, however, improved the intact acrosome when the extender was supplemented with 10% at 96, 120, 144 and 168 hours. The extenders supplemented with the juices moreover had better intact acrosome after post-chilling at 240 hours of storage following the addition of 2.5%, 5% and 7.5% apple juice, and 5% orange juice compared to other treatments and the control ($P<0.05$).

The effects of apple and orange juices on the percentage membrane integrity of chilled spermatozoa of WAD goat bucks are presented in Table 3. Consistently higher values of percentage membrane integrity in 2.5% and 10% apple juice extenders during the 240 hours of storage were observed while consistently higher values of the percentage membrane integrity in the extender supplemented with all levels of orange juice except at 2.5% were observed after 48 up to 240 hours of storage compared to the control group.

Table 3. Membrane integrity (%) of refrigerated spermatozoa in Tris-egg yolk extenders supplemented with juices.

Duration (h)	Control	Apple (%)				Orange (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
n	5	5	5	5	5	5	5	5	5	
0	92.00 ^c	96.00 ^a	94.50 ^b	97.50 ^a	94.50 ^b	94.00 ^b	95.00 ^{ab}	94.50 ^b	95.00 ^{ab}	1.270
24	80.00 ^d	86.00 ^b	80.50 ^d	89.50 ^a	83.00 ^c	78.50 ^d	79.00 ^d	77.00 ^d	80.50 ^d	4.283
48	71.00 ^e	79.50 ^b	72.00 ^d	72.50 ^d	81.00 ^a	69.50 ^e	73.50 ^d	74.50 ^{cd}	76.50 ^c	3.696
72	67.00 ^e	77.00 ^a	61.50 ^f	69.00 ^d	75.00 ^{ab}	62.50 ^f	70.50 ^c	71.50 ^c	73.00 ^b	4.186
96	61.00 ^d	66.50 ^{bc}	56.50 ^e	59.00 ^d	71.00 ^a	61.00 ^d	68.00 ^b	67.50 ^b	70.00 ^a	3.765
120	52.00 ^e	61.00 ^d	52.00 ^e	54.50 ^e	67.50 ^a	53.00 ^e	63.00 ^c	60.00 ^d	65.00 ^b	3.606
144	49.50 ^d	54.00 ^c	46.00 ^{de}	47.00 ^{de}	59.50 ^a	51.50 ^c	57.50 ^{ab}	53.50 ^c	60.00 ^a	2.920
168	44.00 ^c	48.50 ^b	40.00 ^d	44.00 ^c	52.50 ^a	45.00 ^c	53.50 ^a	42.00 ^{cd}	52.00 ^a	2.928
192	38.00 ^e	39.50 ^d	37.50 ^e	38.00 ^e	45.50 ^b	41.50 ^c	45.50 ^b	39.50 ^d	47.00 ^a	2.022
216	32.50 ^d	35.00 ^c	31.00 ^d	34.00 ^c	35.50 ^c	36.00 ^c	40.00 ^a	33.50 ^{cd}	38.50 ^b	1.788
240	27.50 ^d	30.50 ^c	26.50 ^{de}	30.50 ^c	33.00 ^b	33.00 ^b	36.50 ^a	30.50 ^c	33.50 ^b	1.907

a, b, c, d, e, f Values within rows with different superscripts differ significantly ($P<0.05$).

The effects of apple and orange juices on percentage abnormality of chilled spermatozoa of WAD goat bucks are presented in Table 4. The results show a consistently lower percentage abnormality in the extenders supplemented with 7.5% orange and 10% apple juices compared to other inclusion levels and the control group ($P<0.05$).

Table 4. Abnormality (%) of refrigerated spermatozoa in Tris-egg yolk extenders supplemented with juices.

