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Supplementation of Antioxidant BHT to Different Bull Semen Extenders Enhances Semen Quality after Chilling

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ABSTRACT

The effects of adding various concentrations of antioxidant, butylated hydroxytoluene (BHT) on chilled bull semen for 72 h at 4°C in Bioxcell® (BX), Tris egg-yolk- (TEY) and citrate egg-yolk- (CEY) based semen extenders were studied. Twenty-two ejaculates collected from three bulls, each extended using three extenders with BHT at 0 (control), 0.5, 1.0, 1.5, 2.0 and 3.0 mM/mL, were evaluated for sperm quality parameters. Extended semen was packaged into 0.25 mL straws containing 20 x 10^6 spermatozoa and chilled for 72 h. Four random straws each from the control and treatment groups were warmed at 37°C, pooled and evaluated using a computer-assisted semen analyser (IVOS Hamilton Thorne Biosciences) for general and progressive motilities, and for morphology, viability and acrosome damage using eosin-nigrosin stain under phase-contrast microscope. Results showed that sperm morphology, viability and protection of acrosome damage were significantly improved ($p<0.05$) at BHT concentrations of 0.5 in BX and 1.0 mM/mL in TEY and CEY compared to the controls. The BHT also showed a potential positive effect on progressive motility at 0.5 mM/mL in BX and 1.0 mM/mL in TEY and CEY. High concentrations of BHT (2.0 and 3.0 mM/mL), however, produced deteriorative effects on the sperm parameters in all the extenders. In conclusion, BHT when added at 0.5 mM/mL in BX and 1.0 mM/mL concentration to TEY and CEY extenders

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improved the quality parameters of bull chilled semen.

Keywords: antioxidant, bull, butylated hydroxytoluene, extender, oxidation, semen

INTRODUCTION

Reproductive specialists have tried using fresh or chilled semen for artificial insemination (AI) with the view of preserving the quality of spermatozoa. Research revealed that while fresh bull semen was viable at body temperature for a few hours, semen chilled between 4°C and 8°C was viable for up to 24 h at best, without significant decrease in fertilisation rate. The low temperature is needed to reduce sperm metabolism, minimising substrate utilisation by the cell, thereby prolonging its survival time. Though semen has been kept successfully in the short-term by chilling, some adverse effects have been identified, which include depressed motility, decreased viability and structural integrity as well as reduced conception rate (Lemma, 2011).

In an effort to improve semen storage standards and the fertility potential of fresh bull semen, current studies are now focussed on the effects of adding antioxidants such as butylated hydroxytoluene (BHT) to extended semen. Antioxidants primarily protect spermatozoa from debilitation as a result of oxidation, which is caused by higher concentrations of oxidising agents than is needed within the spermatozoa. Oxidising agents such as reactive oxygen species (ROS) are produced by normal cells and small quantities are required for the fertilisation process. The production of ROS is usually increased by injured, dead and phagocytic cells. Low temperature such as experienced during cooling leads to physical injuries to spermatozoa, affecting its structural integrity and resulting in an increase in ROS production and impairment of sperm quality parameters (Medeiros et al., 2002). Natural antioxidants such as superoxide dismutase (SOD) are usually secreted within the sperm plasma to control the excess ROS production but their effect becomes minimal with sperm extension (Ijaz et al., 2009). Butylated hydroxytoluene is a lipid soluble antioxidant and synthetic analogue of Vitamin E. Studies have reported effects on semen quality parameters after addition of BHT into chilled different extenders compared to untreated controls in species such as turkey (Donoghue & Donoghue, 1997), goat (Khalifa et al., 2008) and dog (Sahasha et al., 2011). Being lipophilic gives BHT unique properties that allow it to dissolve easily in sperm cytoplasm, increase intra-cytoplasmic fluidity and exert its effects both from within and outside the sperm cell (Asadpour & Tayefi-Nasrabadi, 2012), thereby improving the viability of spermatozoa by protecting the sperm cell membrane. So far, there has been little information on antioxidant effects of BHT on chilled bull semen. Therefore, the aim of this study was to evaluate the effects of various concentrations of BHT on bull semen's quality such as general motility, progressive motility, morphology, viability and acrosome integrity extended in three different semen extenders.

