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Effects of Exogenous Glutathione Supplementation in Biocell® Extender on Quality of Cryopreserved Buffalo (*Bubalus bubalis*) Semen

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Abstract: The study was designed to investigate the effect of exogenous glutathione supplementation in soybean based extender Bioxcell® extender on post thaw semen quality of buffalo (*Bubalus bubalis*). Split pooled ejaculates (n = 6), possessing >70% visual sperm motility were extended at 37°C with different levels of glutathione (0.0, 0.5, 1 and 2 mM) in Bioxcell® extender. Semen was cooled to 4°C in 2 h, equilibrated at 4°C for 4 h, filled in 0.5 mL straws and frozen in a programmable cell freezer before plunging into liquid nitrogen. Thawing of frozen semen was performed after 72 h at 37°C for 30 sec. Sperm motion characteristics, viability, plasma membrane integrity, acrosome morphology of each semen sample immediately after thawing and incubation for 2 h were assessed by using Computer assisted semen analyzer (SCA), eosin-nigrosin staining, Hypo Osmotic Swelling (HOS) assay and phase contrast microscope, respectively. Results revealed that the addition 0.5 and 1.0 Mm of glutathione in Bioxcell® extender did not present any significant effect on overall and progressive motility as well as sperm motility characteristics (VAP, VSL, VCL, LIN and ALH), compared to the control groups ($p>0.05$). Immediately after thawing the proportion of post thaw sperm viability, plasma membrane integrity and normal apical ridge remained similar in all groups. However, glutathione supplementation of the extender with 2.0 mM concentration decreased sperm motility, viability at 0 and 2 h after thawing in a dose dependent manner compared to the control ($p<0.05$). The integrity of the sperm cells and percentage of spermatozoa with intact acrosome were not affected by the addition antioxidant ($p>0.05$). These results revealed that supplementation of the new commercial in soybean based extender Bioxcell® with glutathione did not improve sperm post thaw motility or acrosomal integrity.

Key words: Buffalo spermatozoa, cryopreservation, glutathione, semen quality, post-thaw motility

INTRODUCTION

There are many efforts in order to substitute egg yolk-based extenders by other synthetic semen diluents. A number of commercially available soya lecithin based extenders containing a substitute for egg yolk like AndroMed®, Biociphos plus® and Bioxcell® have been used for the cryopreservation of bovine, ovine semen that maintained better semen quality (Gil *et al*., 2000; Aires *et al*., 2003; Sariozkan *et al*., 2010; Akhter *et al*., 2011). Cryopreservation of buffalo semen in soya lecithin based extender Bioxcell® maintained the semen quality such motility, plasma membrane integrity and acrosmal integrity and produced acceptable fertility rates (Akhter *et al*., 2010). Keeping in view the poor freezability of bubaline semen attempts have been made to improve the basic buffers developed to minimize the deleterious effects of cryogenic procedures. There are few scattered studies that have used additives such as antioxidants for improvement in post thaw quality of buffalo spermatozoa (Andrabi, 2009). Semen contains appreciable amounts of antioxidants that balance lipid peroxidation and prevent excessive peroxide formation (Lewis *et al*., 1997).

A wide variety of antioxidants such as Glutathione (GSH), oxidized Glutathione (GSSG) have been tested to minimize the damage caused by cooling and freezing thawing on bull (Stradaioli *et al*., 2007; Uysal *et al*., 2007), bufallo (Ansari *et al*., 2010), ram (Bucak and Tekin, 2007) and goat (Sinha *et al*., 1996) semen.

Glutathione, a naturally occurring tri-peptide in semen plays an important role in scavenging reactive oxygen intermediates and other radicals with the help of the glutathione reductase/peroxidase cycle (Meister and Anderson, 1983). Glutathione has many important functions in the cellular physiology and metabolism including the protection of the cell from oxidative stress,

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synthesis of protein and DNA and gamete cell fertilization. Glutathione can influence cell metabolism through detoxication (De Matos and Furnus, 2000) and by preventing the formation of free radicals in spermatozoa (Wijaya, 1996).

It is pertinent to mention that glutathione as additive to semen extender. Glutathione, when added to spermatozoa diluents, decreased or prevented the emergence of free radicals which are harmful for the plasma membranes. As a result, the conceptional and pregnancy rates were improved (Triwulanningish *et al*., 2008).

