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Preservative Effects of Pineapple and Cucumber Juices on Viability of Refrigerated Spermatozoa of West African Dwarf Bucks

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ABSTRACT

This study investigated the preservative effects of pineapple and cucumber juices on the viability of refrigerated spermatozoa of the West African Dwarf (WAD) bucks. Pooled semen from WAD bucks was diluted in Tris-egg yolk extenders containing pineapple and cucumber juices each at 2.5, 5, 7.5 and 10ml/100ml respectively. Microscopic assessments of diluted semen samples were carried out on sperm progressive motility, acrosome and membrane integrities and sperm abnormality after *in vitro* storage at 5^{o}C for 240 hours. The concentration of malondialdehyde (MDA) in the stored semen was measured in thiobarbituric acid reactive substances. The results showed higher ($P<0.05$) sperm progressive motility in extenders supplemented with pineapple and cucumber juices compared to the control. The extenders supplemented with pineapple and cucumber juices had consistent higher ($P<0.05$) acrosome integrity up to 48 hours of post-chilling compared to the control. Higher ($P<0.05$) membrane integrity was obtained in extenders supplemented with fruit juices compared to the control and improved results were obtained in 2.5% pineapple, 2.5% and 5% cucumber fruit juices. The extenders supplemented with 2.5% and 5% cucumber juice had lower ($P<0.05$) abnormality compared to the control The results showed that extenders supplemented with fruit juices had lower ($P<0.05$) MDA concentrations and improved results were obtained at 2.5% and 5% pineapple and 2.5% cucumber fruit juices. The findings indicate preservative potential of pineapple and cucumber juices on sperm viability of chilled semen stored at 5^{o}C.

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INTRODUCTION

Mammalian sperm cells contain a high proportion of polyunsaturated fatty acids, and are susceptible to damage by excessive reactive oxygen species (ROS) causing sperm cells to deteriorate rapidly (Aitken & Fisher, 1994). When semen is stored at 5°C, there is a gradual decline in motility, functional integrity of sperm membranes and fertility (Maxwell & Salamon, 1993; De Lamirande *et al*., 1997). Antioxidants are linked with sperm viability because of their preservative effects against cell damage during preservation (Tai-Wing *et al*., 1991). Goat spermatozoa, like spermatozoa of other mammals, normally contain antioxidants but this endogenous antioxidative capacity may however be insufficient to prevent lipid peroxidation during prolonged storage (Aurich *et al*., 1997). Thus, mammalian spermatozoa lack a significant cytoplasmic component, which contains antioxidants that counteract the damaging effects of reactive oxygen species and lipid peroxidation (Storey, 2007).

Krzyosiak *et al*. (2000) reported that the addition of antioxidants to semen extender improved sperm motility and viability of bovine semen. Natural foods and food-derived antioxidants such as vitamin C and phenolic phytochemicals have received growing attention because they are known to function as chemopreventive agents against oxidative damage (Kiwon *et al*., 2003). Gardner *et al*. (2000) studying the relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices, including orange, grapefruit, pink grapefruit, apple, pineapple and vegetable-juices, found that both vitamin concentrations and total phenolic contents strongly correlated with antioxidant capacity. Improved survival rate of spermatozoa of African Catfish (*Clarias gariepinus*) extended with tomato juice when stored at 5°C (Adeyemo *et al*., 2007) has been observed. In addition, protective effect of orange juice on spermatozoa against the harmful effects of lipid peroxidation of white layer cocks' semen stored for up to 72 hours (Al-Daraji HJ, 2012) has been reported. Information on effects of pineapple and cucumber juices in extender for preserving mammalian spermatozoa is, however, not available in the literature. The objective of this study, therefore, was to determine the preservative effects of these fruit-rich antioxidants on sperm viability of WAD bucks during cold storage.

