LEAKAGE OF TRANSAMINASES DURING CRYOPRESERVATION OF CATTLE AND BUFFALO SEMEN IN EGG YOLK TRIS AND SOYA BEAN MILK BASED EXTENDERS

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ABSTRACT

Three healthy and mature bulls each of Gir cattle and Surti buffalo were used to evaluate the comparative efficacy of standard Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender and soybean based commercially available extenders (Optixcell and Andromed) under split-sample technique for sperm motility and leakage of spermatozoal enzymes transferases (AST-ALT) into the extracellular medium during cryopreservation process. The ejaculates with >70% initial motility were divided into three equal aliquots, and extended @ 100×10^6 sperm/ml at 34°C with three extenders and frozen using biofreezer following 4h of equilibration. Sperm motility was higher ($p<0.05$) in Optixcell followed by TFYG and was least in Andromed for both Gir and Surti bull semen. However, AST-ALT activity was similar ($p>0.05$) between both soyabean based commercial extenders and both had much (2-3 times) lower activities of these enzymes than egg yolk based TFYG extender at all the three stages. In brief, Optixcell gave relatively improved performance during cryopreservation process in both Gir cattle and Surti buffalo semen than the egg yolk based TFYG extender.

Keywords: Cryopreservation, Enzyme leakage, Extender, Gir bull, Semen, Surti buffalo

INTRODUCTION

Sperm progressive motility and seminal enzymes play a critical role in fertilization and are affected owing to cryopreservation. The estimations of the enzymes present in the secretions of accessory sex glands and in spermatozoa give significant information on sperm metabolism and sperm cell damage. The assessment of sperm motility and certain enzyme levels in the seminal plasma are vital in judging the preservability and fertilizing potential of spermatozoa. The best semen extender can boost up the sperm motility/viability and block the leakage of enzymes and other electrolytes from the sperm cell. The estimation of enzymes viz. AST-ALT (GOT-GPT) in sperm or seminal plasma is an important tool to assess the sperm protecting ability of extenders and to evaluate the freezability of semen and fertilizing capacity of the male (Dhami *et al.,* 1995 and Salaam, 2008). However, the comparative information on cryoprotective ability of egg yolk and soya bean based extenders through enzymatic evaluation of bovine semen is meager; hence this study was planned and executed to evaluate these aspects on Gir and Surti buffalo semen.

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MATERIALS AND METHODS

Three healthy mature Gir cattle and three Surti buffalo bulls were used to evaluate comparative efficacy of egg yolk based standard Tris-citric acid-fructose-egg yolk-glycerol (TFYG) extender and soybean based commercially available extenders Optixcell® (IMV, France) and Andromed® (Minitube, Germany) under split-ejaculate technique through sperm motility and seminal plasma transferases, AST-ALT, at different stages of cryopreservation process. The ejaculates (9/bull, total 54) with >70 % initial motility obtained at weekly interval were divided into three equal aliquots, and extended @ 100×10^6

spermatozoa/ml at 34°C with three different extenders. Extenders were reconstituted with Mili-Q water as per the standard formula and as per the instructions of the manufacturers of commercial products, Optixcell (1:2) and Andromed (1:4).

The extended semen samples were soon evaluated for sperm motility and the levels of seminal plasma transferases, and were filled in French mini straws on IS4 system (IMV, France). After gradual cooling over 60-90 min and equilibration for 4 h in cold handling cabinet at 4°C, the straws were frozen in liquid nitrogen vapour using a programmable bio-freezer (IMV, France). The straws of all three extenders were evaluated at pre-freezing (after equilibration) and after 18-24 h of freezing (post-freeze stage) for the above parameters again. Sperm motility was recorded close to 5% grade under phase contrast microscope attached with a biotherm stage at 37°C, while enzymes AST-ALT were estimated in seminal plasma using standard procedures and assay kits procured from Coral Clinical System, Goa, India (Bergmeyer *et al*., 1986). The data generated were analyzed statistically using CRD and students 't' test.

RESULTS AND DISCUSSION

The differences in sperm motility ratings were different ($p<0.05$) between extenders at initial, pre-freeze and post-thaw stage, where Optixcell showed superior results followed by TFYG and Andromed for cattle semen (Table 1). Also, the buffalo semen revealed variation ($p<0.05$) for this trait at initial and pre-freeze stages of preservation, but had no difference ($p>0.05$) between extenders at post-thaw stage (Table 1). In previous studies, higher post-thaw motility in semen extended with egg yolk based TFYG extender than the soya bean based Andromed was also noted (Rehman *et al.,* 2012 and Kumar *et al.,* 2015). Furthermore, the better sperm motility in semen frozen with Optixcell and TFYG than Andromed extender in both cattle and buffalo semen also corroborated well with previous reports (Veerabramhaiah *et al.,* 2011[a] and Chaudhari *et al.,* 2015).

