Effect of fruit-juice on spermatozoa viability and lipid peroxidation of Red Sokoto bucks during liquid storage

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Abstract

Antioxidants are linked with sperm viability because of their protective effects against cell damage during preservation. In order to enhance the life span of refrigerated buck semen, **this study** was carried out to determine the effect of fruit-rich antioxidants on spermatozoa viability and lipid peroxidation (LPO) of buck semen during liquid storage. Pooled semen from five Red Sokoto bucks was diluted with Tris-egg yolk based extender and supplemented each with juices from pawpaw, tomato and watermelon at 0, 2.5, 5, 7.5 and 10/ 100 ml respectively. Following dilution, the semen samples were assessed subjectively after in vitro storage at 5°C for 24, 48, 72 and 96 hours as regards sperm motility, abnormalities, and acrosome status using a phase-contrast microscope. The concentration of malondialdehyde (MDA) as indices of lipid peroxidation (LPO) in the stored semen was measured in thiobarbituric acid reactive substances (TBARS) at 24, 48, 72 and 96 hours. The results showed highest progressive motility in watermelon juice at 2.5% ($P < 0.05$) during the first 24 hours of storage while the lowest progressive motility was recorded at various levels of pawpaw juice ($P < 0.05$). After 48 hours of storage, extender supplemented with watermelon and tomato juices had better progressive motility compared to control except 7.5% and 10% of tomato juice ($P < 0.05$). Irrespective of level of juice in the extender, the percentage of intact acrosome was similar among the various juices and control. The results showed that spermatozoa extended with watermelon juice had the lowest ($P < 0.05$) percentage abnormality compared to other extenders at 24, 48, 72 and 96 hours of storage. Higher ($P < 0.05$) percent spermatozoa abnormality compared to other fruit juices and control was observed at 72 and 96 hours of storage in spermatozoa extended with pawpaw juice. Significant reductions of MDA concentrations were achieved by addition of fruit-rich antioxidants to Tris-egg yolk based extender during the first 72 hours and the reduction was much pronounced in extender supplemented with pawpaw juice compared to control ($P < 0.05$). The findings reveal that fruit-rich antioxidants from watermelon and tomato have protective ability to maintain sperm viability and to reduce concentration MDA of buck semen during liquid storage.

Keywords: Antioxidant, bucks, fruit juice, Lipid peroxidation, spermatozoa viability

Introduction

Goat is the most numerous of the domestic livestock species in Nigeria and presents a great potential to alleviate the problem of protein malnutrition in the country (FAO,

2006). As the demand for these animals in the sub region is constantly high, the prospects for increasing the numbers and productivity need to be utilized. Genetic improvement of goats requires the selection

of superior breeding stock and the application of artificial insemination (AI) technique. The success of AI in goats generally depends on knowledge of semen preservation and insemination techniques (Sugulle *et al.*, 2006). In Nigeria, the lack of a reliable method for short term storage of semen is a limiting factor due to insufficient storage facilities for semen.

Semen preservation would enable producers to keep semen such that it could be used for subsequent AI over extended period of time. The preservation of semen for short-term (liquid) storage is achieved by reducing the metabolism of spermatozoa through reduction in storage temperatures, and for long-term (frozen) storage by arresting the metabolism at sub-zero temperatures. Although the fertilizing capacity of spermatozoa may be prolonged by storage in a liquid or frozen state, the storage processes inevitably reduce the proportion of motile spermatozoa and cause degenerative changes to sperm membrane integrity, which ultimately reduces fertilizing capacity after AI (Maxwell and Watson, 1996). The survival of ejaculated sperm in seminal plasma alone is limited to a few hours. To maintain sperm for longer periods and to cool or cryopreserve semen, dilution with a protective solution is necessary. The extenders must preserve fertilizing capacity of spermatozoa during *in vitro* storage at low temperatures. Notwithstanding, regardless of extenders nature, motility and membrane integrity of spermatozoa deteriorate during the cooling process and storage at low temperatures. The degenerative changes are possible results of lipids peroxidation or excessive production of reactive oxygen species (ROS) during *in vitro* storage (Beconi *et al.*, 1993).

