EFFECT OF THIOGLYCOL ON FERTILITY OF FROZEN NILI-RAVI BUFFALO SEMEN

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ABSTRACT

This study was designed to determine the effect of thioglycol in extender on fertility of cryopreserved buffalo semen under filed conditions. For this purpose, semen from two Nili-Ravi buffalo (*Bubalus bubalis*) bulls of known fertility was split-sampled and diluted with *tris*-citric acid extender either containing thioglycol or without thioglycol (control) at 37°C having approximately 50×10^6 spermatozoa ml^{-1}. Diluted semen was cooled to 4°C in 2 h at the rate of 0.275°C $minute^{-1}$ and equilibrated for 4 h at 4°C. Cooled semen was then split into two aliquots, filled in 0.25 and 0.5 ml straws at 4°C, kept on liquid nitrogen vapours for 10 minutes and plunged in liquid nitrogen for storage. The cryopreserved semen was used to perform artificial insemination in buffaloes and fertility rate based on 90-days first service pregnancy was determined under field conditions. The overall fertility rate was found higher in extender containing thioglycol compared to control 56 vs. 44%, respectively. It is concluded that thioglycol in *tris*-citric acid extender improves the fertility of cryopreserved buffalo semen under field conditions.

Keywords: thioglycol, fertility, frozen semen, buffaloes, artificial insemination

INTRODUCTION

Artificial insemination has been extensively used by developed countries for rapid genetic improvement through exploiting the germplasm of the superior male. The benefits of artificial insemination technique can be fully achieved by successful freezing of semen without compromising its fertility, and so far, this has met with a little success in buffalo (Akhter *et al.*, 2008; Ansari *et al.*, 2010; Ansari *et al.*, 2011; Ansari *et al.*, 2012). The reason for poor fertility rate in buffaloes under field conditions is poor post-thaw characteristics of buffalo semen. This may be the reason that only 7% buffaloes are bred through artificial insemination technique in Pakistan (Livestock Census, 2006).

Cryopreservation is an oxidative process deteriorates the fertility of buffalo bull semen probably by affecting the sperm motility, viability, plasma membrane, acrosomal and DNA integrity (Ansari *et al.*, 2012). Buffalo semen has enzymatic and non-enzymatic antioxidants to protect the spermatozoa from oxidative stress (Akhter *et al.*, 2011). However, it is believed that the indigenous defense system of buffalo semen against the oxidative stress during the process of freezing and thawing is insufficient. This weakness is mainly due to low concentrations of naturally occurring antioxidants in buffalo semen, which is further decreased during

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the extension and freezing process (El-Sisy *et al.*, 2007; Kumar *et al.*, 2011; Ansari *et al.*, 2012).

It was demonstrated that thioglycol had the ability to protect the motility of bovine spermatozoa from deleterious effects of hydrogen peroxide *in vitro*, a major ROS molecule naturally produced in the semen (Bilodeau *et al.*, 2001). The results of another study on buffalo semen also described hydrogen peroxide as blocker of motility (Garg *et al.*, 2009). Moreover, thioglycol protected the bovine embryo from oxidative stress and improved blastocyst quality *in vitro* (Feugang *et al.*, 2004). It is noteworthy to mention that thioglycol can stop the apoptosis in cloned embryos by its antioxidant activity in swine (Park *et al.*, 2004a, b). Beneficial effects of thioglycol were evident for sperm penetration and early embryonic development (Funahashi, 2005). These all studies suggested that thioglycol should be tested in extender for improving the *in vivo* fertility of buffalo cryopreserved semen. Therefore, this study was designed to evaluate the effect of thioglycol in extender on *in vivo* fertility of buffalo bull semen.