As a positive relationship has been reported between glutathione levels and semen quality (Stradaioli *et al*., 2007; Gadea *et al*., 2004) it is suggested that improvement of post thaw quality of bovine semen (Gil *et al*., 2000; Stradaioli *et al*., 2007). Furthermore it has been reported that cryopreserved in Bioxcell® may be attributed to the higher concentrations of glutathione which protect the sperm against oxidative stress and reactive oxygen species. However, for buffalo semen Bioxcell® could not improve semen quality as compared to tris-citric egg yolk extender. During the freezing process, the increase in susceptibility of spermatozoa to lipid peroxidation as affected by cold shock, plays an important role in ageing of spermatozoa, shortening their life span and affecting the preservation of semen. Buffalo spermatozoa contain comparatively more unsaturated fatty acids than in other species, like arachidonic and decosahexaenoic acids which make them more vulnerable to lipid peroxidation (Nair *et al*., 2006).

It is suggested that buffalo bull spermatozoa is more prone to oxidative stress due to high contents of polyunsaturated phospholipids therefore, the level of glutathione in Bioxcell® is insufficient to protect the sperm from oxidative stress during freeze thawing process (Sansone *et al*., 2000). Recently, research has shown that supplementing glutathione in the tris-egg yolk extender medium improves cooled (Ansari *et al*., 2011) and post thaw (Ansari *et al*., 2010) semen quality in buffalo bulls. To the knowledge such a clear effect of glutathione supplementation in soybean lecithin based extenders on motion characteristics, plasma membrane integrity and viability, collectively has not been shown earlier in buffalo spermatozoa. Therefore, the objective of this study was to evaluate and compare to using different level of exogenous glutathione on quality of buffalo bull spermatozoa preserved in soya lecithin based extender such Bioxcell® for cryopreservation of buffalo (*Bubalus bubalis*) semen.

MATERIALS AND METHODS

Semen collection and freezing: The experiment was conducted at the Buffalo Breeding and Extension Training Center, Urmia, West Azerbaijan, Iran during the months of October and December. Bioxcell® was prepared according to manufacturer's instructions (IMV, France).

Two consecutive ejaculates were collected using artificial vagina (at 42°C) from four adult buffalo bulls (*Bubalus bubalis*) of known fertility and similar age (4-5 years) for a period of 6 weeks (replicate). Ejaculated semen from each bull was immediately transferred to laboratory. Sperm progressive motility was determined microscopically (x400; Olympus BX20, Tokyo, Japan) and sperm concentration was determined by digital photometer (IMV, France). To eliminate individual differences, semen samples from the four bulls were pooled. Each pooled sample was split into four equal aliquots at a concentration of 40×10^6 motile spermatozoa/1 mL at 37°C and embedded with different levels of glutathione (glutathione acid sodium alt, Sigma-Aldrich, 1 g), in Bioxcell® extenders (0, 0.5, 1 and 2 mM 100 mL^{-1}). Antioxidant free extender was used as a control. Diluted semen was cooled to 4°C in 2 h, equilibrated for 4 h at 4°C, filled in 0.5 mL French straws (IMV, France) with suction pump at 4°C in a cold cabinet unit (IMV, France) and placed in liquid nitrogen vapors, 5 cm above the level of liquid nitrogen for 10 min. Straws were then plunged and stored into liquid nitrogen (-196°C). After 72 h, four frozen straws from each group were thawed individually at 37°C for 30 sec in a water bath for evaluation.

Semen evaluation

Motility: An aliquot of semen (5 μL) was placed on a prewarmed (37°C) Makler chamber (depth 10 μm) and analyzed for sperm motion characteristics using a computer assisted sperm analyzer (Sperm Class Analyzer, Microptic; Barcelona, Spain). The CASA-derived motility characteristics were analyzed immediately after thawing and 4 h of incubation at 37°C. Four microscopic fields were analyzed in each sample using a phase contrast microscope ((Nikon, Tokyo, Japan) supplied with a prewarmed stage at 37°C and at 100x magnification. The total number of spermatozoa analyzed per sample ranged between 200 and 300. Objects incorrectly identified as spermatozoa were minimized on the monitor by using the playback function. Total motility was defined as the percentage of spermatozoa with mean velocity (VAP) >10 μm sec^{-1}. The CASA derived motility characteristics studied were percentage of motility and progressive motility, straight line velocity (VSL, μm sec^{-1}; the straight line distance from beginning to end of track divided by

time taken), average path velocity (VAP, μm sec^{-1}; the spatial averaged path that eliminated the wobble of the sperm head), curvilinear velocity (VCL, μm sec^{-1}; total distance traveled by a sperm during the acquisition divided by the time taken), Lateral Head Displacement (LHD, μm; deviation of sperm head from the average path), Linearity (LIN, %; VSL/VCL×100) and Straightness (STR, %; VSL/VAP×100) (Mortimer, 2000).