Duration (h)	Control	Apple (%)				Orange (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
n	5	5	5	5	5	5	5	5	5	
0	0.-0.30 ^c	0.83 ^b	0.00 ^e	0.43 ^c	0.33 ^c	1.00 ^a	0.15 ^d	0.30 ^c	0.15 ^d	1.940
24	1.-1.38 ^b	1.13 ^b	1.45 ^a	0.63 ^c	1.30 ^b	1.38 ^b	1.00 ^b	0.70 ^c	0.58 ^c	2.605
48	2.15 ^a	2.28 ^a	2.10 ^a	1.50 ^b	1.85 ^b	1.65 ^b	2.30 ^a	1.10 ^c	1.70 ^b	3.278
72	2.83 ^a	2.90 ^a	2.48 ^a	1.70 ^c	2.18 ^b	1.98 ^{bc}	2.85 ^a	1.43 ^d	2.00 ^b	3.305
96	3-3.20 ^a	3.30 ^a	3.13 ^a	2.10 ^c	2.25 ^c	2.95 ^b	2.80 ^b	1.73 ^d	4-2.48 ^b	2-2.833
120	3.40 ^b	4.00 ^a	3.25 ^b	2.75 ^c	2.90 ^c	3.40 ^b	3.33 ^b	2.53 ^c	2.88 ^c	3.183
144	3.63 ^b	4.50 ^a	3.33 ^c	3.18 ^c	3.28 ^c	4.90 ^a	4.85 ^a	2.88 ^d	3.60 ^b	3.913
168	4.-4.18 ^d	5.08 ^b	5.63 ^a	4.65 ^c	3.58 ^e	4.93 ^c	5.68 ^a	3.40 ^e	4.23 ^d	4.197
192	4.-4.98 ^{cd}	5.30 ^c	6.20 ^a	4.93 ^{cd}	4.30 ^e	5.63 ^b	6.30 ^a	3.68 ^e	4.88 ^{cd}	3.848
216	5.-5.05 ^d	7.65 ^a	7.40 ^{ab}	5.50 ^c	4.38 ^e	6.30 ^c	6.88 ^b	3.68 ^e	6.-6.65 ^b	5.-5.555
240	6.-6.93 ^c	8.08 ^a	7.90 ^a	6.80 ^c	6.13 ^d	7.65 ^{ab}	7.65 ^{ab}	5.50 ^e	7.18 ^c	4.982

a, b, c, d, e Values within rows with different superscripts differ significantly (P<0.05).

Generally, consistently lower (P<0.05) levels of MDA were recorded in the extenders supplemented with fruit juices compared to the control except at 10% at 48 and 192 hours (Table 5). The lower MDA was observed in the extenders supplemented with 10% apple juice compared to other treatments and the control (P<0.05).

Table 5. MDA (nmol/mL) levels of refrigerated spermatozoa in Tris-egg yolk extenders supplemented with juices.

Duration (h)	Control	Apple (%)				Orange (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
n	5	5	5	5	5	5	5	5	5	
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.000
24	0.-0.02 ^c	0.02 ^c	0.03 ^b	0.01 ^d	0.01 ^d	0.02 ^c	0.02 ^c	0.05 ^a	0.03 ^b	0.023
48	0.06 ^a	0.05 ^b	0.05 ^b	0.04 ^c	0.03 ^d	0.05 ^b	0.04 ^c	0.06 ^a	0.06 ^a	0.055
72	0.10 ^a	0.07 ^c	0.07 ^c	0.07 ^c	0.05 ^e	0.06 ^d	0.06 ^d	0.07 ^c	0.09 ^b	0.045
96	0.15 ^a	0.08 ^d	0.09 ^c	0.09 ^c	0.06 ^e	0.10 ^b	0.09 ^c	0.12 ^b	0.10 ^b	0.042
120	0.16 ^a	0.10 ^b	0.12 ^b	0.11 ^b	0.08 ^c	0.13 ^b	0.09 ^c	0.13 ^b	0.12 ^b	0.038
144	0.17 ^a	0.13 ^{cd}	0.14 ^{bc}	0.14 ^c	0.09 ^e	0.15 ^b	0.12 ^d	0.14 ^{bc}	0.14 ^{bc}	0.055
168	1.18 ^a	0.14 ^d	0.15 ^{cd}	0.16 ^c	0.12 ^e	0.16 ^c	0.13 ^{de}	0.17 ^b	0.15 ^{cd}	0.069
192	0.20 ^a	0.15 ^d	0.17 ^c	0.18 ^{bc}	0.14 ^d	0.18 ^{bc}	0.18 ^{bc}	0.19 ^b	0.20 ^a	0.058
216	0.24 ^a	0.17 ^{de}	0.20 ^c	0.19 ^d	0.17 ^{de}	0.22 ^b	0.20 ^c	0.20 ^c	0.22 ^b	0.041
240	0.25 ^a	0.19 ^{de}	0.23 ^c	0.21 ^d	0.22 ^d	0.26 ^a	0.24 ^b	0.22 ^d	0.24 ^b	0.054

a, b, c, d, e Values within rows with different superscripts differ significantly (P<0.05).