MATERIALS AND METHODS

Experimental Site and Animals

The experiment was carried out at the Goat Unit of the Directorate of University Farm, Federal University of Agriculture, Abeokuta, Nigeria located at 7^0 10'N and 3^0 2'E. It lies in the south-western part of Nigeria and has a prevailing tropical climate, a mean annual rainfall of 1,037mm and average temperature of 34.7°C. Six intact WAD bucks ranging from 2.5-3 years of age were used for this study. The animals were kept under intensive management and maintained under a uniform nutritional regimen with concentrate feed supplemented with guinea grass (*Panicum maximum*).

Preparation of Fruit Juices

The fruit juice was prepared according to the procedure of Adeyemo *et al*. (2007). Fresh ripe pineapple was washed thoroughly using distilled water. The pineapple was peeled, cut into pieces, blended for 5 minutes and placed in a sieve and pressure was applied manually to squeeze the juice out from the blended pineapple. In the case of cucumber, fully ripe cucumber was washed thoroughly using distilled water, peeled, cut into pieces and seeds were removed. Little pressure was applied to squeeze out the juice. The juices from pineapple and cucumber were separately collected each into plastic test tubes and centrifuged at 3000rpm for 20 minutes. The supernatant fluid was thereafter decanted into a clean beaker and used immediately for the experiment.

Semen Collection, Dilution and Storage

Semen samples were collected from six WAD bucks with the aid of an artificial vagina. Only ejaculates showing >80% motility were pooled. Pooled semen samples (each pool originating from six bucks) were used. Semen samples were pooled for uniformity and to eliminate individual differences. The extender for the treatment used in this study consisting of Tris-hydroxymethyl-aminomethane (2.42 g), citric acid (1.35 g), glucose (1 g), penicillin (0.028 g), egg yolk (20 ml) and distilled water made up 100ml. The pooled fresh semen was then split into nine equal fractions in different test tubes and diluted with the extenders supplemented with cucumber and pineapple juices at 2.5, 5, 7.5 and 10ml/100 ml of the diluents respectively and the control (no juice) at a final concentration of 235 x 10^6sperm/ml. The pH of the extenders (control: 7.03; pineapple juice: 7.14 and cucumber juice: 6.98) was determined using a digital pH meter. Following dilution, the diluted semen samples were sealed and chilled from 37^{o}C to 5^{o}C at approximately 0.5 ^{o}C/min and maintained at this temperature in a refrigerator for 240 hours and thereafter evaluated for sperm quality characteristics.

Semen Evaluation

Sperm progressive motility. Sperm motility was determined as described by Bearden and Fuquay (1997). Briefly, semen was thawed in Clifton Water bath (Model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) at 37^{o}C and accessed for sperm motility using Celestron PentaView compound microscope (LCD-44348 by RoHS, China) at 400 x magnification. A 5µl sample of semen was placed directly on a heated microscope slide and overlaid with a 22 x 22 mm cover slip. For each sample, five microscopic fields were examined for observe progressive sperm motility by three observers and the mean of the five successive evaluations was recorded as the final motility score.

Acrosomes integrity. The percentage of spermatozoa with intact acrosomes was determined according to Ahmad *et al*. (2003) and Ahmad *et al*. (2014). Briefly, 50µl of each semen sample was added to a 500-µl formalin citrate solution (96 ml 2.9% sodium citrate, with 4ml 37% formaldehyde) and mixed carefully. 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 a Celestron PentaView LCD compound microscope (400 x magnification). Intactness of acrosome characterised by normal apical ridge of 200 spermatozoa were observed and recorded.

Membrane integrity. Hypo-osmotic swelling test (HOST) was used to determine sperm membrane integrity (Jeyengran *et al*., 1884). This was done by incubating 10µl semen in 100µl Hypo-osmotic solution (7.35g sodium citrate [0.0285M] and 13.5g fructose [0.075M]) at 37^{o}C for 30 minutes; 0.1ml of the mixture was spread over a cover slip warm slide and observed under a Celestron PentaView LCD compound microscope (400 x magnification). Two hundred spermatozoa were counted and the percentage of spermatozoa positive to HOST for their swelling characterised by curled tails, indicating intact plasma membrane was determined and those with no swelling characterised by uncurled tails were classified as spermatozoa with abnormal membrane integrity.

