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Use of chelating agent for optimum post thaw quality of buck semen

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ABSTRACT

Ejaculates (35) from adult Sirohi bucks (2–4 years old) were utilized for the present study to find out the freezability of buck semen at different levels of chelating agent used (ethylene diamine tetra acetic acid - EDTA: 0, 0.01, 0.05 and 0.1%) by conventional method of freezing. The ejaculates were collected twice at weekly intervals using artificial vagina and were extended to maintain sperms concentration approximately 100 million / dose (0.25 ml) with tris- citric acid- fructose (TCF) diluent having 10% (v/v) egg yolk and 6% (v/v) glycerol as cryo protecting agent. Filling and sealing of straws were done at 5ºC in cold handing cabinet after 4 h of equilibration period then straws were vapor frozen for 10 min above 2 cm of liquid nitrogen and finally put in to liquid nitrogen. Post thaw motility, live sperm count, abnormalities, acrosomal integrity and hypo osmotic swelling test had been conducted to know freezability. Analysis of data using SPSS 16 revealed that post thaw motility, live sperm count, abnormalities, acrosomal integrity and hypo osmotic swelling positive spermatozoa differed significantly at different levels of EDTA. The post thaw motility, live sperm count, acrosomal integrity and hypo osmotic swelling positive spermatozoa were significantly highest in 0.1% of EDTA used in the present study. So, 0.01% EDTA can be used as an additive in semen dilutor in routine freezing process for better post thaw recovery of buck semen.

Key words: Acrosome, Buck semen, Cryopreservation, Egg yolk, EDTA, Glycerol, HOS

A cryopreservation protocol developed for one species may not be ideal for sperm of other species. Thus to maximize the potential of frozen goat semen production of superior germplasm for wider application, it is essential, to minimize the losses due to poor freezability of samples and also to improve the quality of semen following freezing as well, as an effort of achieving better fertility results following artificial insemination (AI).

Egg yolk is a common component of semen extenders for most of the domestic species (Watson 1990), however, presence of phospholipase A restricts the use of egg yolk to low concentrations in extenders for goat semen (Roy 1957, Ritar and Salamon 1991) or to periods of the year when phospholipase activity is reduced (Tuli and Holtz 1995). For the preservation of buck semen, different extenders are used. When semen is diluted in egg yolk citrate diluents, H_2O_2 is produced by the dead sperm (Shannon and Curson 1972, 1982). Protection against the deleterious effects of H_2O_2 on sperm can be obtained by using cysteine, catalase and EDTA (Mann 1964, Shannon and Curson 1972). EDTA possibly protects spermatozoa by chelating heavy metals which catalyse the oxidising action of H_2O_2. The concentration of EDTA required to achieve the maximum increase in survival was dependent on egg yolk concentration, higher concentrations being required as egg yolk concentration increased (Shannon and Curson 1983). EDTA was as effective as catalase in maintaining fertility of diluted semen up to 36 h of storage at ambient temperatures but was less effective than catalase for longer periods of storage. EDTA does not degrade H_2O_2 which is possibly caused by accumulation of H_2O_2 to levels that could damage sperm even in the presence of EDTA (Shannon and Curson 1983).

The interaction between the Ca^{2+} ion and membrane lipids strongly influences the net surface charge, the orientation of polar heads, the salvation grade and the phase transition with respect to temperature changes (Bakas and Disalvo 1991). Finally, Ca^{2+} may influence the cryoscopic properties of sugars, as EDTA decreases the freezing temperature of disaccharide solutions (Salamon and Maxwell 1995). Ranjan *et al*. (2009b) standardized the egg yolk level in goat semen dilutor and their effect on seminal parameters.

As scanty information is available on the use of EDTA in goat semen extender, the present experiment was designed to optimize the EDTA concentration in goat semen extender for optimum post thaw quality.

MATERIALS AND METHODS

The adult Sirohi bucks aged between 2–4 years old managed under semi-intensive system at CIRG, Makhdoom were used as semen donors for the present study.

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Semen collection, evaluation and dilution: Ejaculates (35) were collected using artificial vagina, twice a week. Immediately after collection, the volume, colour, consistency, and mass motility of ejaculate were assessed. Semen were extended with tris -egg yolk- fructose diluent (Tris- 3.604 g; citric acid- 1.902g; fructose- 1 g; streptomycin- 100 mg; penicillin- 100000 i.u.; triple distilled water- 100 ml; pH- 6.75–6.8) @ 1: 10, having 10% (v/v) egg yolk and glycerol 6% (v/v). Only samples having mass motility +4 and above were taken for study.

EDTA concentration in dilutor: Semen was diluted with Tris-citrate-fructose yolk buffer having 0 %, 0.01 %, 0.05 %, and 0.1% EDTA. Sperm concentrations were adjusted to 100 million / dose (0.25 ml) and diluted semen was equilibrated at 5°C for 4 h before being frozen.