MATERIALS AND METHODS

Animals

Semen samples were collected from three sexually mature and fertile crossbred bulls: Simmental-Brangus, Brangus-Hereford and Kedah-Kelantan-Brangus at the Universiti Putra Malaysia (UPM) farm. The bulls were 5.3±0.3 years old and 649.3±9.7 kg in body weight. All the bulls were maintained under uniform management, fed with Surinam grass (*Brachiaria decumbens*) *ad libitum*, and supplemented with commercial cattle concentrate containing approximately 16% crude protein and 2.6% crude fat, given at a rate of 3 kg/bull/day. They were also given mineral licks and water *ad libitum*.

Semen Collection and Preparation of Extenders

A total of 24 ejaculates were collected during the experiment; two did not pass the inclusion criterion. Therefore, the result representing data from 22 ejaculates were collected using electroejaculation (Electrojac 5. NEOGEN®, Lexington USA), with seven ejaculates from two bulls each and eight from the other. Two ejaculates were collected at four-day intervals. The samples were kept in a Coleman cooler box containing warm water at 37°C for transportation to the laboratory and pre-chilling evaluation. Each ejaculate was processed and treated separately. To minimise a possible effect of individual bull differences, a minimum semen quality criterion as described by Khumran et al. (2015) was followed before processing. Samples were then extended with Bioxcell® (BX), Tris egg yolk (TEY) and citrate egg yolk (CEY), respectively, each of which was further subdivided into six groups. The respective extended semen groups were poured into dried, pre-warmed test tubes containing BHT antioxidant (prepared in ethanol) to make up 0, 0.5, 1.0, 1.5, 2.0 and 3.0 mM/mL BHT concentrations. These mixtures were then left in a water bath at 37°C for 5 min to allow for proper uptake of BHT by spermatozoa before cooling (Ijaz et al., 2009).

Bioxcell®, a commercial semen extender (IMV, France), was diluted at 1:4 in distilled water according to the manufacturer's instructions, while TEY and CEY extenders were prepared according to Bearden et al. (2004) with a penicillin-streptomycin mixture (BP2959-50) as antibiotic at 0.01 mL/mL of extender. All the extenders were adjusted to pH 6.7 using the SevenEasy pH meter (Mettler Toledo Ltd., England). Semen was extended to adjust the concentration of spermatozoa to $20x10^6$ per 0.25 mL mini straws at 37°C, and was then slowly chilled to 4°C over a two-hour period. First, the test tubes containing extended semen were held for 30 min in a beaker of water at 37°C while loaded into the chiller. Secondly, straws were packed with the chilled semen under a 4°C working environment and kept at the same temperature until analysis.

Evaluation of Fresh Semen Quality

Motility and concentration were determined by CASA (Computer Assisted Semen Analyser, IVOS Hamilton Thorne Biosciences). Ten µL of diluted samples in

0.85% normal saline were placed on 20 µm Hamilton Thorne research 2X-cell-glass slides and loaded on the CASA for analysis (Yimer et al., 2011). At least 200 sperm cells from an average of 10 fields were counted per reading.

Sperm morphology was determined by eosin-nigrosin (E & N) stain as described by Memon et al. (2011) and then air dried. Stained slides were viewed under a phase-contrast microscope at 1000x magnification (oil immersion). Two hundred spermatozoa were counted from an average of four microscopic fields. Spermatozoa showing complete normal structures having a smooth-oval head with clearly defined acrosome joined to the tail by the mid-piece without any visible defect were considered normal. Large/small head sizes, abnormal mid-piece, presence of droplets, broken parts, double head and/or tail were considered abnormalities and therefore, sperm cells showing any of these characteristics were considered abnormal (Nagy et al., 2013).

