Preservative effects of apple and orange juices on refrigerated Kalahari Red goat spermatozoa

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Antioxidative mechanisms protect the sperm from the damage caused by free radicals and fruit-rich antioxidants are linked with improved viability of spermatozoa because of their protective effects against cell damage during preservation. This study investigated the effects of apple and orange juices on viability and lipid peroxidation (LPO) of refrigerated spermatozoa of Kalahari Red goats (KRG). Pooled semen from five bucks was diluted with Tris-milk-based extender supplemented with apple and orange juices (natural antioxidants) each at 0.0%, 2.5%, 5.0%, 7.5% and 10.0%. The extended semen samples were assessed at 24 hour intervals up to 216 hours, for sperm motility, acrosome integrity, membrane integrity and sperm abnormalities using a Celestron Penta View LCD digital microscope, after storage at 5 ºC. The concentration of LPO in the stored semen was measured by determining the malondialdehyde in thiobarbituric acid reactive substances. The experimental design was a 2 x 5 x 10 factorial arrangement (2 antioxidants, 5 levels of inclusion and 10 durations of storage). Data were subjected to analysis of variance for the effects of each of the antioxidant source. The highest result of sperm progressive motility (93%) was obtained at the 2.5% inclusion level of extender supplemented with apple juice at 24 hours of storage (P≤0.05). The extenders supplemented with 2.5% and 5.0% concentrations of apple and orange juices had consistently higher (P≤0.05) acrosome integrity from 24 hours up to 216 hours of post-chilling compared to the control. The results showed consistently higher (P≤0.05) membrane integrity in spermatozoa stored in extenders supplemented with fruit juices compared to the control and this was more pronounced in those stored in extenders supplemented with 2.5% and 5.0% of both apple and orange fruit juices after post-chilling. Inclusion levels at 2.5% and 5.0% had similar LPO reactions. The lowest LPO reaction (0.01%) occurred at 2.5% and 5.0% levels of inclusion of orange juice and the same result was obtained at 5.0% inclusion of apple but the highest (0.10%) was recorded in the control (P≤0.05). Spermatozoa stored in the extenders supplemented with 2.5% apple and orange juices had consistently lower (P≤0.05) abnormality compared to the control. This study showed that inclusion of apple and orange juices (natural antioxidants) used in Tris-milk based extender had a protective effect on KRG spermatozoa viability.

Keywords: Lipid peroxidation, Kalahari Red goat, natural antioxidants, fruit juice, spermatozoa viability, malondialdehyde

Preservation and maintenance of biodiversity is one of the most important current concerns of human kind, as the wild species and domestic breeds and strains are disappearing at an alarming rate (Frankham et al. 2002). Therefore, an increasing number of these (cattle, sheep, goats, etc.) require human intervention to guarantee their survival (Corales et al. 2010). Fertility of sperm is the ultimate test of sperm quality. Often it is not possible to measure fertility, but many tests of semen quality in addition to motility and morphology, such as the hypoosmotic swelling test, mucous or gel penetration, and integrity of the DNA have been correlated with fertility (Foote, 2002). Reproductive ability in the male comprises the production of semen containing normal spermatozoa (quality) in the adequate number (quantity), together with the desire and ability to mount (Oyeyemi and Obiogoro 2005).

The introduction of antioxidants in the preservation of semen has been used in an attempt to block or prevent oxidative stress in a variety of cell systems which act in a variety of steps to either scavenge reactive oxidative species (ROS) directly or to prevent the damage of lipid peroxidation in cell membrane (Baran et al. 2009). The storage of diluted goat semen at a refrigeration temperature reduced viability and modified membrane functional status. This may be due to the cold shock occurring during the cryopreservation process of spermatozoa and is associated with the

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oxidative stress induced by ROS generation (Chatterjee et al. 2001). Besides, addition of antioxidants to semen extender improved sperm motility and viability of bovine semen (Krzyzosiak et al. 2000). Natural foods and food-derived antioxidants such as vitamin C, carotenoids and phenolic phytochemicals have received growing attention because they are known to function as chemopreventive agents against oxidative damage (Kiwon et al. 2003). There is a paucity of information on the protective effect of orange juice on spermatozoa against the harmful effects of lipid peroxidation of white layer cock's semen stored for up to 72 hours as reported by Al-Daraji (2012), while information on effect of apple juice for preserving mammalian spermatozoa is, however, not available in the literature. The objective of this study was to determine preservative effects of these fruit juices on sperm viability of Kalahari Red goat bucks during cold storage.