Sperm viability: Eosin-nigrosin staining was used to evaluate sperm viability (Felipe-Perez *et al*., 2008). After thawing one drop of the semen was placed on a tempered glass slide which was mixed with one drop of EoNig solution (0.2 g of eosin and 2 g of nigrosin were dissolved in a buffered saline solution (153 mM NaCl and 9.65 mM NaH_2PO_4, pH 7.4), mixed for 2 h at room temperature and filtered to obtain the staining media. The mixture was smeared on the glass slide and let air dried. The samples were observed under a light microscope. Eosin penetrates in non viable cells which appear red. Nigrosine offers a dark background facilitating the detection of viable, non stained cells. Four smears were performed in each frozen thawed semen sample immediately and after incubation for 4 h for each.

Sperm plasma membrane integrity: Sperm plasma membrane integrity was determined using Hypo Osmotic Swelling (HOS) assay. HOS solution was consisted of 0.73 g sodium citrate and 1.35 g fructose dissolved in 100 mL distilled water (osmotic pressure 190 mn Osmol kg^{-1}). To assess the sperm tail plasma membrane integrity, semen (50 μL) was mixed with of HOS solution (500 μL) and incubated for 30 min at 37°C before examining under phase contrast microscope (x400; Olympus BX20, Tokyo, Japan). Two hundred spermatozoa were assessed for their swelling ability in HOS. The swollen spermatozoa characterized by coiling of the tail were considered to have an intact plasma membrane (Jeyendran *et al*., 1984).

Normal acrosomes: To assess sperm acrosomal integrity, 100 μL of semen sample was fixed in 500 μL of 1% formal citrate (2.9 g tri-sodium citrate dihydrate, 1 mL of 37% solution of formaldehyde, dissolved in 100 mL of distilled water), 100 spermatozoa were examined with a phase contrast microscope (x1000; Olympus BX20, Tokyo, Japan) under oil immersion. Normal acrosome was characterized by normal apical ridge (Andrabi, 2009).

Statistical analysis: Each treatment was replicated 6 times. For each replicate, four straws were thawed and pooled for evaluation of sperm parameters. Analysis of Variance (ANOVA) was used for comparisons of means.

When the ANOVA test showed statistical differences, the mean of the treatments were compared using Duncan's multiple range tests. All the statistical ana lyses were performed using the SAS Software (Version 9.0, SAS Institute Inc., USA) and differences were considered significant at $p<0.05$ level. Results are presented as mean±standard deviation.

RESULTS

Motility: Supplementation of glutathione in semen extender had not significant effect on overall post thaw sperm motility and kinematic parameters as determined subjectively (Table 1). At the onset of incubation, proportions of overall and progressive motility thawed at 37°C for control, 0.5 and 1 mM glutathione extenders were 68.8±1.45, 24.7±1.62, 67.9±1.21% and 24.1±1.91, 65.9±1.87% and 22.5±1.91%, respectively ($p>0.05$). However, supplementation 2 mM of glutathione in semen extender significantly decreased post thaw overall and progressive sperm motility examined after thawing (53.5±1.72 and 18.6±1.13%), ($p<0.05$). After 2 h of incubation, proportions of overall and progressive motility decreased and were 51.7±2.52, 15. 9±2.13, 53.7±1.24% and 11.2±2.16, 49.3±1.6% and 13.9±3.32% and 38.3±1.6, 7.21±1.1% for samples thawed in control, 0.5, 1 and 2 mM glutathione, respectively ($p<0.05$). The negative correlation was not detected between sperm motility and type of extenders after 2 h incubation times (Table 2). The post thaw sperm motility were comparatively superior in soybean lecithin based extenders contain 0.5 and 1 and 2 Mm glutathione at this time ($p<0.05$). All kinematic parameters decreased during incubation but were not differ in all treatment groups at this time (Table 2).