Post-Chilling Evaluation

Samples were evaluated after 24, 48 and 72 h of chilling by randomly selecting four straws from each BHT concentrations treatment and control once each time. The straws were warmed in a water bath at 37°C for 30 s, pooled together and analysed using CASA for general and progressive motilities. The E & N stain was used to determined morphology, viability and acrosome damage. Spermatozoa with clear white heads that did not take up the stain were identified as viable, whereas those that showed partially/completely purple-coloured heads were non-viable. The percentage of acrosome damage was counted according to the method proposed by Yildiz et al. (2000) in order to assess acrosomal morphology.

Statistical Analysis

The statistical analysis system (SAS V 9.1, SAS Inst. Inc., Cary, North Carolina) was used for data analysis. The means of the effects of various concentrations of BHT and control on the chilled semen parameters were compared by factorial ANOVA (using a 3x3x6 design). Duncan's test was used as the post hoc test. The data were checked for normality using the UNIVARIATE procedure of SAS software and the results were presented as means and standard error of the mean. Analyses were conducted at 95% confidence level; therefore, p values<0.05 were considered significant.

RESULTS

Interaction among BHT treatment concentrations, days of chilling and extenders used on sperm parameters (p-values) are presented in Table 1. The table shows that treatments, days and extender all independently influenced general and progressive sperm motility. Significant interaction between treatment, days and extender was noted, however, with regards to morphology, viability and acrosome damage.

Table 2 shows the effects of BHT on chilled bull semen quality parameters in the BX extender after three days of

Table 1
Interaction Effects Among Treatments, Days and Extenders (P Values)

Parameter (%)	Treatment (Trt)	Day (D)	Extender (Ext)	Trt*D	Trt*Ext	D*Ext	Trt*D*Ext
General motility	<.0001	<.0001	<.0001	0.9959	0.5036	0.7169	0.9763
Progressive motility	0.0001	0.0024	<.0001	0.9305	0.2289	0.2791	0.8909
Normal morphology	<.0001	<.0001	<.0001	0.0295	<.0001	0.0025	<.0001
Viability	<.0001	<.0001	<.0001	0.7513	<.0001	0.0674	<.0001
Acrosome damage	<.0001	<.0001	<.0001	0.2090	<.0001	0.0901	<.0001

Values less than 0.05 are considered significant interaction between factors

chilling. General motility was significantly higher ($p<0.05$) in the control and BHT concentrations of 0.5, 1.0 mM/mL compared to 3.0 mM/mL on days 1 and 2. In the control, BHT concentrations of 0.5, 1.0 and 1.5 mM/mL were significantly higher than 3.0 mM/mL on day 3. Progressive motility was best at BHT concentration of 0.5 mM/mL, significantly different with 3.0 mM/mL on day 1. Progressive motility at 0.5 mM/mL was also the same as 1.0, 1.5 mM/mL and control, significantly higher than 2.0, 3.0 mM/mL on days 2 and 3. Normal morphology was also the best in a BHT concentration of 0.5, same as 1.0 mM/mL, and significantly better compared to the control, 1.5, 2.0 and 3.0 mM/mL treatments during quality evaluation over three days. Similarly, BHT concentration of 0.5 was best for viability and acrosome damage, significantly higher compared to the controls and the rest of the treatments during the three days except for 1.0 mM/mL in viability on day 3 and 1.0 mM/mL in acrosome damage on day 1 where they were the same.

Table 2 also indicates that all parameters (except for general motility) were maintained without significant difference in the BX extender for the three days' quality evaluation at BHT concentration of 0.5 mM/mL. This was in opposition to BHT concentration of 3.0 mM/mL, in which all parameters (with the exception of viability) deteriorated daily in quality evaluation over the three days. Progressive motility, viability and acrosome damage were maintained in the control, while general motility and viability deteriorated daily in quality evaluation over the three days.