Materials and methods

Experimental site and animals

The experiment was carried out at the Kalahari goat unit of Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR) and Department of Animal Physiology Laboratory, Federal University of Agriculture, Abeokuta. It is located between latitudes $7^0 18^I 2^{II}$ N and $7^0 18^I 30^{II}$ N and longitudes $3^0 22^I 10^{II}$ E and $3^0 22^I 41^{II}$ E, 76 m above sea level. The climate is humid in the forest zone of South Western Nigeria, with mean precipitation and temperature of 1,112.7 mm and 23.5 ^oC, respectively. Relative humidity averages 81.5% throughout the year. Five intact Kalahari bucks and one matured teaser doe were used for this experiment on the basis of their soundness for breeding purposes. Bucks of 1.5 - 2 years and the teaser doe of the same age group, with body weight between 40 – 45 kg were used. The animals were managed semi-intensively in an open-ventilated pen with concrete floor under natural light and maintained under a uniform and constant nutritional regime. *Chloris gayana* and *Stylosanthes hamata* were fed *ad libitum* and supplemented with concentrate feed composed as follows: maize (15%), soybean meal (5%), groundnut cake (4%), palm kernel cake (23.5%), wheat Offal (50%), bone meal (1%), sodium chloride, salt (1%), premix (0.5%) with crude protein (16.49%), dry matter (88.19%), ether extract (2.48%), crude fibre (27.50%), ash (10.98%), nitrogen free extract (41.74%), organic matter (89.02%) and metabolisable energy (12.27 MJ/kg DM); water was also given *ad libitum.*

Preparation of apple juice

The fruit was prepared in accordance with the procedure of Adeyemo et al. (2007). Five fresh medium size (85g) apples were washed thoroughly using distilled water. Each apple was cut into two pieces and their seeds were removed. The apples were blended for 5 minutes to extract the juice. The blended apple was placed in a sieve and pressure was applied to extract the juice. In the case of orange, five fully ripe fruits were washed thoroughly using distilled water. The oranges were peeled, each was cut into two pieces and seeds were removed. Light pressure was applied to extract the juice from the fruit. The juices from both apple and orange were collected into plastic test tubes, which were then placed inside the centrifuge. The centrifuge was set at 3000 revolutions per minute and left to operate for 25 minutes. The test tubes were later removed and the supernatant fluid decanted into a clean beaker and used immediately for the experiment.

Semen collection

Semen samples were collected using an artificial vagina. The water jacket of the artificial -vagina was filled with hot water at a

temperature of 40 – 42°C by opening the nostle while graduated semen collection tube was fixed to the narrow end of the artificial vagina hose, and fastened by a rubber band.

Semen dilution and chilling

Dilution and chilling of the samples were done for uniformity and to minimize individual semen differences, only ejaculates showing greater than 80% motility were pooled. Pooled semen (each originating from the five males) was diluted by the use of Tris-milk-based extender – tris (2.42g), citric acid (1.36g), glucose (1g), penicillin (0.028g), skim milk (10g), and supplemented with either apple or orange juice at 2.5, 5.0, 7.5, 10.0ml. The control treatment was not supplemented with juice. Distilled water was added to make up 100ml, for both control and others, at a ratio of 1:20 (1 part semen to 20 parts extender) in the semen extender prepared from dairy skimmed milk supplemented with natural antioxidants. Immediately after the dilution, the semen samples were dispensed into 5ml tubes that were then sealed and chilled from 37ºC to 5ºC at approximately 0.5ºC/minute and maintained at this temperature for 0, 24, 48, 72, 96, 120, 144, 168, 192 or 216 hours. At each of these times sperm quality characteristics were evaluated.

pH of the extender

The pH determination of the extender was carried out by using a digital pH meter and this was measured at the time of semen collection. The extenders' pH were slightly alkaline ranging between 7.1 - 7.3 (control: 7.1; apple: 7.3 and orange: 7.2).