MATERIALS AND METHODS

Preparation of extenders

The stock extender was consisted of 1.56 g citric acid (Fisher Scientific, UK), 3.0 g *tris*–(hydroxymethyl)-aminomethane (Research Organics, USA), 0.2% fructose (Scharlau, Spain), 7% glycerol (Riedel-deHaen, Germany) and 20% egg yolk in 74 ml distilled water. Two experimental extenders were prepared by adding thioglycol 0.0, and 1.0 mM in stock extender.

Collection and initial evaluation of semen

Semen was collected from two Nili-Ravi buffalo bulls (*Bubalus bubalis*) with artificial vagina at 42°C, transferred to the laboratory immediately for initial evaluation (volume, motility, concentration). Sperm progressive motility (%) was assessed (200X) with phase contrast microscope. Sperm concentration was measured with bovine photometer ACCUCELL (IMV, France). Qualifying semen ejaculates [motility >60%, volume >3.0 ml, concentration >0.5 billion/ml] were split into two aliquots for dilution in experimental extenders.

Processing of Semen

Semen aliquots were diluted with one of the five experimental extenders at the rate 50 × 10^6 motile spermatozoa ml^{-1} approximately at 37°C and cooled to 4°C in 2 h and equilibrated ion for 4 h at 4°C. Cooled semen was filled in 0.5 ml French straws with suction pump at 4°C in the cold cabinet unit and kept on liquid nitrogen vapours for 10 minutes. Straws were then plunged into liquid nitrogen (-196°C) for storage till artificial insemination.

Artificial insemination and pregnancy diagnosis

A total of 400 inseminations performed with cryopreserved experimental semen were recorded, in Tehsil Chichawatani, District Sahiwal and Tehsil Kahore Pacca, District Lodhran. The inseminations were preformed over three months during the peak breeding season (October to December). All the experimental inseminations were performed approximately 24 h after onset of heat. The artificially bred animals were examined for pregnancy through rectal palpation at least 90 days post-insemination under field conditions.

Statistical analysis

The data on fertility rate were compared

by using chi-square statistics (MINITAB® Release 12.22, 1998).

RESULT AND DISCUSSION

The objective of the study was to identify the effect of thioglycol addition to semen extender for in vivo fertility of buffalo bull semen. The data on conception rate in buffaloes inseminated with cryopreserved semen containing either thioglycol or without thioglycol (control) is given in Table 1. In present study, thioglycol improved the in vivo fertility rate in buffaloes under field conditions compared to control 56 vs. 44%, respectively. It is known that thioglycol addition in *in vitro* maturation media improved the quality of buffalo embryos (Shang *et al.*, 2007). It is believed that thioglycol addition in the *in vitro* culture media protected the bovine embryos from oxidative stress and improved the quality of blastocysts by reducing apoptosis induction (Feugang *et al.*, 2004). Moreover, an *in vitro* study reveled that the addition of thioglycol increased the number of cumulus cells attached to oocytes and cell numbers of blastocysts by influencing ATP metabolism (Tsuzuki *et al.*, 2005). Funahashi (2005) reported beneficial effect of thioglycol for sperm penetration and early embryonic development. Thioglycol presence in maturation media also improved the maturation and M II stage of canine oocytes *in vitro* (Kim *et al.*, 2004). It is suggested that improvement in fertility of buffalo semen cryopreserved in extender containing thioglycol was observed due to ability of thioglycol for protecting the mammalian gametes and embryos from oxidative stress during early embryonic developmental stages.

CONCLUSION

It is concluded that addition of thioglycol to semen extender improves the in vivo fertility of buffalo bull spermatozoa under field conditions.

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Table 1. Fertility rate of buffalo bull semen cryopreserved in extender containing thioglycol.

Bull No.	Extender Type	No. of inseminations recorded	Pregnancies Achieved	Chi-square	P-value
1	Control	100	43 (43%)	4.50	0.03
	Thioglycol	100	58 (58%)		
2	Control	100	45 (45%)	1.62	0.20
	Thioglycol	100	54 (54%)		
Overall	Control	200	88 (44%)	5.76	0.02
	Thioglycol	200	112 (56%)		

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