The most common ROS are superoxide anion, hydrogen peroxide, peroxy radicals,

hydroxyl radicals, nitric oxide and peroxy nitrite anion (Sikka, 1996). ROS can cause alterations in sperm plasma membrane and reduction in motility and fertilizing ability of spermatozoa (Maxwell and Stojanov, 1996; Dalvit *et al.*, 1998; Chatterjee and Gagnon, 2001; Raina *et al.*, 2002). The addition of natural antioxidants such as vitamin E in the freezing diluents exerts a protective effect against lipid peroxidation, thereby preserving the metabolic activity and cellular viability of spermatozoa (Beconi *et al.*, 1993). A greater protective effect against lipid peroxidation has been observed in bovine semen samples frozen with vitamin E and then incubated with vitamin E after thawing versus samples incubated without the antioxidant (Beconi *et al.*, 1991). Another non-enzymatic antioxidant (vitamin C) has been proposed as electron donor for some trans plasma membrane redox systems and vitamin C may act as an oxidant at low concentrations and as an antioxidant at high concentrations (Affranchino *et al.*, 1991; Breininger *et al.*, 2005). Vitamin B, vitamin C, vitamin E, carotenoids and phenolic compounds are the most abundant antioxidants present in plant fruits (Hernández *et al.*, 2006; Lim *et al.*, 2007). Therefore, the present research was undertaken to investigate the effect of fruit-rich antioxidants from pawpaw, tomato and watermelon on sperm viability and LPO of RS buck spermatozoa during liquid storage.

Materials and Methods

The study was carried out at the Goat Unit of the Teaching and Research Farm Division, Federal University of Agriculture, Abeokuta, located in the tropical rain forest zone of South Western Nigeria within 7° 10'N and 3° 2'E. Five intact Red Sokoto bucks ranged between

2.5-3 years of age and kept under semi intensive management system were used for this study. Watermelon and ripe tomato fruits were blended separately for one minute each, while ripe pawpaw fruit was wreathed to remove the juice. The juice from each fruit was collected into different plastic test tubes and centrifuged at 3000 revolutions per minute for 10 minutes. The clear supernatant fluid of each fruit was thereafter decanted into clean separate beakers. Tris-egg yolk based extender (Kumar and Atreja, 2012) used for this study consisted of Tris (2.42g), citric acid (1.36g), glucose (1g), penicillin (0.028g), egg yolk (20ml) and distil water made up 100ml as control. The extender was supplemented with pawpaw, tomato and watermelon juice each at 0, 2.5, 5, 7.5 and 10/100ml respectively. Pooled semen sample (each pool originating from six males) was diluted at ratios of 1:5 (semen: extender v/v) with the extender. Following dilution, the semen samples were drawn into eppendorf tubes, sealed and gradually cooled from 37°C to 5°C and maintained at this temperature for 96 hours.

Assessment of sperm motility: Following dilution, the semen samples were assessed subjectively after *in vitro* storage at 5°C for 24, 48, 72 and 96 hours as regards sperm motility using a phase-contrast microscope (400x magnification) with a warm stage maintained at 37°C. A wet semen mount was made using a drop of semen placed on a microscopic slide and for each sample five microscopic fields were examined. The mean of the five successive evaluations was recorded as the final motility score.

Assessment of Acrosome status and spermatozoa abnormality: Acrosome status

and spermatozoa abnormality were evaluated with eosin-nigrosin smears. At the end of every 24 hours of storage, 3µl of semen sample was placed on a microscopic slide, 2µl of eosin-nigrosin was dropped on it, and a smear was made using a microscopic slide. The proportion of sperm cells with intact acrosome was estimated under a phase-contrast microscope. Morphological examination of the sperm cells was carried out (Bearden and Fuquay, 1997) and abnormalities of sperm cells were observed under a phase-contrast microscope.

Determination of Lipid Peroxidation: At the end of every 24 hours, the level of malondialdehyde (MDA) in the chilled semen was measured by determining the thiobarbituric acid reactive substances (TBARS) according to Buege and Steven (1978). For this assay, 0.1ml of sperm suspension was incubated with 0.1 ml of 150 mM Tris-HCl (pH 7.1) for 20 minutes at 37°C. Subsequently, 1ml of 10% trichloroacetic acid (TCA) and 2 ml of 0.375% thiobarbituric acid was added followed by incubation in boiling water bath for 30 minutes. Thereafter, it was centrifuged for 15 minutes at 3000 rpm inside the blank tube. The absorbance was read in Spectrophotometer at 532 nm.