Semen evaluation

Sperm motility

Sperm motility was determined as described by Bearden and Fuquay (1997). Semen was thawed in a Clifton Water Bath (model: 74178 by Nickel Electro Ltd, Weston-S-Mare Somerset, England) and assessed for sperm motility using a Celestron Penta View LCD digital microscope with a warm stage maintained at 37ºC. Semen was thawed at 37ºC for 3 minutes. A semen mount was made using 5 µl semen and the semen was placed directly on a microscope slide and covered with a cover slide. For each sample, five microscopic fields were examined two times by two different observers and their mean was recorded as the final score for sperm cells that were progressively moving in a straight line.

Acrosome integrity

This followed the procedure of Ahmed et al. (2014) to determine the percentage of spermatozoa with intact acrosome. This was determined by adding 50 µl of each sperm sample to a 500 µl formalin citrate solution (96 ml 2.9% sodium citrate, with 4ml 37% formaldehyde) followed by careful mixing. A small drop of the mixture was placed on a microscope slide and a total of 200 spermatozoa were counted for live sperms with intact acrosome (apical piece) in at least four different microscopic fields for each sample, using a Celestron Penta View LCD digital microscope.

Membrane integrity

The hypo-osmotic swelling test (HOST) was used to determine sperm membrane integrity and this was done by incubating 10 µl semen in a 100 µl hypo-osmotic solution (fructose and sodium citrate) at 37 ºC for 30 minutes, 0.1 ml of the mixture was spread over a warm slide, fitted with a cover slip, and observed under the Celestron Penta View LCD digital microscope.

Malondialdehyde (MDA)

MDA which is the indice of lipid peroxidation (LPO) in the stored semen was first determined

and LPO was carried out following the procedure of Buege and Steven (1978) and Bansal and Bilaspuri (2008). For this assay, 0.1 ml of sperm suspension was incubated with 0.1 ml of 150 mM Tris-HCL (pH 7.1) for 20 minutes at 37ºC. Subsequently, 1ml of 10% trichloroacetic acid (TCA) and 2ml of 0.375% thiobarbituric acid were 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. The absorbance was read with a spectrophotometer at 532 nm. The results were converted to lipid peroxidation concentration (M) with a conversion factor:

$M = (absorbance/1.56*10^5)/cm$

Morphological abnormality**:** abnormalities were determined as described by Bearden and Fuquay (1997) with the use of eosin-nigrosin smears. A thin smear of the mixture was drawn across the slide, and the slide was dried. A cover slip was applied to the preparation and the sample was examined under the Celestron Penta View LCD digital microscope. The sperm cells that were alive at the time when the slide was being prepared were not stained and looked white or clear. The dead cells stained pink (Silvestre et al. 2004). Sperm cells with abnormal head, mild-piece and tail were observed.

Statistical analysis

Data obtained were subjected to a 2 x 5 x 10 factorial arrangement, using SAS (1999).
The model used to analyse the data is stated below:

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

where,

μ = population mean
A_i = Effect of i^{th} antioxidants (1, 2)
L_j = Effect of j^{th} different level of inclusions (0, 2.5, 5.0, 7.5, 10).
T_k = Effect of k^{th} duration of storage / time intervals (0 - 216 hours).
$(AL)_{ij}$ = ij^{th} effects due to interaction between antioxidants and different level of inclusions.
$(AT)_{ik}$ = ik^{th} effects due to interaction between antioxidants and duration of storage.
$(ALT)_{ijk}$= ijk^{th} effects due to interaction between antioxidants, different level of inclusions, and duration/time of storage.
$\sum_{ijk}$ = residual error.