Abnormality. Sperm morphological abnormalities were determined as described by Bearden and Fuquay (1997) with the use of eosin-nigrosin smears. Briefly, a thin smear of mixture of semen and eosin-nigrosin solution was drawn across the slide and dried. The percentage of morphologically abnormal spermatozoa with defects in the head, midpiece and tail were observed under a Celestron PentaView LCD compound microscope (400 x magnification).

MDA concentrations. At the end of every 24 hours, the levels of malondialdehyde (MDA) as indices of lipid peroxidation in the stored semen were measured in a thiobarbituric acid reactive substance (TBARS) according to Buege and Steven (1978), Armstrong and Browne (1994) and Yagi (1998). For this assay, 0.1ml of sperm suspension was incubated with 0.1ml of 150mM Tris-HCl (pH 7.1) for 20 minutes at 37°C. Subsequently, 1ml of 10% trichloroacetic acid (TCA) and 2ml of 0.375% thiobarbituric acid was added followed by incubation in a boiling water bath for 30 minutes. Thereafter, it was centrifuged for 15 minutes at 3000 rpm inside the blank tube and the absorbance was read with a spectrophotometer at 532nm. The concentration of MDA was calculated as follows: The concentration of malondialdehyde MDA (nmol/ml) = AT – AB/1.56 × 10^5; where: AT = the absorbance of the sample serum, AB = the absorbance of the blank, 1.56 × 10^5 molar absorptivity of MDA.

Statistical Analysis

Data obtained were subjected to a two-way analysis of variance (ANOVA) using SPSS version 16 and means separated by the Duncan Multiple Range Test (Duncan, 1955) in the model below:

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

where,

Y_{ijkl}= Dependent variables

μ = Population mean

A_i= effect due to i^{th} fruit juices, i = 1, 2

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

T_k= effect due to k^{th} duration of storage, k = 0, 24, 48, 72, 96 …….240

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

$(AT)_{ik}$= effect due to ik^{th} interaction between fruit juices and storage duration

$(LT)_{jk}$= effect due to jk^{th} interaction between levels of inclusion and storage duration

$(ALT)_{ijk}$= effects due to ijk^{th} interaction between fruit juices, levels of inclusion and storage duration

$\sum_{ijkl}$= Experimental error

RESULTS AND DISCUSSION

The results (Table 1) showed higher ($P<0.05$) sperm progressive motility in extenders supplemented with pineapple and cucumber juices compared to the control group. The inclusion of pineapple and cucumber juices in tris-egg yolk extender for *in vitro* cold storage of semen obtained from WAD bucks in this study indicated that these fruit juices have the ability to sustain progressive motility. This could be attributed to the high level of vitamin C, E and other antioxidants present in these fruits (Gebhardt & Thomas, 2002; Djuric & Powell, 2001). Reza *et al*. (2011) showed that vitamin E or C supplementation in stored semen improved motility of spermatozoa. The wholesome effects on sperm viability that accompanied supplementation of semen extender with the fruit juices could be on account of their potential source of vitamin C, a water-soluble antioxidant (Martin *et al*., 2002; Mermeistein, 1999) and vitamin E, both naturally occurring free radical scavengers known to scavenge superoxide anions and singlet oxygen and protect lipoproteins from detectable peroxidative damage (Wainer *et al*., 1986, Donnelly *et al*., 1999). In contrast however, Aurich *et al*. (1997) reported that addition of ascorbic acid did not improve the maintenance of motility of cooled equine spermatozoa during the 96-hour storage period. The antioxidant capacity of the juices might not, however, be due to the vitamin C only but could also arise from some phenolic compounds present in these fruits (Spanos & Wrolstad, 2004). The major phenolic compound in fruits is ferulic acid (Augustin & Williams, 2000), that neutralises free radicals known as superoxide, hydroxyl radical and nitric oxide and in addition acts synergistically with other antioxidants, giving them extra potency to reduce free radical damage to cell membranes (Zuo *et al*., 2002). Progressive motility recorded at various levels of juices could therefore be associated with the concentrations of the vitamins (USDA, 2009) and phenolic compounds present in the juices (Cutler *et al*., 2008). Moreover, in the present study, the concentrations of fruit juices as antioxidants used might have been optimum for preserving buck sperm progressive motility, as effects of pineapple and cucumber juices varied with the level