Table 3 shows the effects of BHT on chilled bull semen quality using a TEY extender after quality evaluation of the semen over the three days. Normal morphology, viability and acrosome damage were better at a BHT concentration of 1.0 mM/mL, significantly higher compared to the controls and BHT concentrations

Table 2
Effect of BHT on Chilled Bull Semen Quality in Bioxcell® Extender Kept for 3 Days

Parameter %	Day	Control	BHT concentrations (mM/mL)				
			0.5	1.0	1.5	2.0	3.0
General motility	D1	68.87±4.28^{a,y}	68.80±4.40^{a,y}	67.27±4.38^{a,y}	62.80±5.19^{ab}	56.80±6.48^{ab,y}	49.40±6.16^{b,x}
	D2	61.40±3.70^{a,xy}	61.73±5.10^{a,xy}	62.27±5.10^{a,xy}	54.13±5.15^{ab}	43.40±5.18^{bc,xy}	35.20±5.38^{c,xy}
	D3	54.13±4.93^{a,z}	52.07±4.90^{a,z}	51.00±4.93^{ab,z}	47.73±5.36^{ab}	36.47±4.74^{bc,z}	29.53±4.78^{c,z}
Progressive motility	D1	23.89±2.84^{ab}	27.78±4.29^{a}	27.00±3.54^{a}	25.44±3.59^{ab}	17.56±2.85^{ab}	16.11±2.22^{b,x}
	D2	20.56±2.80^{abc}	28.78±7.01^{a}	22.78±2.81^{ab}	18.56±3.02^{abc}	14.11±2.14^{bc}	10.33±1.83^{c,xy}
	D3	19.22±2.72^{a}	20.89±2.65^{a}	20.56±2.76^{a}	17.67±2.90^{ab}	11.22±2.34^{bc}	7.11±1.98^{c,y}
Normal morphology	D1	84.33±0.57^{b,x}	92.67±1.19^{a}	89.33±0.71^{ab,x}	86.67±1.07^{b,x}	78.67±2.79^{c,x}	77.83±2.63^{c,x}
	D2	82.50±0.90^{b,xy}	90.67±1.18^{a}	87.00±0.92^{ab,xy}	84.33±0.81^{b,xy}	72.33±2.67^{c,xy}	70.00±2.89^{c,y}
	D3	81.50±0.93^{b,y}	90.50±0.88^{a}	86.17±0.86^{ab,y}	83.67±0.81^{b,y}	69.17±2.85^{c,y}	66.83±2.33^{c,y}
Viability	D1	76.83±0.81^{cb}	85.67±1.02^{a}	79.83±1.21^{b}	78.33±1.93^{b}	72.33±2.80^{cd}	71.17±2.38^{d}
	D2	76.50±0.87^{b}	85.83±0.93^{a}	79.67±1.38^{b}	76.17±1.88^{b}	68.33±3.06^{c}	66.83±3.24^{c}
	D3	76.17±0.98^{b}	83.67±1.81^{a}	78.83±1.49^{ab}	76.83±1.53^{b}	65.83±3.10^{c}	64.00±3.30^{c}
Acrosome damage	D1	16.17±1.02^{a}	3.17±0.93^{c}	5.17±1.15^{bc,x}	8.67±1.29^{b,x}	17.00±1.55^{a,x}	19.33±1.61^{a,x}
	D2	17.33±0.92^{b}	3.50±1.00^{d}	7.00±0.97^{d,xy}	11.17±1.43^{c,xy}	21.50±2.02^{a,xy}	21.83±1.34^{a,y}
	D3	17.50±1.11^{c}	5.00±1.11^{f}	9.17±0.77^{e,y}	13.50±1.14^{d,y}	24.83±1.50^{b,y}	28.33±1.10^{a,y}

Data are expressed as Mean ±Standard Error (SE) n=22. Different superscripts a-f within rows and x-z within columns denote significant differences ($p<0.05$)

Table 3
Effect of BHT on Chilled Bull Semen Quality in Tris Egg Yolk Extender Kept for 3 Days