Results

Table 1 shows higher sperm progressive motility in extenders supplemented with apple and orange juices in a tris-milk based extenders when Kalahari Red goat semen was stored at refrigerated temperature. The highest result was obtained at 2.5% inclusion level of extender supplemented with apple juice (93.00) at 24 hours of storage ($P \leq 0.05$).

Table 2 shows that highest acrosome integrity was observed at 2.5% inclusion level of apple juice (80.0%) at 72 hours of storage and the value was not significantly different at 5% and 7.5% at the same hours of storage ($P \geq 0.05$). The highest acrosome integrity was observed at 2.5% inclusion level of orange juice (83.0%) at 24 hours of storage. Also, at 10.0% and 2.5% inclusion level of apple (80.0%) and orange (83.0%) juices at 48 and 72 hours of storage respectively ($P \leq 0.05$). The extenders supplemented with 2.5% and 5.0% concentrations of apple and orange juices had consistently higher ($P \leq 0.05$) acrosome integrity commenced from 24 hours up to 216 hours of post-chilling compared to the control (Table 2). Table 3 shows consistently higher ($P \leq 0.05$) membrane integrity in extenders supplemented with fruit juices compared to the control and this was more pronounced in extenders supplemented with 2.5% and 5.0% of both apple and orange fruit juices after post-chilling.

Table 4 shows that inclusion levels at 2.5% and 5.0% had similar lipid peroxidation reaction but the lowest occurred at 2.5% and 5.0% levels of inclusion (0.01%) of orange juice and the same result was obtained at 5.0% inclusion of apple but highest (0.10%) was recorded in the control ($P \leq 0.05$).

The extenders supplemented with 2.5% apple and orange juices had consistently lower ($P \leq 0.05$) abnormality compared to the control (Table 5).

Discussion

It was shown in this study that the supplementation with fruit juices not only improved motility but also enhanced acrosome integrity and membrane integrity and agreed with the findings of Daramola and Adekunle (2015). The beneficial effect of antioxidants from apple and orange 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 and Wrolstad 2004; Reza et al. 2011). In contrast, Shoae and Zamiri (2008) showed that excessive amount of antioxidants caused high fluidity of plasma membrane above the desired point, making sperm more prone to acrosomal damages. However, the survival of spermatozoa in Kalahari buck semen increased when the dosage of vitamin C added to the extenders increased. Differences in preservation protocols and constituents of extenders, the time of addition/exposure of sperm to antioxidant, concentration of antioxidants and species variations may explain, at least in part, this difference (Shoae and Zamiri 2008).

The finding indicated that supplementation of milk-based extender with fruit juices from apple and orange possibly reduced the suppressive effects of lipid peroxidation on the metabolic activity of Kalahari buck spermatozoa and this is in agreement with Daramola and Adekunle (2015).

The lower percentage of sperm abnormality observed in the extenders supplemented with different levels of apple and orange juices compared to the control after post-chilling suggested that the supplementation had beneficial effects on sperm morphology. The percentage sperm abnormalities observed were within the range for post-thawed goat semen as reported by the Brazilian College of Animal Reproduction (Henry and Neves 1998) in extender supplemented with the fruit juice and control.

Table 1: Effects of duration of storage (hours), type of natural antioxidant sources and levels of inclusion on mean percentage sperm motility (SEM ± 2.5) of Kalahari Red goat semen