Comparison of post-thaw sperm viability, plasma membrane integrity and acrosomal ridge: The data on

Table 1: Effect of Glutathione (GSH) supplementation in the Bioxcell® extender on motility and kinematics parameters of buffalo bull spermatozoa immediately after thawing at 37°C

	Groups (Glutathione mM^{-1})			
Parameters	Control	0.5	1	2
Motility (%)	68.8±1.450^a	67.9±1.21^a	65.9±1.87^a	53.5±1.72^b
PM (%)	24.7±1.620^a	24.1±1.43^b	22.5±1.91^b	18.6±1.13^b
VCL (μm sec^{-1})	39.3 ±1.79	36.3±1.95	35.1±1.74	33.3±0.88
VSL (μm sec^{-1})	15.7±2.310	16.1±2.59	16.6±3.59^c	13.1±1.00
VAP (μm sec^{-1})	21.5±2.620	20.3±2.94	21.1±4.23	17.8±1.09
LIN (%)	34.8±1.080	35.7±1.19	40.1±1.41	37.9±1.48
STR (%)	68.5±2.620	66.5±2.93	76.6±2.29	73.5±1.96
ALH (μm)	1.77±0.12	1.82±0.12	1.67±0.09	1.55±0.03
BCF (Hz)	14.2±1.530	14.3±1.71	14.1±1.68	13.3±1.13

Data are means±SEM; PM: Progressive Motile spermatozoa; VSL: Straight path Velocity; VCL: Curvilinear Velocity; VAP: Average path Velocity; LIN: Linearity; STR: Straightness; ALH: Amplitude of the Lateral movement of the Head, BCF: Beat Frequency; $^{a-c}$Values in the same row with different superscripts differ significantly ($p<0.05$)

Table 2: Effect of Glutathione (GSH) supplementation in the Bioxcell® extender on postthaw motility and kinematics parameters of buffalo spermatozoa after incubation for 2 h at 37°C

	Groups (Glutathione mM^{-1})			
Parameters	Control	0.5	1	2
Motility (%)	51.70±2.52[a]	53.70±1.24[b]	49.3±1.60[a]	38.30±1.60[b]
PM (%)	15.90±2.13[a]	11.20±2.16[a]	13.9±3.32[a]	7.20±1.21[b]
VCL (μm sec^{-1})	29.00±1.92	23.10±1.86	27.2±3.01	22.60±1.73
VSL (μm sec^{-1})	9.11±1.27	8.72±1.10	9.62±2.19	6.11±1.11
VAP (μm sec^{-1})	13.36±1.37	11.37±1.28	13.1±2.45	10.10±1.31
LIN (%)	29.50±2.48	26.40±2.08	32.6±3.62	27.60±2.79
STR (%)	67.40±2.22	57.20±1.55	63.1±2.45	59.70±2.86
ALH (μm)	1.49±0.07	1.58±0.21	2.01±0.71	1.57±0.21
BCF (Hz)	10.50±1.21	16.10±1.18	10.3±1.58	11.90±1.47

Data are means±SEM; PM: Progressive Motile spermatozoa; VSL: Straight path Velocity; VCL: Curvilinear Velocity; VAP: Average path Velocity; LIN: Linearity; STR: Straightness; ALH: Amplitude of the Lateral movement of the Head, BCF: Beat Frequency; [a-c]Values in the same row with different superscripts differ significantly ($p<0.05$)

Table 3: Effect of Glutathione (GSH) supplementation in the Bioxcell® extender on viability, plasma membrane integrity and normal apical ridge of buffalo spermatozoa after thawing at 37°C

	Groups (Glutathione mM^{-1})			
Parameters (%)	Control	0.5	1	2
Viability	67.9±3.8[a]	63.6±0.6[a]	62.7±1.5[a]	55.7±2.51[a]
PMI	47.5±2.5	50.5±2.4	51.5±1.3	49.5±1.30
NAR	78.7±2.3	76.5±0.3	74.7±1.8	71.7±3.80

Table 4: Effect of Glutathione (GSH) supplementation in the Bioxcel® extender on postthaw viability, plasma membrane integrity and normal apical ridge of buffalo spermatozoa after incubation for 2 h at 37°C