The improved sperm viability that accompanied supplementation of semen extenders with apple and orange juices in this experiment has revealed that the fruit juices have the ability to maintain motility and this might be on account of their excellent source of antioxidants such as vitamins C and E present in these fruits (Mermeistein, 1999; Djuric and Powell, 2001; Gebhardt and Thomas, 2002; Martin et al., 2002). The findings agree with Ball et al. (2001), Reza et al. (2011) and Adikwu and Deo (2013) that vitamin E or C additions in preserved semen improved sperm motility. Vitamins C and E as antioxidants are known to eliminate superoxide anions and singlet oxygen and protect lipoproteins from peroxidative damage (Michael et al., 2008; Swaran and Flora, 2009). The results of this experiment agree with the previous works which have revealed that vitamin C exerted an antioxidative effect during freezing and thawing of bovine sperm (Beconi et al., 1993; Mermeistein, 1999). This, however, contradicts Aurichet et al. (1997), who reported that the addition of ascorbic acid did not improve motility of cooled equine spermatozoa during the 96-h storage period. Besides the vitamin C content in the fruits, the antioxidant properties of phenolic compounds present in these fruits might also be implicated in the improved motility observed (Spanos and Wrolstad, 2004). The important phenolic compound in orange juice is ferulic acid (Augustin and Williams, 2000) that acts synergistically with other antioxidants to reduce an adverse effect of free radicals on the external and internal membranes of cells (Zuo et al., 2002). The vitamins and phenolic compounds present in the juices could therefore be linked with progressive motility observed at various inclusions of juices (Mullen et al., 2007; USDA, 2009). Moreover, in the present study, the level of fruit juices seemed to be optimal for maintaining buck sperm motility, as effects of apple and orange juices varied with the level of supplementation in the extenders; namely, at higher concentrations, sperm motility was maintained and better preserved in the extenders with the addition of 10% and 7.5% apple and orange juices respectively. The juices seemed to play the role of oxidants at low levels and of antioxidants at higher levels. Apple juice, however, surpassed orange juice as it maintained sperm motility better at the higher level of supplementation. Previous studies, however, have shown that a stimulating effect of vitamin E on oxidation is more pronounced at higher concentrations while vitamin C is known to be effective as an oxidant at low concentrations (Breininger et al., 2005; Mullen et al., 2007).

Worthy of note in this experiment is that the addition of fruit juices to Tris-egg yolk extenders did not only improved sperm motility, but also maintained acrosome integrity and membrane integrity. In addition, the fruit juice extenders also reduced both percentage abnormality and MDA concentration in the chilled semen. A beneficial effect of antioxidants on intact acrosome and membrane integrities in the extenders with the addition of the fruit juices during 240 hours of refrigeration compared to the control group could possibly be linked to

vitamin C and other antioxidants in these juices (Mermeistein, 1999; Spanos and Wrolstad, 2004). The results of the present experiment therefore indicate that the combined action of these vitamins and phenolic compounds in the juices improved sperm parameters in the extenders supplemented with the fruit juices. The findings have corroborated the previous findings that observed a beneficial effect of ferulic acid on sperm viability and reduced lipid peroxidative damage to sperm membranes (Zheng and Zhang, 1997). The antioxidant ability of ferulic acid is linked to its structural characteristics (Kheradmand and Babaei, 2006; Marimuthu et al., 2007). Moreover, it is the synergistic activity of vitamin C, vitamin E and other antioxidants that stimulate the protective effects against lipid peroxidation and preservation of cell membrane integrity (Donoghue and Wishart, 2000; Rahman, 2007).