Data Analysis: Data obtained were subjected to 2-way analysis of variance and means separated by Duncan Multiple Range Test (Duncan, 1955) in SPSS version 16 using the following model:

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

Where,

Y_{ijkl} = Dependent variables

μ = Population mean

A_i = effect due to i^{th} fruit juices, $i = 1, 2, 3$

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, $k = 0, 24, 48, 72, 96$

$(AL)_{ij}$ = 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

$ijkl$ = Experimental Error

Results

Progressive motility

The effect of different fruit juices on spermatozoa motility of post-chilled RS buck spermatozoa is presented in Table 1. The results showed highest progressive motility in watermelon juice at 2.5% during the first 24 hours of storage while the lowest progressive motility was recorded at various levels of pawpaw juice and the reduction was more pronounced at the higher levels ($P < 0.05$). Similarly, higher levels of tomato (7.5% and 10%) and watermelon (10%) resulted in reduced motility ($P < 0.05$). The results showed that 5% and 2.5% tomato and watermelon juices respectively had higher ($P < 0.05$) progressive motility after 48 hours of storage compared to pawpaw juice and the control. Progressive motility in watermelon juice was higher ($P < 0.05$) at various levels after 48, 72 and 96 hours of storage compared to pawpaw and tomato juices and the control except at 10%. The results showed that 5% tomato juice had the highest progressive motility ($P < 0.05$) among the various levels of tomato juice

inclusion in the extender. Progressive motility at 96 hours was zero for the control and pawpaw juice except at 5% compared to semen extended with the tomato and watermelon fruit juices ($P < 0.05$). After 48 hours of storage, extender supplemented with watermelon and tomato juices had better progressive motility compared to control except 7.5% and 10% tomato juice ($P < 0.05$).

Acrosome Status: The effect of different fruit juices on acrosome status of post-chilled RS buck spermatozoa is presented in Table 2. Irrespective of level of juice in the extender, the percentage of intact acrosome was similar among the various juice and control.

Percentage abnormalities: The effect of different fruit juices on percentage abnormalities of post-chilled RS buck spermatozoa is presented in Table 3. Percentage abnormalities varied among the fruit juices and the control. The results showed that spermatozoa extended with watermelon juice had the lowest ($P < 0.05$) percentage abnormalities compared to other extenders and control at 24, 48, 72 and 96 hours of storage. At 72 and 96 hours of storage, spermatozoa extended with pawpaw juice had higher percent of spermatozoa abnormality compared to the other fruit juice and control.

Lipid peroxidation: The effect of different fruit juices on concentration of malondialdehyde (MDA) as indices of LPO of post-chilled RS buck semen is presented in Table 4. Significant reductions in MDA concentrations were achieved by addition of fruit-rich antioxidants to Tris-egg yolk based extender during the first 72 hours and

Table1: Progressive motility (%) of Red Sokoto buck spermatozoa extended with tris-egg yolk extender supplemented with fruit juices

Duration (h)	Contro 1	Pawpaw (%)					Tomato (%)					Watermelon (%)					SEM
		2.5	5	7.5	10		2.5	5	7.5	10		2.5	5	7.5	10		
24.00	73.33 ^b	33.33 ^c	10.00 ^{ef}	0.00 ^f	0.00 ^f		66.67 ^c	76.66 ^b	66.66 ^c	53.33 ^d		83.33 ^a	70.00 ^b	73.33 ^b	69.67 ^b	2.85	
48.00	63.33 ^b	26.67 ^d	6.67 ^e	0.00 ^f	0.00 ^f		53.33 ^c	66.67 ^a	40.00 ^c	50.00 ^c		66.66 ^a	66.67 ^a	70.00 ^a	62.66 ^b	3.72	
72.00	53.33 ^b	0.00 ^e	6.67 ^d	0.00 ^e	0.00 ^e		50.00 ^b	60.33 ^a	35.33 ^c	46.67 ^e		63.33 ^a	62.67 ^a	63.00 ^a	50.00 ^b	3.34	
96.00	0.00 ^f	0.00 ^e	3.33 ^c	0.00 ^f	0.00 ^f		10.00 ^d	50.00 ^b	16.67 ^d	33.33 ^c		53.33 ^a	56.66 ^a	53.33 ^a	26.67 ^e	3.33	