	Groups (Glutathione mM^{-1})			
Parameters (%)	Control	0.5	1	2
Viability	37.6±2.8[a]	32.5±2.1[a]	35.5±2.5[a]	11.6±2.1[b]
PMI	21.5±1.8	19.0±2.1	20.1±2.3	18.7±2.5
NAR	54.3±2.5	50.2±4.3	56.2±1.9	51.3±2.2

Data are means±SEM; [a-c]Values in the same row with different superscripts differ significantly ($p<0.05$)

viability, plasma membrane integrity and acrosomal ridge of buffalo bull spermatozoa are shown in Table 3. Immediately after thawing, the proportion of post thaw sperm viability (67.9±3.8, 63.6±0.6, 62.7±1.5), plasma membrane integrity (47.5±2.5, 50.5±2.4, 51.5±1.3) and normal apical ridge (78.7±1.3, 76.5±0.3, 71.7±3.8) remained similar for samples thawed in control, 0.5, 1 and 2 mM glutathione, respectively ($p>0.05$). However, supplementation 2 mM of glutathione in semen extender significantly decreased viability in comparison with others groups examined after thawing (55.7±2.51%), ($p<0.05$). The post thaw sperm viability dramatically decreased during incubation at 37°C for 2 h (Table 3). The negative correlation was detected between sperm viability and type of extenders after 2 h incubation time. The post thaw sperm viability were comparatively superior in soybean lecithin based extenders contain 0.5, 1 and 2 mM glutathione at this time (37.6±2.8 and 32.5±2.4 vs. 11.6±2.1) ($p<0.05$) (Table 4). The percentage of plasma membrane integrity and normal acrosomal rings after the incubation at 37°C for 2 h was not different between the groups but dramatically decreased at this time (Table 4). The type of extender as well as the interaction between extenders and incubation time had not significant effects.

DISCUSSION

The present study for the first time describes systematically the effect supplementation exogenous glutathione in commercially available soybean lecithin based extender Bioxcell® on post thaw sperm kinematic parameters using CASA in buffalo bull semen.

The present study showed that supplementation of glutathione in Bioxcell® extender had not significant effect on overall post thaw sperm motility and kinematic parameters (Table 1). These findings are in close agreement with earlier study on boar humman (Donnelly *et al*., 1999), bull (Foote *et al*., 2002; Sariozkan *et al*., 2010) and ram (Bucak *et al*., 2008) who observed post thaw motility of semen were not improved after the fortification of extenders with glutathione.

Similarly, Tuncer *et al*. (2010) reported that the addition 0.5 or 2 mM Glutathione (GSH) in soybean lecithin extender Laiciphose® extender did not present any significant effect on the percentages of post thaw sperm morphology such as acrosome and total abnormalities, subjective, CASA and progressive motilities as well as sperm motility characteristics (VAP, VSL, VCL, LIN and ALH), compared to the control groups. Contrary to the results, Ahmed (2008) found that addition of Glutathione (GSH) to extender of frozen buffalo semen improved sperm characteristics. Ansari *et al*. (2010) reported that glutathione supplementation up to 2.0 mM in the tris egg yolk extender improves the post thaw quality of buffalo bull spermatozoa.

Furthermore, it has been reported that post thaw sperm motility in bovine (El-Nenaey *et al*., 2006; Foote *et al*., 2002; Munsi *et al*., 2007; Bilodeau *et al*., 2000) and caprine semen (Sinha *et al*., 1996) were increased after the fortification of extenders with glutathione. In present study, supplementation 2 mM of glutathione in semen extender significantly decreased post thaw overall and progressive sperm motility examined after thawing. El-Harairy *et al*. (2011) reported that the frozen-thawed bull semen added with 0.4 mM glutathione/100 mL is more efficient in maintaining post thawing sperm motility, freezability, acrosomal integrity, maintaining enzymatic activities and subsequent fertilizing efficiency of bull spermatozoa than other levels such as 0.2 and 0.8 mM concentration or free glutathione medium. The addition of glutathione to ram sperm diluents

improved sperm motility, viability and plasma membrane characteristics and improved sperm survival following 6 h cooling storage at 5°C (Bucak and Tekin, 2007).

Freezing and thawing processes also lead to the generation of Reactive Oxygen Species (ROS). Excess production of ROS during cryopreservation has been associated with reduced post thaw motility, viability, membrane integrity, antioxidant status, fertility and sperm functions (Aitken, 1995; Bilodeau *et al*., 2000; White, 1993; Zhao and Buhr, 1995).

