EFFECTS OF ADDITION OF TAURINE TO SEMEN EXTENDERS ON MICROSCOPIC FACTORS OF BUFFALO BULLS SPERM AFTER THAWING

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ABSTRACT

The aim of this study was to evaluate the effects of the addition of different levels of antioxidant (taurine) on buffalo spermatozoa cryopreserved in an egg yolk based extender. Ejaculates were collected from five mature buffalo bulls (four ejaculates per buffalo) and were evaluated and diluted at 37°C in one of the following experiments: tris-egg yolk extender (control), or the same extender supplemented with either 10, 25, 50, 75 or 100 mM taurine. The semen was loaded into 0.5 ml straws, cooled and frozen in a programmable freezer and subsequently stored in liquid nitrogen. Prior to evaluation, frozen straws were thawed in a water bath (37°C for 20 s). Freezing extenders supplemented with 25 and 10,100 mM taurine led to higher and lower sperm motility values (respectively), compared to the control ($P<0.05$) and following the freeze-thawing process. The addition of antioxidants did not affect acrosomal integrity compared to the control.

Keywords: buffaloes, *Bubalus bubalis*, semen, extender, taurine, microscopic parameters

INTRODUCTION

The buffalo is second-ranked milk producer in world and produces more than a third of Asian milk (Bandyopadhyay *et al.*, 2003). About 23% of Iran's buffalo are in Azerbaijan Province. Buffalo nurturing is difficult because of the innate susceptibility of this animal to environment tensions that cause mild heat and quiescent heat and increase days open and cause heavy losses to the buffalo husbandry industry (Ingawale *et al.*, 2004). The increasing importance of buffaloes has led to the introduction of artificial insemination to increase the breeding efficiency of this species. One of the important characteristics of mammalian sperm is its high density of unsaturated fatty acids on phospholipids structures that are distributed asymmetrically throughout the lipid layers of the plasma membrane (Aitken *et al.*, 1993; Aitken., 1995 and Gadella *et al.*, 1999). Buffalo sperm is more susceptible to cold stress than that of other species such as bull, rabbit and human (Fise *et al.*, 1989 and Watson., 1981). Of course, different reactions to temperature tensions results from differences in membrane lipid composition as buffalo sperm has high levels of saturated and unsaturated fatty acids and has lowest ratio of clostrol/phospholipid compared with other species (Evans, 1988). The low fertility of frozen semen compared with fresh

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semen and natural mating is because of injuries due to freezing and thawing, and in a comparison of frozen semen with fresh semen of buffalo, about 8 times more frozen semen was required to achieve the best fertility (Shannon *et al.,* 1995). The motility of frozen-thawed semen is lower in the buffalo than in the bull: in the buffalo, it is about 10-20 percent whereas in the bull, it is 30-35 percent (Nandi *et al.,* 2006 and Totey *et al.,* 1992). Sperm cells are very susceptible to peroxidation of membrane lipids after thawing, because they have high amounts of unsaturated fatty acids in their structures which cause metabolic alterations and loss of special sperm functions such as motility, solidarity of sperm membrane, and fertility (Alvarez *et al.,* 2005; Cassani *et al.,* 2005; Lenzi *et al.,* 2002; Storey, 1997 and Wishart, 1984). During the freezing through of the membrane, unsaturated fatty acids peroxidation produces reactive oxygen (Alvarez *et al.,* 2005 and Jones *et al.,* 1997). Addition of amino acids to the extender has positive effects on motility after thawing of sperm and protection of sperm membrane integrity in different species (Chen *et al.,* 1993; Pena *et al.,* 1998 and Sanchez *et al.,* 1997). Taurine and hypotaurine are required for capacitation and fertility of sperm (Guerin *et al.,* 1995). Positive effects of taurine on protection of membrane function and structural integrity of the acrosome membrane has been established (Aruoma *et al.,* 1998; Fellman *et al.,* 1985 and Pasantes *et al.,* 1989).

MATERIALS AND METHODS

Materials

All materials used in this study such as taurine were supplied from the Sigma Company.

Animals and semen collection:

Five mature buffalo bulls (3-4 years old) were selected in the Northwest Buffalo Research and Nurturing Center. Collection of semen samples was done in the spring by artificial vagina and after teasing of selected buffalo bulls via a constricted female buffalo. The 20 ejaculations of the five native buffalo bulls were collected (four replications for each animal and the volume of each ejaculation was 2 ml) for twice in one week.

Fresh and post-thawing semen samples evaluation:

Rapidly after collection of the samples, the semen was transported to the lab and primary evaluation was done of concentration and motility aspects. One semen droplet was placed on a slide and sperm motility percentage was assayed by hot plate microscope (200×).

Also sperm concentration of each ejaculation was designated via spectrophotometer. Those ejaculations were selected which had 1-2 ml volumes, sperm motility more than 70% and concentration more than $2.5x10^9$ sperm per ml. In this study, we used tris as basic extender. Collected semen samples were divided into 11 identical portions, and at 37˚C were mixed with basic extenders concomitant with five levels of taurine (10, 25, 50, 75 and 100 mM) and basic extender without antioxidant (final sperm concentration was $5x10^6$ sperms/ml). Diluted semen samples were transferred to 0.5 ml straws and were sealed with polyvilin alcohol powder and then frozen. After one month, from each group, five straws were selected randomly and thawed for 20 seconds at 37˚C in a water bath. Percentage motility was assessed using a phase-contrast microscope (x40). After supravital staining, viability was evaluated by microscope with 400x magnification as a second index of

sperm quality. For assessment of acrosome health and natural head structure, 500 µl of thawed semen samples was mixed with 50 µl citrate formaldehyde 1% and citrate tri sodium dehydrate 2.9% to fix sperms. After fixing, prepared slides were assessed through a phase-contrast microscope.