The lower level of MDA recorded in the fruit juice extenders compared to the control could probably be on account of flavonoids and ferulic acid in these fruits. This study agrees with the findings of Zheng and Zhang (1997) that orange and apple juice constituents particularly vitamin C, flavonoids and ferulic acid suppressed a damaging effect of lipid peroxidation during liquid storage of rooster's semen. The reduction in MDA agrees with the previous reports (Aurich et al., 1997; Arabi and Seidaie, 2008) that vitamin C protected the sperm cells from endogenous oxidative DNA and membrane damages. Fruits generally are rich in flavonoids, ferulic acid and vitamin C, which makes apple and orange juices potentially good sources of antioxidants for semen preservation. Moreover, major activities of antioxidants in fruits are from phenolic compounds (Cao et al., 1997). Therefore, the present study suggests that phenolics could play a vital role in antioxidant properties in addition to other hydroxycinnamic derivatives such as dicaffeoylquinic and chlorogenic acids in the fruits (Zhang and Hamauzu, 2004). This information further indicates that all improvements in semen parameters when treated with the fruit juices were linked to flavonoids and ferulic acid found in the juices in addition to vitamin C; and they acted synergistically to protect sperm cells from lipid peroxidation during refrigeration. The results of the present study therefore support the Fenton reaction (O'Flaherty et al., 2003) that antioxidants influence the removal of hydrogen peroxide to produce hydroxyl radicals. This effect explains the improved motility, acrosome and membrane integrities, reduced abnormality and MDA concentration when apple and orange juices were added to the extenders.

Conclusion

The findings indicate that the additions of the fruit juices to semen extenders were the most suitable and best at 10 ml/100 ml and 7.5 ml/100 ml of apple juice and orange juice respectively in maintaining sperm motility of buck spermatozoa

during liquid storage. The present results indicate that the additions of apple and orange juices to semen extenders are suitable agents for preserving semen quality in cold storage for up to 240 hours.

Acknowledgements

The authors are grateful to the Head of the Department of Animal Physiology and the laboratory technologists for granting us permission to use the laboratory.

References

- Adeyemo, O.K., Adeyemo, O.A., Oyeyemi, M.O., & Agbede, S.A. (2007). Effect of semen extenders on the motility and viability of stored African Catfish (*Clarias gariepinus*) spermatozoa. *Journal of Applied Science & Environmental Management*, 11 (1), 13-16.
- Adikwu, E., & Deo, O. (2013). Hepatoprotective effect of vitamin C (Ascorbic Acid). *Pharmacology and Pharmacy*, 4 (1), 84-92.
- Ahmad, Z., Anzar, M., Shahab, M., Ahmad, N., & Andrabi, S.M.H. (2003). Sephadex and sephadex ion-exchange filtration improves the quality and freezability of low-grade buffalo semen ejaculates. *Theriogenology*, 59 (5-6), 1189-202.
- 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 Journal Shahrekord*, 2, 39-46.
- Augustin, S., & Williams, G. (2000). Dietary intake and bioavailability of polyphenols. *Journal of Nutrition*, 130, 2073-2085.
- Aurich, J.E., Schönherr, U., Hoppe, H., & Aurich, C. (1997). Effects of antioxidants on motility and membrane integrity of chilled-stored stallion semen. *Theriogenology*, 48 (2), 185-92.
- Ball, B.A., Medina, V., Gravance, C.G., & Baumbe, J. (2001). Effect of antioxidants on preservation of motility, viability and acrosomal integrity of equine spermatozoa during storage at 5 degrees C. *Theriogenology*, 56 (4), 577-89.
- Bearden, H.J., & Fuquay, J.W. (1997). Semen Evaluation. In *Applied Animal Reproduction*, 4th ed., (pp. 158-169). New Jersey: Prentice Hall.
- Beconi, M.T., Francia, C.R., Mora, N.G., & Affranchino, M.A. (1993). Effect of natural antioxidants on frozen bovine semen preservation. *Theriogenology*, 40 (4), 841-51.
- Bilodeau, J.F., Blanchette, S., Cormier, N., & Sirad, M.A. (2002). Reactive oxygen species-mediated loss of bovine sperm motility in egg yolk Tris-extender: Protection by pyruvate, metal chelators and bovine liver or oviductal fluid catalase. *Theriogenology*, 57, 1105-1112.
- Breining, E., Beorlegui, N.B., Flaherty, C.O.M., & Beconi, M.T. (2005). Alpha-tocopherol improves biochemical and dynamic parameters in cryopreserved boar semen. *Theriogenology*, 63 (8), 2126-35.
- Cao, G., Sofic, E., & Prior, R.L. (1997). Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radicals Biology and Medicine*, 22, 749-760.
- Cutler, G.J., Nettleton, J.A., Ross, J.A., Harnack, L.J., Jacobs, D.R., Scrafford, C.G., Robien, K. (2008). Dietary flavonoid intake and risk of cancer in postmenopausal women: The Iowa Women's Health Study. *International Journal of Cancer*, 123 (3), 664-71.
- Daramola, J.O., Adekunle, E.O., Onagbesan, O.M., Oke, O.E., Ladokun, A.O., Abiona, J.A., Adeleke, M.A. (2016). Protective effects of fruit-juices on sperm viability of West African Dwarf goat bucks during cryopreservation. *Animal Reproduction*, 13 (1), 7-13.
- Djuric, Z., & Powell, L.C. (2001). Antioxidant capacity of lycopene-containing foods. *International Journal of Food Science and Nutrition*, 52, 143-149.