of supplementation in the extender. Sperm progressive motility was maintained longer at concentrations of 5% of pineapple and 7.5% cucumber juices for 192 and 144 hours respectively.

The extenders supplemented with various concentrations of pineapple and cucumber juices had consistently higher ($P<0.05$) acrosome integrity up to 48 hours of post-chilling compared to the control (Table 2). The results (Table 3) showed consistently higher ($P<0.05$) membrane integrity in extenders supplemented with fruit juices compared to the control and this was more pronounced in extenders supplemented with 2.5% pineapple, 2.5% and 5% cucumber fruit juices after post-chilling. Interestingly, in the present study, the extenders supplemented with fruit juices had not only improved sperm progressive motility, but also enhanced the acrosome integrity and membrane integrity. The beneficial effect of antioxidants from pineapple and cucumber juices on intact acrosome and membrane integrity in the extenders supplemented with fruit juices during cold storage compared to control group observed in this study could be linked to vitamin C and other antioxidative compounds in these juices (Spanos & Wrolstad, 2004; Reza *et al*., 2011). The finding corroborates previous report (Zheng & Zhang, 1997) that ferulic acid was beneficial to sperm viability and reduction of lipid peroxidative damage to sperm membranes. The antioxidant potential of ferulic acid is attributed to its structural characteristics because its phenolic nucleus and unsaturated side chain readily forms a resonance stabilised radical, which accounts for its potent antioxidant activity (Marimuthu *et al*., 2007).

The extenders supplemented with 2.5% and 5% cucumber juice had consistent lower ($P<0.05$) abnormality compared to the control (Table 4). The lower percentage of sperm abnormality observed in the extenders supplemented with these levels of cucumber juice compared to the control after post-chilling suggested that the supplementation had beneficial effects on sperm morphology. It is relevant to mention that semen processing does not necessarily increase the proportion of sperm abnormalities (Revell, 2003). Moreover, the percentage sperm abnormalities observed were within the range for post-thawed goat semen as per the Brazilian College of Animal Reproduction (Henry & Neves, 1998) in extender supplemented with the fruit juice and control.

The results (Table 5) showed that extenders supplemented with fruit juices had lower ($P<0.05$) MDA concentrations compared to the control, and improved results were obtained at 2.5% and 5% pineapple and 2.5% cucumber fruit juices. Flavonoids have antioxidant capacity that is stronger than that of vitamin C and E used to prevent free radical production (Alía *et al*., 2003). The finding indicated that supplementation of tris-egg yolk extender with fruit juices from pineapple and cucumber possibly reduced the suppressive effects of lipid peroxidation on the metabolic activity of buck spermatozoa.

TABLE 1
Progressive Motility (%) of Buck Spermatozoa Chilled with Tris Egg Yolk Extenders Supplemented with Juices