With regard to leakage of aminotransferases, in the present study, the activities of enzyme AST-ALT were 2-3 times higher ($p<0.05$) for semen extended in egg yolk based TFYG as compared to soyabean based Optixcell and Andromed extenders in both Gir and Surti bull semen at different stages of cryopreservation (Table 1). This may be due to inherent biological quality of egg yolk used in TFYG extender and absence of egg yolk in soya based extenders. There was gradual increase in the seminal plasma AST-ALT activity from initial, pre-freeze to post-thaw stages in semen of both the species in all three extenders, indicating its leakage from sperm cells into the extracellular media, the differences in values between initial and post-thaw stage were significant in all dilutors. However, statistically the differences in values between initial and pre-freeze stages; and between pre- and post-freeze stages were not significant, due to overall much higher values with greater standard errors, as compared to the values and trend documented in many of the above studies. This could be due to variations in inherent enzyme activity of accessory glands of animals, cryo-resistance of sperms, freezing protocols, extender quality, rate of dilution, and assay technique and unit of expression used in different studies. The results of the current study, however, are in contradiction to lesser enzyme leakage documented in the semen extended with Tris than Biociphos-Plus extender (Veerabramhaiah *et al.,* 2011[b]).

Based on reports of various authors, it appears that different extenders have different defensive ability. In our study, the ready to use commercial soybean based extender 'Optixcell' was better than TFYG in terms of microscopic visualization and sperm motility to some extent and enzyme activity as well. On the other side, though Andromed extender is also a soya protein based extender, could not excel even at par with TFYG extender for cattle or buffalo semen cryopreservation in respect of sperm progressive motility and was proved to have less cryoprotective capacity. Ideal soya lecithin concentration in the extender is prerequisite for protection of spermatozoa

Table 1: Sperm motility and leakage of AST-ALT enzymes from Gir cattle and Surti buffalo bull spermatozoa into the plasma at different freezing stages of cryopreservation of semen in three extenders (TFYG, Tris-citric acid-fructose-egg yolk-glycerol; Opti., Optixcell; Andro., Andromed)

Stage	Extender	Sperm motility, %		AST/GOT activity, U/L		ALT/ GPT activity, U/L	
		Gir	Surti	Gir	Surti	Gir	Surti
Initial	TFYG	75.0±0.9^{a}	80.4±0.7^{ab*}	1382.8±32.0^{b}	1367.8±27.8^{b}	1388.3±37.8^{b}	1425.2±26.3^{b}
	Opti.	78.3±0.8^{b}	82.1±0.6^{b*}	492.3±26.4^{a}	537.7±20.0^{a}	562.9±19.2^{a}	606.9±14.1^{a}
	Andro.	76.2±0.9^{ab}	79.8±0.6^{a*}	460.2±26.1^{a}	535.0±22.7^{a}	519.3±21.3^{a}	573.9±17.4^{a}
Pre-freeze	TFYG	69.4±0.9^{a}	74.4±0.8^{ab*}	1451.4±33.6^{b}	1436.6±28.5^{b}	1501.7±31.4^{b}	1520.4±23.7^{b}
	Opti.	73.1±0.7^{b}	76.0±0.7^{b}	537.8±27.0^{a}	583.2±18.8^{a}	607.2±22.2^{a}	649.4±14.5^{a}
	Andro.	70.8±0.9^{ab}	73.5±0.7^{a}	530.1±26.5^{a}	596.1±22.7^{a}	567.1±22.2^{a}	650.9±14.4^{a}
Post-thaw	TFYG	40.4±1.5^{ab}	39.6±1.8	1564.1±37.9^{b}	1543.2±28.8^{b}	1592.6±32.3^{b}	1625.1±21.5^{b}
	Opti.	42.1±1.6^{b}	42.9±1.8	592.3±28.3^{a}	651.7±21.6^{a}	659.4±24.5^{a}	712.8±13.8^{a}
	Andro.	37.1±1.4^{a}	38.5±1.9	589.1±28.2^{a}	682.9±23.3^{a}	639.1±24.9^{a}	725.5±15.2^{a}

*p<0.05; Means bearing different superscripts (a,b) between extenders at each stage differ significantly (p<0.05)

during temperature variations (Singh *et al.,* 2013). The concentration of soybean below or above the optimal may be damaging and this might be the situation with Andromed extender.

In the present study, Optixcell gave relatively improved performance than the TFYG extender. This might be due to presence of antioxidant and unknown additives in Optixcell extender, which favoured the performance of this extender. However, we cannot underestimate TFYG extender, as it gave satisfactory results in the present study and was reported by others (Dhami and Kodagali, 1990; Dhami and Sahni, 1993; Chaudhari *et al.,* 2015). The only problem with regard to TFYG extender is the need to prepare fresh daily and the presence of egg yolk which is of animal origin and varies in quality depending upon the source and storage time, and disease freedom of egg, thereby, probable threat of introducing the xenobiotic contamination in the extended semen. Despite the inconsistency in day to day quality and considering the cost, TEFY extender is still a preferred choice on most semen stations, until the newer ready to use extenders prove superiority through *in vitro* and *in vivo* tests with affordable cost for enhancing conception rates in bovines.

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