- Donoghuea, A.M., Wishart, G.J., Jungnickel, M.K., Molinia, F.C., Harman, A.J., & Rodger, J.C. (2000). Storage of poultry semen. *Animal Reproduction Science*, 62 (1-3), 213-32.
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, 11 (1), 1-42.
- Food and Agriculture Organization (FAO). (2006). A system of integrated agricultural censuses and surveys, volume 1, World programme for the census of Agriculture. In Statistical Development Series. No 11.
- Gebhardt, S.E., & Thomas, R.G. (2002). Nutritive value of foods. US Department of Agriculture, Agriculture Research Service. Home and Garden Bulletin, 72,
- Henkel, R.R. (2011). Leukocytes and oxidative stress: Dilemma for sperm function and male fertility. *Asian Journal of Andrology*, 13 (1), 43-52.
- Kheradmand, A., & Babaei, H. (2006). Comparative evaluation of the effects of antioxidants on the chilled-stored ram semen. *Iran Journal of Veterinary Research*, 17 (4), 40-45.
- Krzyzosiak, J., Evenson, D., Pitt, C., Jost, L., Molan, P., & Vishwanath, R. (2000). Changes in susceptibility of bovine sperm to in situ DNA denaturation during prolonged incubation at ambient temperature under conditions of exposure to reactive oxygen species and nuclease inhibitor. *Reproduction, Fertility and Development*, 12 (6), 251-261.
- Srinivasan, M., Sudheer, A.R., & Menon, V.P. (2007). Recent advances in Indian herbal drug research guest editor: Thomas Paul Asir Devasagayam Ferulic Acid: Therapeutic potential through its antioxidant property. *Journal of Clinical Biochemistry and Nutrition*, 40 (2), 92-100.
- Martin, A., Cherubini, A., Anders-Lacueva, A., Paniagua, C., & Joseph, J.A. (2002). Effects of fruits and vegetable on levels of vitamin E and C in the brain and their association with cognitive performance. *Journal of Nutrition Health and Aging*, 6, 392-404.
- Mermeistein, N.H. (1999). High-pressure pasteurization of juice. *Food Technology*, 53, 86-90.
- Michael, A.J., Alexopoulos, C., Pontiki, E.A., Hadjipavlou-Litina, D.J., Saratsis, P., Ververidis, H.N., Rawlings, N.C. (2008). Quality and reactive oxygen species of extended canine semen after vitamin C supplementation. *Theriogenology*, 70 (5), 827-35.
- Mullen, W., Marks, S.C., & Crozier, A. (2007). Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *Journal of Agricultural and Food Chemistry*, 55 (8), 3148-57.
- O'Flaherty, C., Beorlegui, N., & Beconi, M.T. (2003). Participation of superoxide anion in the capacitation of cryopreserved bovine sperm. *International Journal of Andrology*, 26 (2), 109-114.
- Ondei, L.S., Silveira, L.M., Leite, A.A., Souza, D.R.S., Pinhel, A.A.S., Percário, S., Bonini-Domingos, C.R. (2009). Lipid peroxidation and antioxidant capacity of G6PD-deficient patients with A-(202G>A) mutation. *Genetic & Molecular Research*, 8 (4), 1345-1351.
- 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*, 105280, 1-8.
- Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging*, 2 (2), 219-36.
- Reza, A., Razi, J., & Hossein, T. (2011). Influence of Added Vitamin C and Vitamin E on Frozen-Thawed Bovine Sperm Cryopreserved in Citrate and Tris-Based Extenders. *Veterinary Research Forum*, 2 (1), 37-44.
- Saleh, R.A., Agarwal, A., Kandirali, E., Sharma, R.K., Thomas, A.J., Nada, E.A., Alvarez, J.G. (2002). Leukocytospermia is associated with increased reactive oxygen species production by human spermatozoa. *Fertility and Sterility*, 78 (6), 1215-24.
- Spanos, G.A., & Wrolstad, R.E. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition*, 79 (5), 727-747.
- Sugulle, A.H., Bhuiyan, M.M.U., & Shamsuddin, M. (2006). Breeding soundness of bulls and the spermatozoa from three strains of mice. *Cryobiology*, 35, 255-269.
- Flora, S.J. (2009). Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxidative Medicine and Cellular Longevity*, 2 (4), 191-206.