This increase in sperm motility with glutathione supplementation might be due to a decrease in oxidative stress and ROS production that is associated with lipid peroxidation of the sperm plasma membrane causing poor sperm motility (Triwulanningsih *et al*., 2008).

It was recently reported that in the domestic bull Bioxcell® has the ability to maintain glutathione content in cryopreserved sperm because it contains higher concentrations of glutathione than tris-egg yolk extender (450 vs. 40 umoles) and it is suggested that improvement of post thaw quality of bovine semen cryopreserved in Bioxcell® may be attributed to the higher concentrations of glutathione which protect the sperm against oxidative stress and reactive oxygen species (Gil *et al*., 2000; Stradaioli *et al*., 2007). However, for buffalo semen Bioxcell® could not improve semen quality as compared to tris-citric egg yolk extender (Akhter *et al*., 2010). Excessive production of ROS during cryopreservation has been associated with the reduced postthaw motility, viability, membrane integrity, antioxidant status and fertility and sperm functions. The postthaw motility of the cryopreserved buffalo semen is poor and the success rate of IVF with buffalo sperm is only 10-20% as compared to cattle which is 30-35% (Bansal and Bilaspuri, 2011).

Physico-chemical properties of a diluent can affect the pattern of sperm motility and in this study these properties might have been altered by the addition of glutathione in the semen extender before freezing (Ansari *et al*., 2010). Foote *et al*. (2002) demonstrate that while antioxidants added to semen extenders may have beneficial effects on bull sperm under some conditions, unlike tris-citric egg yolk, a substantial array of antioxidants such as glutathione were not beneficial to bull sperm cryopreserved in whole milk extender.

Ansari *et al*. (2011) reported that addition glutathione up to 1.0 mM in tris-citric egg yolk extender improves the quality of buffalo bull spermatozoa stored at 5°C for 5 days of storage. However, higher concentrations of glutathione >1.0 mM were non-beneficial for improvement of semen quality parameters. Immediately after thawing the proportion of post thaw sperm viability remained similar for samples thawed in control, 0.5, 1 and 2 mM glutathione (Table 3). However, supplementation 2 mM of glutathione in semen extender significantly decreased viability in comparison with others groups examined after thawing (Table 4).

In contrast to the finding addition of GSH to the semen extender improved the number of viable sperms in post thaw caprine semen (Sinha *et al*., 1996) while a decrease in sperm viability was associated with decreased glutathione content of the porcine semen (Gadea *et al*., 2004). Moreover, an improvement in sperm viability has been reported to be highly correlated with glutathione content of the bull spermatozoa (Stradaioli *et al*., 2007) and buffalo (Ansari *et al*., 2010).

The present study observed that supplementation of semen extender with glutathione did not show any improvement in the percentage of spermatozoa with intact plasmalemma and normal acrosomes after thawing and incubation for 2 h (Table 4). In the similar study on buffalo semen, Ansari *et al*. (2010) reported that fortification of tris-egg yolk extender with glutathione up to 2.0 mM significantly increased the sperm plasma membrane integrity and normal acrosomes evaluated at 0, 3 and 6 h after thawing, however at the dose of 3.0 mM did not show any improvement in the percentage of that parameters. However, an addition of GSH in bovine semen has been reported to result in a higher number of acrosome intact spermatozoa (Munsi *et al*., 2007). A high correlation between the percentage of intact acrosome and fertility of frozen bovine spermatozoa was reported after 2 and 4 h of post thaw incubation. Since, glutathione plays an important role in scavenging reactive oxygen intermediates and other radicals with the help of glutathione reductase (Meister and Anderson, 1983), it is a possibility that glutathione might protect the spermatozoa from membrane damage by inhibiting the lipid peroxidation process (Sinha *et al*., 1996). The presence of normal acrosome on a spermatozoon is essential for the acrosomal reaction that is required at the proper time to facilitate fertilization and acrosomal integrity assessment can be an effective tool to predict the fertilizing ability of buffalo bull spermatozoa (Bailey *et al*., 2000; Ansari *et al*., 2010).

CONCLUSION

These results revealed that supplementation of the new commercial in soybean based extender Bioxcell® with glutathione did not improve sperm post thaw motility or acrosomal integrity. however, glutathione supplementation up to 2.0 mM in the Bioxcell® extender decrease post thaw quality of the buffalo bull spermatozoa.

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