		Apple (%)				Orange (%)			
Duration (hr)	Control	2.5	5	7.5	10	2.5	5	7.5	10
0	80.0^{bA}	86.0^{aA}	88.0^{aA}	78.5^{bA}	84.0^{aA}	87.0^{aA}	80.0^{bA}	82.5^{aA}	82.0^{aA}
24	70.5^{cB}	93.0^{aA}	77.0^{cB}	72.5^{cA}	77.0^{cB}	84.5^{bA}	76.5^{cA}	75.5^{cA}	78.5^{bA}
48	61.5^{cC}	82.0^{aB}	75.0^{bB}	59.5^{dB}	67.0^{cC}	76.5^{bB}	71.0^{bB}	72.5^{bB}	71.0^{bB}
72	62.0^{bC}	79.5^{aB}	73.0^{aB}	73.0^{aA}	63.0^{bC}	74.0^{aB}	68.0^{bB}	73.0^{aB}	61.5^{bC}
96	50.0^{dD}	81.5^{aB}	75.0^{aB}	74.0^{bA}	67.5^{bC}	76.0^{aB}	70.5^{bB}	62.5^{cC}	58.0^{cC}
120	45.5^{dE}	76.5^{aB}	64.0^{bC}	55.0^{cB}	51.5^{cD}	73.5^{aB}	73.0^{aA}	57.5^{bC}	59.0^{bC}
144	33.5^{cF}	66.5^{aC}	51.0^{bD}	39.5^{cC}	31.5^{dE}	50.5^{bC}	45.0^{bC}	40.5^{cD}	33.5^{cD}
168	17.0^{dG}	59.5^{aC}	45.5^{bD}	36.5^{cC}	31.5^{cE}	42.0^{bD}	43.5^{bC}	38.5^{bD}	29.5^{cD}
192	9.5^{Eh}	56.5^{aD}	39.5^{bE}	36.0^{bC}	27.0^{cE}	35.5^{bD}	33.0^{bD}	31.0^{cE}	21.5^{dE}
216	2.0^{eI}	48.5^{aE}	32.5^{bE}	26.5^{bD}	20.0^{cF}	27.5^{bE}	23.0^{cE}	23.0^{cF}	15.5^{dD}

^{abcde} values within rows and ^{ABCDEFGHI} columns with different superscript(s) differ significantly ($P \leq 0.05$). SEM: standard error of means

Table 2: Effects of duration of storage (hours), natural antioxidant sources and levels of inclusion on mean percentage acrosome integrity (SEM ± 1.4) of Kalahari Red goat semen

		Apple (%)				Orange (%)			
Duration (hr)	Control	2.5	5	7.5	10	2.5	5	7.5	10
0	83.5^{aA}	76.0^{cB}	79.0^{bA}	84.5^{aA}	84.0^{aA}	76.0^{bB}	74.0^{cB}	76.5^{cA}	76.5^{cA}
24	65.8^{cB}	76.0^{bB}	78.0^{bA}	63.5^{dD}	78.0^{bB}	83.0^{aA}	75.0^{bA}	74.5^{bB}	74.0^{bA}
48	62.5^{cC}	76.5^{aB}	75.5^{bB}	75.5^{bB}	80.0^{aA}	79.5^{aA}	75.5^{bA}	61.5^{cC}	59.5^{cC}
72	59.5^{dC}	85.5^{aA}	80.5^{bA}	79.5^{bA}	71.0^{cC}	83.0^{aA}	76.5^{bA}	79.0^{bA}	67.5^{cB}
96	60.0^{eD}	79.5^{aB}	63.5^{cD}	69.0^{bC}	76.5^{aB}	77.0^{aB}	65.0^{bC}	53.5^{dD}	62.5^{cC}
120	59.0^{dE}	79.0^{aB}	71.0^{bC}	62.0^{cD}	62.0^{cD}	71.5^{bC}	79.0^{aA}	65.5^{cC}	71.0^{bB}
144	52.5^{eF}	65.0^{aC}	57.0^{bE}	46.5^{dE}	49.0^{cE}	53.5^{bD}	51.0^{cD}	43.5^{dF}	42.5^{dD}
168	48.0^{eG}	66.5^{aC}	50.0^{bE}	46.0^{cE}	42.0^{dF}	53.5^{bD}	51.0^{bD}	48.5^{cE}	42.5^{dD}
192	46.0^{fG}	68.5^{aC}	51.0^{bE}	47.5^{bE}	39.0^{dF}	24.5^{eF}	44.0^{cE}	39.5^{dF}	38.0^{dE}
216	39.5^{fH}	57.5^{aD}	45.5^{bF}	36.5^{cF}	26.0^{eG}	34.5^{cE}	30.5^{dF}	28.5^{dG}	23.0^{eF}