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
0	80.00^{c}	90.00^{a}	84.00^{b}	86.00^{b}	84.00^{b}	92.00^{a}	94.00^{a}	92.00^{a}	86.00^{b}	1.171
24	60.00^{d}	88.00^{a}	82.00^{b}	74.00^{c}	50.00^{e}	84.00^{b}	90.00^{a}	88.00^{a}	82.00^{b}	2.242
48	50.00^{e}	80.00^{b}	70.00^{c}	64.00^{d}	46.00^{e}	74.00^{c}	90.00^{a}	84.00^{b}	76.00^{c}	2.557
72	40.00^{d}	78.00^{a}	70.00^{b}	50.00^{c}	44.00^{d}	74.00^{b}	82.00^{a}	80.00^{a}	70.00^{b}	2.714
96	16.00^{d}	76.00^{a}	68.00^{b}	42.00^{c}	12.00^{d}	70.00^{ab}	70.00^{ab}	72.00^{ab}	68.00^{b}	3.571
120	0.00^{f}	60.00^{b}	66.00^{a}	40.00^{d}	10.00^{e}	58.00^{c}	64.00^{b}	70.00^{a}	66.00^{a}	3.685
144	0.00^{f}	52.00^{b}	64.00^{a}	40.00^{c}	10.00^{e}	42.00^{c}	38.00^{d}	68.00^{a}	36.00^{d}	3.571
168	0.00^{f}	32.00^{c}	58.00^{a}	38.00^{b}	4.00^{e}	30.00^{c}	16.00^{d}	36.00^{b}	32.00^{c}	2.857
192	0.00^{e}	30.00^{b}	50.00^{a}	20.00^{c}	0.00^{e}	30.00^{b}	14.00^{d}	34.00^{b}	30.00^{b}	2.457
216	0.00^{d}	22.00^{b}	26.00^{a}	12.00^{c}	0.00^{d}	30.00^{a}	12.00^{c}	30.00^{a}	22.00^{b}	1.900
240	0.00^{e}	6.00^{c}	11.00^{ab}	6.00^{c}	0.00^{e}	8.00^{c}	10.00^{b}	12.00^{a}	4.00^{cd}	1.104

^{abcdef} Values within rows with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Means

TABLE 2
Acrosome Integrity (%) of Buck Spermatozoa Chilled with Tris Egg Yolk Extenders Supplemented with Juices

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
0	92.75^{b}	97.25^{a}	99.00^{a}	98.00^{a}	99.00^{a}	98.00^{a}	97.75^{a}	97.00^{a}	98.50^{a}	0.408
24	92.25^{b}	96.75^{a}	97.50^{a}	98.00^{a}	97.50^{a}	97.25^{a}	97.25^{a}	96.70^{a}	97.00^{a}	0.370
48	88.50^{b}	94.50^{a}	94.00^{a}	94.50^{a}	93.00^{a}	93.25^{a}	94.67^{a}	93.75^{a}	93.75^{a}	0.352
72	84.25^{b}	91.00^{a}	91.50^{a}	84.00^{b}	89.00^{a}	91.00^{a}	91.25^{a}	89.75^{a}	90.75^{a}	0.382
96	80.00^{b}	86.00^{a}	88.50^{a}	82.00^{b}	83.00^{b}	88.00^{a}	87.25^{a}	86.70^{a}	84.00^{a}	0.450
120	75.00^{b}	81.00^{ab}	83.89^{ab}	78.50^{b}	78.50^{b}	82.50^{ab}	82.00^{ab}	81.50^{ab}	79.75^{ab}	0.440
144	71.50^{c}	79.00^{a}	69.50^{c}	71.50^{c}	75.00^{b}	79.00^{a}	77.25^{a}	78.00^{a}	75.50^{b}	0.446

TABLE 2 (*continued*)

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
168	65.50^{b}	68.00^{a}	61.50^{c}	60.20^{c}	69.00^{a}	70.00^{a}	68.75^{a}	68.00^{a}	69.50^{a}	0.506
192	58.75^{b}	58.50^{b}	56.00^{b}	58.50^{b}	60.00^{a}	62.50^{a}	62.00^{a}	63.00^{a}	58.75^{b}	0.581
216	53.50^{b}	52.50^{b}	49.00^{c}	55.50^{a}	53.00^{b}	55.75^{a}	55.50^{a}	57.00^{a}	53.00^{b}	0.501
240	45.50^{b}	49.25^{a}	41.00^{c}	47.50^{a}	42.00^{c}	46.75^{a}	47.25^{a}	47.00^{a}	44.75^{b}	0.448

^{abcdef} Values within rows with different superscripts differ significantly (P<0.05). SEM: Standard Error of Means