^{abcde f} Values within rows with different superscripts differ significantly (P<0.05), SEM = Standard Error of Means

Table2: Acrosome status (%) of Red Sokoto bucks spermatozoa extended with tris-egg yolk extender supplemented with fruit juices

Duration (h)	Contro 1	Pawpaw (%)					Tomato (%)					Watermelon (%)					SEM
		2.5	5	7.5	10		2.5	5	7.5	10		2.5	5	7.5	10		
24.00	98.00	95.27	93.33	93.00	98.00		93.33	97.00	96.67	96.66		96.66	98.00	97.00 ^a	96.66	0.96	
48.00	93.33	91.93	90.00	90.33	96.67		86.67	96.67	91.90	90.00		96.67	96.67	90.00 ^{ab}	96.67	1.07	
72.00	93.33	89.68	88.57	80.00	93.33		86.67	86.67	83.33	90.00		93.33	93.33	90.00 ^{ab}	93.33	1.60	
96.00	83.33	86.67	76.67	76.67	90.00		83.33	73.33	70.00	78.57		83.87	86.67	87.77 ^b	86.67	1.65	

SEM = Standard Error of Means

Table 3: Percentage abnormality of Red Sokoto bucks spermatozoa extended with tris-egg yolk extender supplemented with fruit juices

Duration (h)	Control	Pawpaw (%)				Tomato (%)				Watermelon (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	2.5	5	7.5	10	
24.00	2.67 ^c	6.67 ^b	3.00 ^c	10.00 ^a	10.00 ^a	3.33 ^c	2.33 ^c	4.67 ^b	4.66 ^b	1.67 ^c	3.33 ^c	2.67 ^c	3.33 ^c	0.44
48.00	4.66 ^b	7.33 ^a	9.66 ^a	10.00 ^a	96.67	4.66 ^b	4.67 ^b	8.00 ^a	7.33 ^a	4.67 ^b	3.33 ^b	3.00 ^b	3.33 ^b	0.46
72.00	6.33 ^c	13.33 ^a	13.00 ^a	13.33 ^a	10.00 ^a	9.00 ^b	5.66 ^c	8.66 ^b	10.00 ^b	4.67 ^c	4.00 ^c	3.67 ^c	5.00 ^c	0.88
96.00	16.66 ^b	26.67 ^a	15.67 ^b	20.00 ^a	13.33 ^a	7.00 ^c	10.00 ^b	14.00 ^b	12.67 ^b	7.33 ^c	5.33 ^c	4.67 ^c	7.33 ^c	1.44

^{a b c} Values within rows with different superscripts differ significantly ($P < 0.05$), SEM = Standard Error of Means

Table 4: MDA (nmol/ml) levels of Red Sokoto bucks spermatozoa extended with tris-egg yolk extender supplemented with fruit juices

Duration (h)	Control	Pawpaw (%)				Tomato (%)				Watermelon (%)				SEM
		2.5	5	7.5	10	2.5	5	7.5	10	2.5	5	7.5	10	
24.00	0.73 ^{ab}	0.74 ^{ab}	0.62 ^{ab}	0.55 ^b	0.35 ^c	0.56 ^b	0.36 ^c	0.56 ^b	0.8 ^{ab}	0.85 ^{ab}	1.00 ^a	0.86 ^{ab}	0.82 ^{ab}	0.07
48.00	0.63 ^a	0.39 ^c	0.38 ^c	0.54 ^b	0.37 ^c	0.2 ^c	0.39 ^c	0.67 ^a	0.45 ^a	0.56 ^b	0.87	0.53 ^a	0.51 ^a	0.11
72.00	0.93 ^{ab}	0.51 ^c	0.58 ^c	0.39 ^d	0.42 ^d	0.71 ^b	0.81 ^{ab}	0.74 ^b	0.53 ^c	0.93 ^{ab}	0.67 ^b	0.80 ^{ab}	1.02 ^a	0.18
96.00	0.77 ^{ab}	1.35 ^a	1.01 ^a	1.29 ^a	0.78 ^{ab}	1.69 ^a	0.95 ^{ab}	1.82 ^a	0.96 ^{ab}	1.32 ^a	1.24 ^a	1.50 ^a	1.12 ^a	0.34

^{a b c d} Values within rows with different superscripts differ significantly ($P < 0.05$), SEM = Standard Error of Means

the reduction was much pronounced in extender supplemented with pawpaw juice compared to control ($P < 0.05$).