Sperm staining and evaluation: Diluted semen (10 µl) was placed on a clean grease free warm slide (37°C) with cover slip and observed under 40 × magnification of phase contrast microscope for assessing the progressive motility. The average values of 2 experts were considered for calculating the progressive motility. For calculating the live and dead sperm count, a method of Hancock (1951) using Eosin- Nigrosine stain was followed. Abnormal sperms were counted with the same staining technique. Giemsa stain was used to assess the acrosomal integrity of frozen thawed buck spermatozoa as per Watson (1975). Hypo osmotic swelling test was carried out by Ranjan *et al*. (2009a).

Statistical analysis: Data were analyzed by two-way analysis of SPSS package 16. The factorial model included the effect of EDTA concentration as independent variables and percent post thawed motility and live sperm count, abnormalities, acrosome intact sperm and hypo osmotic swelled sperm as dependent variables.

RESULTS AND DISCUSSION

Ejaculates (35) from adult Sirohi bucks were collected and data were analysed by using SPSS 16. The effects of EDTA on the cryoprotective action of base extender in frozen semen were evaluated and the percentages of motile spermatozoa live and dead spermatozoa, hypo osmotic swelled spermatozoa and acrosome integrity for each EDTA concentration were averaged. The effect of the different concentration of EDTA in diluents on post thaw sperm quality was summarized in Table 1.

The results showed that the progressive motility, live sperm count, abnormality, acrosomal integrity and hypo osmotic swelling positive spermatozoa (mean±SE) were 78.82±0.37, 86.75±0.43, 2.26±0.12, 88.38±0.44 and 81.19±0.71 respectively in fresh semen. The data also showed that all the parameter calculated in the present study was within acceptable limit for freezing process.

The post thaw quality of semen frozen in dilutor having different percent of EDTA as a chelating agent was found suitable for storage and further use in artificial insemination programme. The results showed that the progressive motility, live sperm count, abnormality, acrosomal integrity and hypo osmotic swelling positive spermatozoa (mean±SE) were highest in 0.01% EDTA followed by 0.05, 0.0 and 0.1%. The details are presented in Table 1. The data also revealed that the post thaw quality was significantly differed ($P<0.05$) with different concentration of EDTA used in present study. The progressive motility and live sperm count was significantly highest in 0.01% EDTA followed by0.05%, 0.0% and 0.1%. The abnormal sperm percent was found highest in 0.1% EDTA and there was no significant difference ($P<0.05$) among 0, 0.01 and 0.05% of EDTA. The acrosome intact spermatozoa was found significantly highest in 0.01%, followed by 0.0, 0.05 and 0.1% of EDTA used and there was no significant difference ($P<0.05$) between 0 and 0.05% of EDTA. The hypo osmotic swelling positive spermatozoa were significantly highest in 0.01% EDTA followed by 0.05%, 0.0% and 0.1% of EDTA used in dilutor.

Significant features of the results (Table 1) were that in diluents containing 0.1% EDTA had negative effect on post thaw quality then control ($P<0.05$). However, 0.01% EDTA was significantly more effective than higher concentrations of EDTA ($P<0.05$). The difference may be caused by elevated H_2O_2 levels with semen diluted in 10% egg yolk (Shannon and Curson 1982). The effect of increasing H_2O_2, by the more percent of dead sperm/ml in higher concentration of 0.1% EDTA (Table 1) caused poor post thaw quality.

The addition of EDTA to the diluents may eliminate the inhibitory action of Ca^{2+} on membrane protection. Other workers had also demonstrated improved protective properties of EDTA in diluents. The post-thaw quality were ameliorated ($P < 0.01$) by addition of EDTA to the extender Khalifa and El-Saidy (2006). Ca^{2+} reduced sperm viability and affected acrosome morphology during cooling (Bailey and Buhr 1995). Calcium also plays a role in signaling pathways in acrosomal reaction, which is reduced in the presence of EDTA (Roldan *et al.* 1994). Accordingly, in the present study also higher percentages of acrosome integrity with EDTA in post-thawing evaluations was

Table 1. Effect of different concentrations of EDTA in goat semen dilutor on post thaw quality of Sirohi buck semen

Concentration of EDTA (%)	Progressive motility (%)	Live (%)	Abnormality (%)	Acrosome intact (%)	HOS (%)
0.0 (control)	36.14±0.41^{c}	55.14±1.00 ^{c}	2.73±0.10 ^{b}	70.67±1.01^{b}	49.26±1.04^{c}
0.01	44.86±0.48^{a}	63.13±0.95 ^{a}	2.56±0.09 ^{b}	75.00±0.89 ^{a}	59.74±1.08^{a}
0.05	42.79±0.60^{b}	58.66±0.92 ^{b}	2.76±0.09 ^{b}	71.35±0.86 ^{b}	55.04±0.82^{b}
0.1	29.28±0.92^{d}	44.76±1.14 ^{d}	3.93±0.77^{a}	66.71±1.03^{c}	38.11±1.08^{d}

obtained (Table 1). The addition of EDTA confers the highest cryopreserving activity tested for post-thawing resistance, possibly by removing calcium from the medium thereby preventing cation competition for membrane-binding sites.

It was concluded that 0.01% EDTA is the best suited in goat semen dilator for optimum post thaw quality, and advocate using this in routine semen cryopreservation practice.

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