^{abcdef} values within rows and ^{ABCDEFGH} columns with different superscript(s) differ significantly (P≤0.05). SEM: standard error of means

Table 3: Effects of duration of storage (hours), natural antioxidant sources and levels of inclusion on mean percentage membrane integrity (SEM ± 2.5) of Kalahari Red goat semen

		Apple (%)				Orange (%)			
Duration (hr)	Control	2.5	5	7.5	10	2.5	5	7.5	10
0	82.5^{aA}	67.5^{cB}	87.5^{aA}	80.0^{aA}	85.0^{aA}	75.0^{bA}	70.0^{bA}	72.5^{bA}	70.0^{bA}
24	85.0^{aA}	82.5^{aA}	77.5^{aB}	77.5^{aA}	80.0^{aA}	72.5^{bA}	72.5^{bA}	65.0^{bA}	72.5^{bA}
48	62.9^{bB}	75.0^{aA}	72.5^{aB}	67.5^{aB}	70.0^{aB}	75.0^{aA}	67.5^{aA}	60.0^{bB}	57.5^{bB}
72	52.9^{bC}	67.5^{aB}	67.5^{aC}	62.5^{aB}	70.0^{aB}	57.5^{bB}	47.5^{cB}	45.0^{cC}	40.0^{cC}
96	42.5^{bD}	65.0^{aB}	50.0^{bD}	42.5^{bC}	47.5^{bC}	65.0^{aB}	50.0^{bB}	50.0^{bC}	50.0^{bB}
120	37.5^{bD}	52.5^{aC}	40.0^{bE}	35.0^{bC}	35.0^{bD}	47.5^{aC}	32.5^{bC}	50.0^{aC}	32.5^{bC}
144	32.5^{bE}	45.0^{aC}	40.0^{aE}	32.5^{bD}	30.0^{bD}	47.5^{aC}	32.5^{bC}	32.5^{bD}	32.5^{bC}
168	25.5^{bE}	37.5^{aD}	27.5^{bF}	27.5^{bD}	27.5^{bD}	42.5^{aC}	35.0^{aC}	30.0^{bD}	30.0^{bD}
192	25.5^{bE}	40.0^{aD}	30.0^{bF}	25.0^{bD}	30.0^{bD}	45.0^{aC}	32.5^{bC}	30.0^{bD}	27.5^{bD}
216	25.5^{aE}	30.0^{aE}	15.0^{bG}	20.0^{bE}	20.0^{bE}	25.0^{aD}	22.5^{aD}	25.0^{aD}	25.0^{aD}

^{abc} values within rows and ^{ABCDEFG} columns with different superscript(s) differ significantly (P≤0.05). SEM: standard error of means

Table 4: Effects of duration of storage (hours), natural antioxidant sources and levels of inclusion on mean percentage lipid peroxidation (SEM ± 0.001) of Kalahari Red goat semen

		Apple (%)				Orange (%)			
Duration (hr)	Control	2.5	5	7.5	10	2.5	5	7.5	10
0	0.00^{aG}	0.00^{aE}	0.00^{aF}	0.00^{aD}	0.00^{aG}	0.00^{aG}	0.00^{aF}	0.00^{aF}	0.00^{aE}
24	0.02^{bF}	0.02^{bD}	0.01^{cC}	0.02^{bE}	0.01^{cF}	0.01^{cF}	0.01^{cE}	0.02^{bE}	0.03^{aC}
48	0.05^{aE}	0.04^{bB}	0.03^{cC}	0.03^{cD}	0.04^{bC}	0.02^{dE}	0.02^{dD}	0.03^{cD}	0.04^{bB}
72	0.05^{aE}	0.04^{bB}	0.03^{cC}	0.02^{dE}	0.05^{aB}	0.03^{cD}	0.04^{bB}	0.04^{bC}	0.04^{bB}
96	0.06^{aD}	0.05^{bA}	0.03^{dC}	0.02^{eE}	0.05^{bB}	0.04^{cC}	0.05^{bA}	0.05^{bB}	0.03^{dC}
120	0.09^{aB}	0.04^{dB}	0.06^{bA}	0.04^{dC}	0.05^{cB}	0.09^{aA}	0.04^{dB}	0.03^{eD}	0.03^{eC}
144	0.05^{aE}	0.03^{bC}	0.02^{dD}	0.03^{cD}	0.02^{dE}	0.03^{cD}	0.03^{cC}	0.04^{bC}	0.03^{cC}
168	0.10^{aA}	0.05^{cA}	0.04^{dB}	0.09^{aA}	0.06^{bA}	0.05^{cB}	0.05^{cA}	0.06^{bA}	0.05^{cA}
192	0.08^{aC}	0.04^{bB}	0.04^{bB}	0.05^{bB}	0.05^{bB}	0.03^{dD}	0.04^{cB}	0.03^{cD}	0.02^{eD}
216	0.06^{aD}	0.03^{bC}	0.02^{cE}	0.02^{cE}	0.03^{bD}	0.02^{cE}	0.02^{cD}	0.03^{bD}	0.03^{bC}