Discussion

Progressive motility: The supplementation with fruit-rich antioxidants to preservation medium was performed to protect the sperm against damages caused by ROS to motility and viability (Bilodeau *et al.*, 2002). The inclusion of pawpaw, water melon and tomato juices in this study for preservation of Red Sokoto bucks spermatozoa indicated that the juices had ability to sustain sperm motility. This could probably be attributed to the high level of antioxidants such as Vitamin C and E present in these fruits (Djuric and Powell, 2001; Gebhardt and Thomas, 2002). This is in agreement with the finding of Reza *et al.* (2011) that sperm motility was significantly higher in semen samples extended with Vitamin C compared with the control group. Vitamin C protects the spermatozoa by preventing endogenous oxidative DNA and membrane damages. Moreover vitamin C is known to work by scavenging superoxide anions and singlet oxygen, and can protect lipoproteins from detectable peroxidative damage (Donnelly *et al.*, 1999). Furthermore, Raina *et al.* (2002) found that incorporation of vitamin C or E in tris-citric acid (TCA) based extender improved the motility of liquid buffalo bull semen. Yildiz and Daskin (2004) reported a positive effect on the maintenance of motility during cooled storage of ram semen after addition of ascorbic acid. Bhakat *et al.* (2011) also reported better motility compared to the control at the inclusion of Vitamin E in Karan Fries bulls semen. Supplementation of ascorbic acid and α -tocopherol in semen extender has also been reported to improve the quality of cryopreserved Nili-Ravi buffalo semen (Andrabi *et al.*, 2008).

Better progressive motility recorded at the inclusion of watermelon compared to tomato and pawpaw juice could probably be due to high level of lycopene present in watermelon. Watermelon (*Citrullus lanatus*) is a member of the Cucurbitaceae family. It is an excellent source of the potent carotenoid antioxidant "lycopene". This powerful antioxidant travels through the body neutralizing free radicals (Erhardt *et al.*, 2003). A cup of watermelon provides 24.3% of the daily value of vitamin C, and through its beta carotene, 11.1% of the daily value of vitamin A (Edwards *et al.*, 2003). The antioxidant function of lycopene is its ability to help protect cells and other structures in the body from oxygen damage and protection of DNA inside white blood cells has been linked to antioxidative role of lycopene (Edward *et al.*, 2003).

Higher level of vitamin A, lycopene and β -carotene (lipophilic antioxidants) found in watermelon (USHHS, 1995) is implicated for the higher motility observed in watermelon juice compared with pawpaw juice and tomato juice at 96 hours of storage. The reason for the low sperm motility when pawpaw juice was added to the semen particularly at the higher levels is unclear. Ripe pawpaw was used in this present study, hence activity of enzyme papain in pawpaw could not have been accounted for the reduced motility as the papain, a protein-dissolving enzyme are found in unripe papaya fruit (Hewitt *et al.* 2002), and has been reported to dissociate cells in the first step of cell culture preparations, breaks down extracellular matrix molecules holding the cells together and its activity lead to complete lysis of cells (Lopes *et al.* 2007). One possible reason however may be that its activity

possibly reached the higher level above necessary level to promote an improvement in sperm motility. The results suggest that the higher levels of pawpaw juice might have been toxic to the integrity of sperm membrane. Futino *et al.* (2010) reported that despite having a protective effect, some substances in semen diluents at higher concentrations can become harmful to spermatozoa due to their potential toxicity. This is also evidenced in semen samples extended with higher levels of tomato and watermelon juices. Moreover several studies reported that papaya fruits contained low phenolic compounds and low antioxidant capacity (Patthamakanokporn *et al.*, 2008). In contrast, Harris (2008) reported that pawpaw contains compounds such as phenolics, polyphenolics like flavonoids, ascorbic acid and phenolic compounds when tested *in vitro* for antioxidative activity and have been positively correlated with radical scavenging and reducing ability. The reducing potential and radical scavenging ability are highest in ripe and lowest in overripe pulp (Harris, 2008). Pawpaw, a climacteric fruit, ripens very quickly and ripeness level of the pawpaw has an effect on the quantity of antioxidative compounds such as phenolics (Harris, 2008). Harris (2008) reported that as the pawpaw ripens, the concentrations of these compounds decline and so the ability of the antioxidants to scavenge radicals and reduce metal ions. Pawpaw used in this study was fully ripe which probably accounted for the low spermatozoa motility observed.