TABLE 3
Membrane Integrity (%) of Buck Spermatozoa Chilled with Tris Egg Yolk Extenders Supplemented with Juices

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
0	89.75^{b}	89.00^{b}	91.00^{a}	92.00^{a}	90.00^{a}	93.00^{a}	93.00^{a}	92.00^{a}	92.50^{a}	0.456
24	88.75^{a}	87.00^{a}	86.00^{a}	86.50^{a}	83.50^{b}	86.50^{a}	87.00^{a}	85.00^{a}	84.00^{b}	0.420
48	81.75^{b}	85.50^{a}	80.00^{b}	82.50^{b}	79.00^{b}	81.00^{b}	83.00^{a}	81.50^{b}	81.50^{b}	0.375
72	74.75^{c}	80.00^{a}	78.00^{a}	77.00^{b}	76.00^{b}	78.00^{a}	79.50^{a}	77.00^{b}	77.50^{b}	0.346
96	71.50^{b}	75.00^{a}	74.00^{a}	75.00^{a}	73.50^{a}	75.50^{a}	74.50^{a}	72.00^{b}	73.00^{a}	0.326
120	65.50^{c}	73.00^{a}	70.00^{a}	73.00^{a}	69.00^{b}	74.00^{a}	73.50^{a}	70.00^{a}	71.00^{a}	0.485
144	59.50^{c}	70.50^{a}	65.00^{b}	69.00^{a}	61.50^{c}	70.00^{a}	70.00^{a}	66.00^{b}	68.50^{a}	0.561
168	55.00^{c}	65.00^{a}	58.50^{b}	63.00^{a}	54.33^{c}	65.50^{a}	64.00^{a}	63.00^{a}	63.50^{a}	0.548
192	49.25^{c}	60.00^{a}	54.00^{b}	50.40^{c}	50.00^{c}	61.50^{a}	59.50^{a}	57.00^{a}	50.00^{c}	0.600
216	43.50^{c}	55.00^{a}	49.00^{b}	48.00^{b}	44.00^{c}	56.50^{a}	54.50^{a}	50.00^{b}	48.00^{b}	0.635
240	38.50^{d}	50.00^{a}	43.50^{bc}	44.00^{b}	33.00^{e}	50.50^{a}	49.50^{a}	46.00^{b}	40.00^{c}	0.620

^{abcdef} Values within rows with different superscripts differ significantly (P<0.05). SEM: Standard Error of Means

TABLE 4
Abnormality (%) of Buck Spermatozoa Chilled with Tris Egg Yolk Extenders Supplemented with Juices

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
0	1.50^{a}	1.00^{a}	1.50^{a}	0.25^{b}	0.50^{b}	0.50^{b}	1.50^{a}	0.25^{b}	1.00^{a}	0.276
24	2.00^{a}	2.00^{a}	3.00^{a}	2.70^{a}	3.00^{a}	1.25^{b}	1.50^{b}	1.50^{b}	1.25^{b}	0.273
48	3.50^{a}	3.00^{a}	3.50^{a}	3.50^{a}	3.70^{a}	1.50^{b}	2.70^{b}	2.50^{b}	2.75^{a}	0.241
72	4.70^{a}	3.50^{a}	4.00^{a}	4.50^{a}	4.00^{a}	2.00^{b}	2.80^{b}	3.00^{b}	3.50^{a}	0.233
96	5.50^{a}	4.75^{a}	4.70^{a}	5.50^{a}	4.50^{a}	3.00^{b}	3.20^{b}	3.50^{b}	4.50^{a}	0.233
120	6.00^{a}	5.00^{a}	5.50^{a}	6.00^{a}	5.25^{a}	4.50^{b}	3.70^{b}	4.50^{b}	4.75^{b}	0.236
144	7.00^{a}	5.70^{b}	6.25^{a}	6.50^{a}	5.50^{b}	5.00^{b}	4.50^{c}	6.00^{a}	5.00^{b}	0.196
168	7.75^{a}	6.00^{a}	6.50^{a}	6.50^{a}	6.00^{a}	5.70^{b}	5.70^{b}	7.00^{a}	6.00^{a}	0.160
192	8.00^{a}	7.00^{a}	6.75^{b}	7.40^{a}	6.50^{b}	6.00^{b}	6.70^{b}	7.50^{a}	6.50^{b}	0.151
216	8.50^{a}	7.50^{a}	7.00^{a}	8.00^{a}	6.67^{b}	6.50^{b}	7.00^{b}	8.00^{a}	7.70^{a}	0.143
240	9.50^{a}	8.00^{b}	7.50^{bc}	8.50^{b}	8.00^{b}	7.00^{c}	8.00^{b}	8.75^{b}	8.00^{b}	0.126