Parameter %	Day	Control	BHT concentrations (mM/mL)				
			0.5	1.0	1.5	2.0	3.0
General motility	D1	58.47±4.00^{x}	61.93±3.81^{x}	61.07±4.22^{x}	55.87±4.16^{x}	53.33±4.38^{x}	51.73±4.69^{x}
	D2	44.60±3.34^{y}	50.47±4.21^{xy}	50.53±4.41^{xy}	41.67±5.48^{xy}	38.07±5.54^{y}	35.40±5.17^{y}
	D3	42.00±4.22^{y}	42.87±4.54^{y}	43.93±4.67^{y}	38.73±5.32^{y}	32.87±5.61^{y}	29.67±5.65^{y}
Progressive motility	D1	23.00±2.61	25.33±2.52	29.00±2.06	28.22±2.61	25.11±2.80	20.78±2.73
	D2	24.11±2.84	23.22±2.77	27.44±2.41	25.89±3.41	20.56±2.56	18.56±2.65
	D3	21.11±3.12	21.56±2.64	27.11±2.53	24.33±3.03	19.33±2.32	37.67±22.81
Normal Morphology	D1	91.67±0.77^{b,x}	90.83±0.68^{b}	94.50±0.54^{a}	91.33±1.15^{b}	87.83±0.82^{c,x}	86.83±0.59^{c,x}
	D2	89.00±1.14^{bc,xy}	89.50±1.40^{b}	93.17±0.71^{a}	90.67±1.08^{ab}	86.33±0.83^{cd,x}	84.83±0.51^{dy}
	D3	86.83±1.26^{b,y}	87.50±1.18^{b}	93.17±0.68^{a}	90.50±0.90^{a}	82.50±0.79^{c,y}	81.50±0.69^{c,z}
Viability	D1	59.50±2.73^{bc}	66.83±2.59^{a}	67.83±1.26^{a}	65.00±2.58^{ab}	54.67±0.93^{cd,x}	49.67±0.51^{d,x}
	D2	59.17±2.46^{b}	65.83±2.41^{a}	68.17±1.74^{a}	63.33±2.85^{ab}	51.00±0.65^{c,y}	47.00±0.65^{c,y}
	D3	56.83±2.36^{b}	63.17±1.97^{a}	67.00±1.71^{a}	64.67±1.75^{a}	47.67±0.75^{c,x}	43.00±0.43^{d,z}
Acrosome damage	D1	9.83±2.28^{bc}	12.67±2.44^{ab}	4.50±0.97^{c}	9.17±2.13^{bc}	16.67±1.45^{a,x}	17.17±1.60^{a,x}
	D2	12.67±2.04^{b}	13.50±2.27^{b}	5.33±1.48^{c}	10.33±2.39^{cb}	20.67±1.64^{a,xy}	21.83±1.51^{a,xy}
	D3	15.83±1.98^{b}	15.00±2.16^{b}	7.50±2.00^{c}	13.17±1.97^{cb}	24.50±2.03^{a,y}	26.33±1.99^{a,y}

Data are expressed as Mean ±Standard Error (SE) n=22. Different superscripts a-f within rows and x-z within columns denote significant differences ($P<0.05$)

of 2.0 and 3.0 mM/mL for all three days. Even though there was no significant difference between treatments and controls for general and progressive motilities, BHT concentration of 1.0 gave the highest percentage during the three days of quality evaluation. All semen quality parameters deteriorated daily with BHT concentrations of 2.0 and 3.0 mM/mL, as well as all treatments in general motility over the three days of evaluation.

Table 4 shows the effects of BHT on chilled bull semen quality using the CEY extender after three days of quality evaluation. Progressive motility was not significantly affected by either treatment or days but the BHT concentration of 1.0 mM/mL gave the highest percentage among the groups in the entire duration of semen quality evaluation. Higher viability and less acrosome damage were obtained at the BHT concentration of 1.0 mM/mL, significantly different when compared to the controls, BHT concentrations of 2.0 and 3.0 mM/mL during the entire duration of semen quality evaluation. This was followed by general motility on day 3.