Statistical analysis:

The study was replicated three times. Results are expressed as the mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) was used to assess differences among treatments on motion characteristics, sperm viability and normal acrosome morphology. When the F ratio was significant ($P<.05$), Tukey's post hoc test was used to compare treatment means (Version 12.0; SPSS, Chicago, IL).

RESULTS

Sperm parameters

sperm motility: As shown in Table 1, among treatments, maximum motility were achieved in 25 mmol of taurine and minimum were achieved in 10 and 100 mmol of taurine.

sperm acrosomal integrity: Among treatments, best acrosomal condition was seen in control group but, differences between groups was not significant.

sperm viability: According to Table 1, about antioxidant treatments, treatment of 75 mmol of taurine had maximum increasingly effect on viability percentage of sperm after thawing and increasing of antioxidant amounts for more than 50 mmol has negative effects on sperm viability.

DISCUSSION

Improvement of semen freezing methods requires knowledge about biochemical and physiological processes that occur during the freezing, thawing and extender characteristics (Holt., 1997; King *et al.,* 2004 and Sariozkan *et al.,* 2009). Antioxidant capability in sperm cells is limited because of a deficiency cytoplasmic components having antioxidant effects to expunge

Table 1. Effect of different concentrations of Taurine on buffalo-bull semen characteristics after freezing-thawing.

Live sperm (%)	Acrosomal integrity (%)	Motility (%)	Treatments
52.77 ± 0.77^{de}	68.33 ± 0.23	46.72 ± 0.38^{c}	Taurine 10 mM
65.22 ± 0.75^{b}	68.52 ± 0.29	58.13 ± 0.17^{a}	Taurine 25 mM
73.98 ± 0.62^{a}	67.57 ± 0.33	56.90 ± 0.19^{a}	Taurine 50 mM
59.48 ± 0.98^{c}	68.50 ± 0.23	53.41 ± 0.22^{b}	Taurine 75 mM
53.77 ± 0.49^{d}	67.07 ± 0.27	46.80 ± 0.42^{c}	Taurine 100 mM
52.13 ± 0.40^{e}	68.63 ± 0.09	45.08 ± 0.11^{d}	Control

Values are (mean ± standard error of mean).

Different letters within a column indicates significant differences (P<0.05).

reactive oxygen. Thus, mammalian sperm does not have enough ability to counter peroxidation during the freezing and thawing processes (Alvarez *et al.,* 2005; Bilodeau *et al.,* 2000 and Lapointe *et al.,* 2003). Reactive oxygen may be responsible for the loss of motility, acrosomal membrane integrity, and fertility and for sperm metabolic alterations. In recent years, adding antioxidants to semen extenders for improvement of sperm quality have been studied. Addition of antioxidants with maintenance of acrosome and mitochondrial integrity against cold increases post-thawing sperm motility. Viability and acrosomal membrane continuity are important in evaluation of semen quality, because the sperm motility test is not sufficient for evaluation of viability after freezing and thawing. Improvement of semen parameters after adding antioxidants (such as taurine and trehalose) to extenders in bull, pig, sheep, goat and dog has been reported. Taurine acts as non enzymatic antioxidant which hase important role in protecting sperms against reactive oxygen species. Eleshestavi *et al.*, 2008 were reported that adding special amino acids (glutamine, glycine, alanine and systein) to extenders (tris, citrate, fructose and glycerol) before freezing, causes improvement of semen quality after thawing. In the current study, addition of different levels of the antioxidant to the extender before freezing caused significant improvement in sperm quality such as forward motility and viability of sperms compared to the control group ($P<0.05$) but no significant differences in acrosomal integrity between treatment and control groups weres seen. Only in the t 100 mmol taurine treatment was a significant difference in acrosomal health seen: 100 mmol of taurine caused significant decreases in acrosomal integrity and finally decreasing fertility. The results achieved in this study in improvement of sperm motility after thawing were similar with results achieved in sheep, rabbit and pig sperms (Atessahin *et al.,* 2008; Bucak *et al.,* 2007 and Molinia *et al.,* 1994). On the other hand, adding taurine to extenders has led to no improvement in motility in bull and goat sperm after thawing, probably because of species diversity, extender composition and antioxidant density. Taurine has a protective effect against lipid peroxidation and losing motility in ram sperm and also has positive effects on viability and membrane continuity of ram sperm after thawing. Chen *et al.*, 1993, Pena *et al.*, 1998 and Sanchez *et al.*, 1997 reported that sperm motility significantly increased with the addition of antioxidants to extenders after thawing; this is compatible with the current research results but is inconsistent with our research results with regard to acrosomal condition.. Some studies revealed that adding taurine and cysteine to extenders before freezing causes improvement in forward motility of sperm after thawing in pig, bull, human, sheep and goat that is compatible with our research results (Atessahin *et al.,* 2008; Bucak *et al.,* 2008; Funahashi *et al.,* 2005; Uysal *et al.,* 2007).

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