^{abcdef} Values within rows with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Means

TABLE 5
MDA Concentrations (nmol/ml) of Buck Spermatozoa Chilled with Tris Egg Yolk Extenders Supplemented with Juices

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
24	0.08^{a}	0.01^{e}	0.02^{c}	0.02^{c}	0.04^{b}	0.01^{e}	0.03^{d}	0.03^{d}	0.04^{b}	0.006
48	0.17^{a}	0.02^{c}	0.03^{b}	0.03^{b}	0.03^{b}	0.01^{d}	0.01^{d}	0.02^{c}	0.03^{b}	0.013
72	0.20^{a}	0.08^{d}	0.10^{c}	0.10^{c}	0.11^{b}	0.05^{e}	0.11^{b}	0.07^{e}	0.09^{d}	0.012
96	0.23^{a}	0.13^{c}	0.13^{c}	0.14^{b}	0.15^{b}	0.11^{d}	0.12^{d}	0.11^{d}	0.13^{c}	0.009
120	0.24^{a}	0.13^{e}	0.16^{c}	0.17^{c}	0.19^{b}	0.13^{e}	0.15^{d}	0.12^{e}	0.15^{d}	0.010
144	0.25^{a}	0.15^{e}	0.17^{d}	0.19^{c}	0.21^{b}	0.13^{f}	0.14^{e}	0.14^{e}	0.16^{d}	0.009
168	0.27^{a}	0.17^{e}	0.19^{d}	0.23^{c}	0.24^{b}	0.16^{e}	0.17^{e}	0.18^{d}	0.19^{d}	0.008

TABLE 5 (*continued*)

Duration (h)	Control	Pineapple (%)				Cucumber (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	
192	0.50^{a}	0.32^{c}	0.32^{c}	0.32^{c}	0.37^{b}	0.26^{e}	0.27^{e}	0.28^{e}	0.30^{d}	0.020
216	0.55^{a}	0.42^{d}	0.43^{d}	0.44^{c}	0.45^{b}	0.37^{e}	0.37^{e}	0.40^{e}	0.40^{e}	0.014
240	0.76^{a}	0.45^{e}	0.46^{e}	0.51^{c}	0.64^{b}	0.46^{e}	0.50^{d}	0.50^{d}	0.52^{c}	0.025

^{abcdef} Values within rows with different superscripts differ significantly ($P<0.05$). SEM: Standard Error of Means

The results of the present study, therefore, suggest that the antioxidants from pineapple and cucumber juices probably work by removing hydrogen peroxide from the medium, thus preventing the generation of hydroxyl radicals, which are powerful oxidants by the Fenton reaction (O'Flaherty *et al*., 2003). This effect may explain the current findings of improved progressive motility, acrosome and membrane integrity, lower abnormalities and reduced MDA when pineapple and cucumber juices were added to the extenders.

CONCLUSION

The improved progressive motility, acrosome and membrane integrities, reduced abnormalities and MDA following supplementation with pineapple and cucumber juices in the extenders indicate that antioxidant properties of these juices may be involved in beneficial effect on the sperm viability and the fruit juices may be used for sperm preservation of chilled West African Dwarf buck semen.

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