DISCUSSION

In this study, the effects of BHT added to BX, TEY and CEY on chilled bull semen were examined. It was found that optimum results were achieved when a certain amount of BHT was applied to a particular extender. Based on the results, 0.5 mM in BX and 1.0 mM/mL in TEY and CEY concentrations were found to increase sperm plasma membrane protection by improving the cells' normal morphology and viability and prevent acrosome damage when compared with the control groups. On the other hand, high BHT concentrations tended to produce opposing effects on the spermatozoa parameters in all the extenders. These findings (that BHT is useful at lower and detrimental at higher concentrations) are consistent with a number of previous studies done on different animals. BHT has been found to protect chilled semen in goats (Khalifa et al., 2008). Specifically, addition of BHT or other exogenous antioxidants have been found useful in chilled semen of various species of animals. Chilled turkey sperm viability was improved and loss of motility was reduced by addition of BHT (Donoghue & Donoghue, 1997). It was also reported that vitamin E, which is a natural analogue of BHT, improved viability of chilled canine spermatozoa (Michael et al., 2009). Moreover, selected antioxidants such as glutathione, cysteine and hypotaurine have been reported to improve viability and functional status in chilled boar semen (Funahashi & Sano, 2005).

Some researchers reported significantly increased motility in chilled semen of other animal species such as goat (Khalifa et al., 2008) and turkey (Donoghue & Donoghue, 1997) using BHT compared to the controls. In the present study, there was no significant difference between the treated groups and the control for general motility. However, the fact that BHT maintained consistently higher values of progressive motility at 0.5 mM in BX and 1.0 mM in TEY and CEY than the control may imply its potential

Table 4
Effect of BHT on Chilled Bull Semen Quality in Citrate Egg Yolk Extender Kept for 3 Days

Parameter %	Day	Control	BHT concentrations (mM/mL)				
			0.5	1.0	1.5	2.0	3.0
General motility	D1	49.80±5.46^{ab,x}	60.87±5.13^{a,x}	57.60±5.65^{ab,x}	52.33±4.67^{ab,x}	45.20±5.99^{ab,x}	42.47±5.65^{b,x}
	D2	36.27±3.94^{ab,y}	43.07±3.99^{a,y}	40.00±4.41^{ab,y}	33.80±3.78^{abc,y}	29.33±2.74^{bc,y}	23.33±3.18^{c,y}
	D3	22.33±3.30^{abc,z}	27.60±3.52^{ab,z}	29.87±3.08^{a,y}	24.27±3.44^{ab,y}	19.27±2.23^{bc,y}	14.20±2.54^{c,y}
Progressive motility	D1	23.00±3.46	25.67±2.66	27.44±3.16	25.11±3.60	18.78±3.32	17.00±3.73
	D2	18.11±3.43	18.89±3.16	21.67±4.35	18.11±4.15	13.00±3.31	11.44±3.51
	D3	15.44±4.02	17.89±3.89	18.89±3.95	15.78±4.21	11.89±3.45	10.22±3.33
Normal morphology	D1	89.17±0.21^{a,x}	90.00±0.25^{a}	89.00±0.43^{a}	88.33±0.71^{a}	69.17±2.35^{b,x}	64.17±1.28^{c,x}
	D2	86.00±0.98^{b,y}	89.83±1.35^{a}	87.67±0.57^{ab}	88.83±0.41^{ab}	63.33±1.50^{c,y}	58.67±0.38^{d,y}
	D3	83.33±1.44^{b,y}	86.33±1.85^{ab}	87.00±1.40^{a}	88.33±0.38^{a}	56.33±0.57^{c,z}	52.00±0.85^{d,z}
Viability	D1	58.00±1.50^{d,x}	67.00±0.65^{e,x}	78.33±1.88^{a}	73.00±1.50^{b,x}	57.33±0.99^{d,x}	54.83±1.17^{d,x}
	D2	50.67±0.28^{d,y}	65.33±0.62^{c,x}	74.83±1.24^{a}	69.00±1.76^{b,xy}	53.67±0.99^{d,y}	50.67±1.81^{d,x}
	D3	51.67±0.71^{d,y}	62.67±0.57^{c,y}	74.00±1.09^{a}	67.33±1.73^{b,y}	49.33±1.36^{d,z}	44.17±1.83^{e,y}
Acrosome damage	D1	17.33±1.58^{bc,x}	14.33±0.99^{cd,x}	9.17±0.77^{e,x}	11.67±0.14^{de,x}	19.33±1.26^{b,x}	24.33±1.48^{a,x}
	D2	20.33±1.78^{bc,y}	17.33±1.68^{c,y}	10.83±0.37^{d,y}	13.17±0.51^{d,y}	24.00±1.48^{ab,y}	27.33±1.36^{a,y}
	D3	25.67±1.86^{bc,y}	22.67±1.36^{c,y}	12.67±0.54^{e,y}	18.33±0.75^{d,y}	28.67±1.36^{b,z}	33.67±1.44^{a,y}