Acrosome Status: Irrespective of level of

juice in the extender, the percentage of intact acrosome was similar among the various juice and control. Appreciable values though comparable to the control group were however obtained for intact acrosome following the inclusion of fruit juices in semen extender and indicated reduced damage to the sperm cells during storage. The results obtained could be attributed to the protective ability of vitamin C, vitamin E and β -carotene in the diluents. This agreed with the work of Thuwanut *et al.* (2008) who reported that cysteine or vitamin E supplementation of tris-egg yolk extender improved motility and integrity of the sperm membrane and DNA of frozen-thawed epididymal cat spermatozoa. Furthermore, supplementing the freezing extender with antioxidants, especially vitamin E, has been reported to have beneficial effects on acrosome integrity (Andrabi *et al.*, 2008).

Percentage abnormalities: In this study, sperm abnormalities varied in all the experimental extenders. This is in contrast to the report of Revell (2003) that semen processing does not necessarily increase the proportion of spermatozoa abnormality. However, the percentage spermatozoa abnormalities observed were within the range for post-thawed goat semen as per the Brazilian College of Animal Reproduction (Henry and Neves, 1998) in all the treatments and control. Moreover, watermelon juice surpassed other treatments with respect to reduced sperm abnormality. At the cellular level, hydrophilic antioxidants are found in the cytoplasm, whereas lipophilic antioxidants are found in cell membranes (Halliwell and Gutteridge, 2006). Watermelon is better enriched in lipophilic antioxidants (USHHS, 1995), this makes watermelon juice a more potential good source of

antioxidants for semen preservation compared with pawpaw and tomato juice.

Lipid peroxidation: Significant reductions of MDA concentrations were achieved by addition of fruit-rich antioxidants to Tris-egg yolk based extender compared to control in this study. This reduction following inclusion of the fruit juices comparable to control indicated the high anti-oxidant protective ability of these fruit juices and this was probably due to carotenoids present in these fruits. It also supports and extends previous reports suggesting that carotenoids have a protective effect against ROS mediated perturbation in sperm quality (Hekimoglu *et al.*, 2009). Carotenoids such as beta-carotene and lycopene form an important component of the antioxidant defense (Gupta and Kumar, 2002). Beta-carotenes have been reported to protect the plasma membrane against lipid peroxidation (Martin *et al.*, 1996).

Therefore, it is possible that the presence of carotenoid (source of vitamin A) in the fruit juices of *watermelon*, *tomato* and *pawpaw* attenuated lipid peroxidation and its associated oxidative stress in this study by two pathways in line with Al-Reza *et al.* (2009): either by increasing the activity of glutathione peroxidase followed with rapid conversion of H_2O_2 to H_2O and subsequent preventing H_2O_2 accumulation or by quenching the hydroxyl radicals that trap HO leading to oxidative breakdown of the carotenoid molecule (Al-Reza *et al.*, 2009). It thus seems that these fruit juices may protect the membrane of sperm cells against oxidative damage. In this context, our finding further indicates that supplementation of Tris-egg yolk extender with fruit juice from watermelon, tomato and pawpaw significantly prevents LPO suppressive effects on the metabolic activity of buck spermatozoa.

Supplementation of cryopreservation extenders with antioxidants has been shown to provide a cryoprotective effect on bull, ram, goat, boar, canine, and human sperm quality, thus improving semen parameters after thawing (Bucak *et al.*, 2010). The results of the present study therefore suggest that antioxidants from these fruits possibly worked 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) and consequently, the chilling of semen in the extenders containing fruit juices possibly prevented premature capacitation and subsequent cell death. This effect may explain the current findings of higher motility and lower LPO when *watermelon* and *tomato* fruit juices were added to the extender.

Conclusion

The findings of this study showed that extension of spermatozoa-with watermelon and tomato fruit juices had better progressive motility, reduced spermatozoa abnormality and reduced LPO compared to control. Fruit-rich antioxidants from watermelon and tomato have protective and preservative ability to maintain sperm viability of RS buck semen during liquid storage.

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