^{abcde} values within rows and ^{ABCDEFG} columns with different superscript(s) differ significantly (P≤0.05). SEM: standard error of means

Table 5: Effects of duration of storage (hours), natural antioxidant sources and levels of inclusion on mean percentage morphology (SEM ± 1.0) of Kalahari Red goat semen

		Apple (%)				Orange (%)			
Duration	Control	2.5	5	7.5	10	2.5	5	7.5	10
0	12.5^{aH}	7.5^{bF}	11.0^{aG}	9.0^{bH}	8.0^{bG}	9.0^{bG}	7.5^{bH}	9.5^{bH}	6.0^{cG}
24	16.0^{bG}	7.0^{dF}	19.0^{aF}	19.0^{aF}	7.5^{dG}	3.5^{eH}	11.0^{cG}	8.0^{dH}	19.0^{aF}
48	29.5^{cE}	21.0^{eD}	29.0^{cD}	45.0^{aC}	41.5^{bD}	18.5^{fE}	25.0^{dD}	19.0^{eF}	21.5^{eF}
72	30.5^{aE}	11.5^{fE}	19.5^{dF}	23.0^{cE}	27.5^{bE}	12.5^{fF}	23.5^{cD}	15.5^{eG}	31.5^{aE}
96	24.5^{bF}	9.0^{fF}	13.0^{dG}	15.5^{dG}	20.5^{cF}	12.5^{eF}	19.0^{cE}	23.5^{bE}	30.0^{aE}
120	41.5^{aD}	13.0^{fE}	26.0^{dE}	34.5^{bD}	27.0^{dE}	16.5^{eE}	15.0^{eF}	32.0^{bD}	30.0^{cE}
144	53.0^{aC}	29.5^{fB}	38.0^{dC}	45.5^{cC}	55.0^{aC}	35.0^{eD}	43.0^{dC}	46.5^{cC}	51.0^{bD}
168	64.0^{aB}	26.5^{eC}	40.5^{dC}	44.5^{cC}	55.0^{bC}	42.0^{dC}	41.5^{dC}	46.0^{cC}	55.5^{bC}
192	72.0^{aA}	31.0^{fB}	49.0^{eB}	53.5^{dB}	60.5^{cB}	54.5^{dB}	55.0^{dB}	47.0^{eB}	68.0^{bB}
216	70.5^{bA}	39.5^{gA}	55.0^{fA}	59.0^{eA}	71.5^{aA}	62.0^{dA}	66.0^{cA}	71.5^{aA}	73.5^{aA}

^{abcdef} values within rows and ^{ABCDEFGH} columns with different superscript(s) differ significantly ($P \leq 0.05$). SEM: standard error of means

Conclusion

Supplementation of apple and orange juices resulted in an improved progressive motility, acrosome and membrane integrities, reduced abnormalities and lipid peroxidation, it is evident that these fruit juices can be used for preservation of sperm cells of Kalahari Red goat with beneficial effects.

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