- U.S. Department of Agriculture (USDA). (2009). USDA National Nutrient Database for Standard Reference. Nutrient Data Laboratory. Release 22. Retrieved from <http://www.nal.usda.gov/fnic/foodcomp/search/>
- Vishwanath, R., Shannon, P., Hammon, D.S., Wang, S., Holyoak, G.R., Gomez, E., & Diez, C. (2000). Storage of bovine semen in liquid and frozen state. *Animal Reproduction Science*, 62 (1-3), 23-53.
- Zhang, D.L., & Hamazu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry*, 88 (4), 503-509.
- Zheng, R.L., & Zhang, D.L. (1997). Effects of ferulic acid on fertile and asthenozoospermic infertile human sperm motility, viability, lipid peroxidation, and cyclic nucleotides. *Free Radical Biology and Medicine*, 22 (4), 58.
- Zubair, M., Lodhi, A.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.
- Zuo, Y., Wang, C., & Zhan, J. (2002). Separation, characterization, and quantitation of benzoic and phenolic antioxidants in American cranberry fruit by GC-MS. *Journal of Agricultural and Food Chemistry*, 50 (13), 3789-94.

Received: February 6, 2017

Accepted: December 28, 2017

UTICAJI SOKOVA OD JABUKE I POMORANDŽE NA KVALITET
RASHLAĐENE SPERME JARČEVA

Ezekiel O. Adekunle¹, James O. Daramola¹, Olusiji S. Sowande²,
John A. Abiona¹ i Monsuru O. Abioja^{1*}

¹Odsek za fiziologiju životinja,
Federalni poljoprivredni univerzitet, Abeokuta, Nigerija
²Odsek za proizvodnju i zdravlje životinja,
Federalni poljoprivredni univerzitet, Abeokuta, Nigerija

R e z i m e

Ovim istraživanjem se proučavaju uticaji sokova od jabuke i pomorandže na kvalitet rashlađenih spermatozoida jarčeva. Uzorci sperme zapadnoafričkih patuljastih (engl. *West African Dwarf* – WAD) jarčeva razređeni su tris-razređivačima na bazi žumanceta jajeta, pri čemu je svaki od njih dopunjen sokovima od jabuke i pomorandže u količinama 0, 2,5, 5, 7,5 i 10/100 ml razređivača. Procenjena je životna sposobnost sperme i koncentracija malondialdehida (MDA) uzoraka razređene sperme posle skladištenja *in vitro* tokom 240 sati na 5°C. Sposobnost da se održi pokretljivost spermatozoida bila je viša kod razređivača sa 7,5% sokom od pomorandže, a zatim je sledio razređivač sa 10% sokom od jabuke u poređenju sa drugim tretmanima ($P < 0,05$). Razređivači u kojima je dodat 2,5%, 5% i 7,5% sok od jabuke, i 5% sok od pomorandže imali su viši netaknuti akrozom u poređenju sa drugim tretmanima i kontrolom ($P < 0,05$). Višeprocentni integritet membrane uočen je pri dodavanju 10% soka od pomorandže u poređenju sa drugim tretmanima. Dosledni i smanjeni ($P < 0,05$) nivoi MDA uočeni su kod razređivača dopunjenim voćnim sokovima, a niži nivoi MDA su zabeleženi u razređivačima dopunjenim 10% sokom od jabuke u poređenju sa drugim tretmanima i kontrolom ($P < 0,05$). Rezultati pokazuju da su dodavanja voćnih sokova u razređivače sperme kako bi se održala životna sposobnost spermatozoida bila najbolja pri koncentracijama 10 ml/100 ml soka od jabuke i 7,5 ml/100 ml soka od pomorandže.

Ključne reči: antioksidansi, jarčevi, voćni sok, skladištenje tečnosti, životna sposobnost sperme.

Primljeno: 6. februara 2017.
Odobreno: 28. decembra 2017.

* Autor za kontakt: e-mail: dimejiabioja@yahoo.com