Data are expressed as Mean±Standard Error (SE) n=22. Different superscripts a-f within rows and x-z within column denote significant differences ($P<0.05$)

positive effect on progressive motility. Nevertheless, it is worth noting that bull semen is specifically more sensitive to refrigeration temperatures (between 4 and 8oC) compared with turkey and goat semen and, even though egg yolk is an excellent cold shock protectant, it cannot completely prevent cold damage in bulls (Parks, 2013) due to its high density lipoproteins. Therefore, we theorised that the damage caused by the low temperature might have affected sperm motility in this study, thereby masking the effect of BHT. Similarly, the measure of acidity or alkalinity (pH) is another factor that determines extracellular sperm motility (Carr & Acott, 1984). Even though decreases in sperm motility due to pH can be reversed by controlling the pH, it is unlikely that pH changes affected the differences observed in the current study since all the semen extenders were set at the common pH of 6.7 (De Pauw et al., 2003 ; Brannigan & Lipshultz, 2008).

The results of this study showed that most parameters were maintained without significant deterioration for three days at BHT concentrations of 0.5 in BX and 1.0 mM in TEY and CEY extenders, as opposed to other treatment groups and controls, where most parameters deteriorated daily. In this research, better results were achieved at a lower BHT concentration in BX than in TEY and CEY. This could be because while TEY and CEY extenders contain egg yolk as a cold shock protectant (Pace & Graham, 1974), Bioxcell® contains soybean lecithin instead, substituting egg yolk. Soybean-lecithin-based semen extenders such as AndroMed® and Biociphos Plus® have been reported to be superior in bull semen storage when compared with egg-yolk-based extenders (Akhter et al., 2010). This is not unconnected with the fact that soybean lecithin has a high content of low density lipoprotein, which makes it a better shock protectant and may require little synergism from an antioxidant. Moreover, density of the lipoproteins in the egg yolk used may affect sperm quality: low density lipoproteins protect spermatozoa from damage due to cold shock while high density lipoproteins in egg yolk result in reduction of respiration and sperm motility (Amirat et al., 2005). Since the quality of the egg yolk is not constant, the deleterious effect due to high density lipoproteins might have affected the spermatozoa in the TEY and CEY extenders. Furthermore, Khalifa et al. (2008) suggested that it is possible that BHT has higher solubility to egg lipids rather than the plasma membrane of the spermatozoa. Therefore, when BHT is added, much of its potency would affect the egg yolk, and very little would affect the spermatozoa membrane. This phenomenon may explain why higher concentrations of BHT were required for optimum effect in the egg-yolk based extender. However, if the BHT concentration had risen much higher than required such as 2.0 and 3.0 mM/mL in the current study, perhaps the BHT would have caused high fluidity within the cytoplasm of the spermatozoa beyond a critical limit, causing it to rupture.

The present study indicated less sperm damage during preservation in the TEY

than in the CEY semen extender. Even though the reason is not well understood, this is in line with previous studies carried out in other species such as buffalo bulls and rams, where significantly better sperm quality in TEY compared to CEY in chilled spermatozoa was seen (Akhter et al., 2010; Rakha et al., 2013).

CONCLUSION

In conclusion, addition of BHT at 0.5 concentration in Bioxcell and 1.0 mM/mL to Tris egg yolk and citrate egg yolk extenders improved chilled bull semen quality parameters, signalling that addition of BHT at this concentration has potential for protecting sperm progressive motility.

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