EFFECT OF BIOXCELL AND TRIS CITRIC EGG YOLK EXTENDER ON POST THAW SEMEN QUALITY AND *IN VIVO* FERTILITY IN CROSS BRED JERSEY BULL

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ABSTRACT

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Present investigation was carried out to compare the commercially available extender Bioxcell® with tris citric egg yolk (TEYG) extender for post thaw quality and *in-vivo* fertility of jersey Red Sindhi cross bred bulls (CBJ). Twenty four ejaculates of semen samples were collected from four fertile CBJ bulls (5-6 years) by artificial vagina maintained at 41°C.Qualifying ejaculates (>60% motility) from each bull were divided into two aliquots and diluted with TEYG or Bioxcell® extender. Diluted semen was cooled, equilibrated at 4°C for 4hrs and filled in 0.5ml straws. Straws were cryopreserved in liquid nitrogen for 24 hrs. and then thawed at 37°C for 30 seconds to assess post thaw motility (PTM), livability (PTL), plasma membrane integrity (HOSr), and percent intact acrosome (PIA). For comparison of in vivo fertility cows were inseminated under field condition and fertility rate was calculated on conception rate (CR) basis. The PTM, PTL, HOSr and PIA were found to be 51.05 ± 0.25 and 51.82 ± .26, 56.47 ± 0.41 and 56.46 ± 0.42, 67.94 ± 0.46 and 67.99 ± 0.45 and 69.53 ± 0.52 and 69.55± 0.52 in TEYG and Bioxcell® extender respectively. The C.R. recorded for TEYG was 53.20% while in Bioxcell® extender it was 52.67%. It is inferred that Bioxcell® can be alternatively used for cryopreservation of bull semen with comparable post thaw sperm parameters with that of TEYG extender.

Key words - TEYG, Bioxcell®, Bull semen, Cryopreservation, Yolk free commercial semen extender.

INTRODUCTION

Artificial insemination is most valuable and widely implemented technology for selection and breeding of cattle (Gravance *et al.,* 2009). Every step of an ejaculate processing starting from collection to production of A.I doses is very important and affects final quality. Out of all steps involved in semen processing, dilution of sperm, filling of straw, their cooling and freezing for producing frozen semen doses, have significant effect on sperm motility and the effect of the extender used is especially important. (Siddique *et al.*, 2006).

Egg yolk has been used as a basic component of extenders for bull ejaculate since 1939 (Amirat *et al.*, 2004).Though addition of egg yolk, changes the composition of an extender, still it is recommended because of the excellent protection effect on sperm cells, that it provides (celeghini *et al.,* 2008).It contains a low density lipoprotein which has a cryoprotective effect on the integrity of plasma membrane as well as on the percentage of normal spermatozoa and sperm motility. In spite of all the benefits of egg yolk on semen cryopreservation, sometimes it serve as a potential source of microbiological contamination which compromises with the quality of cryopreserved semen and its standardization. Hence, world organization for animal health (OIE) in 2003, recommended that the products of animal origin used in semen processing should be free of any biological risk. Therefore as an alternative to replace the component of animal origin in semen extenders now-a-days, commercial extenders such as AndroMed®, Biociphos plus® and Bioxcell® are used by many semen laboratories. Most of these commercial semen extenders have got soya lecithin which contains phosphatidyl choline and saturated fatty acids which maintains the structural stability of cells during cryopreservation (Oke *et al.*,

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2010). Keeping in the view of the above facts, the present investigation was carried out to compare the efficacy of commercial extender Bioxcell® with that of routinely used TEYG on basis of the post thaw quality and *in vivo* fertility.

MATERIALS AND METHODS

The present study was conducted in the year 2013 at Department of Animal Reproduction, Gynaecology and obstetrics, O.U.A.T, Bhubaneswar on four CBJ bulls (CB200, CB204, CB210, CB238) of similar age group (5-6years) belonging to frozen semen bank, Cuttack under state A.H. Dept., Odisha. A total number of 24 ejaculates were collected by artificial vagina maintained at 41°C. Immediately after collection, the ejaculates were transferred to laboratory and evaluated for sperm motility and sperm concentration. Ejaculates having more than 60% motile sperm from each bull were selected and split into two aliquots. One aliquot was diluted with TEYG extender while the other with Bioxcell® extender (IMV, France) at 37°C having final concentration of 80×10^6 cells /ml. Diluted semen was cooled from 37°C to 4°C, equilibrated at 4°C for 4 hours and filled into 0.5 ml French mini straws. Semen straws were kept over liquid N_2 vapors for 15 minutes, then plunged and stored in liquid nitrogen. After 24 hours of storage, semen straws are thawed at 37°C for 30 seconds to assess PTM, PTL, HOSr and PIA.

Post thaw motility test was done by putting a drop of semen on a pre-warmed microscope slide and examined under high power magnification (400X) with thermostatically controlled phase contrast microscope. The sperm livability was assessed by using eosin and nigrosin stains. Sperm plasma membrane integrity was assessed from the hypo osmotic swelling reaction as described by Revel and Mrode (1994). For acrosomal integrity 500µl of each semen sample was fixed in 50µl of 1% solution of formal citrate containing 2.9% trisodium citrate dehydrate and 1% commercial formaldehyde. Two hundred spermatozoa were counted with a phase contrast microscope (1000X) for their normal apical ridge. A total number of 480 inseminations were performed with cryopreserved bull semen in TEYG and Bioxcell extender from CBJ bulls. All the inseminations were performed during mid-estrus stage and animals were examined for pregnancy through rectal examination at least 45 days post insemination under field condition. The data collected was analyzed statistically as per method suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The data on post thaw sperm parameters like PTM, PTL, HOSr and PIA, are presented in Table 1.The overall percentage of PTM was 51.05 ± 0.25 and 51.82 ± 0.26 when extended with TEYG and Bioxcell® respectively. In the present study, difference in PTM could not be observed observed between two extenders. Similar values of PTM was recorded by Singh (2005) while Stradaioli *et al.*,(2007) observed higher values in Bioxcell compared to *TEYG* extender. The higher sperm motility in Bioxcell® might be due to higher content of glutathione as compared to TEYG. Over all PTL when extended with TEYG was 56.40± 0.41 while in Bioxcell® it was was 56.46± 0.42. Comparison among the values indicated no significant differences between two groups. The present finding corroborates with the finding of Giri (2010) while much higher values of PTM in Bioxcell® have been reported by Perumal (2008).The inconsistent egg yolk composition (Muller Schlosser *et al.*,2001) and the effect of egg yolk on sperm chromatin structure might have resulted in poor post thaw sperm viability in his case.

The post thaw plasma membrane integrity of spermatozoa usually is assessed with ease by hypo osmotic swelling test. The percentage of HOS reacted sperm in TEYG and Bioxcell® extended semen recorded an overall value of 67.94 ± 0.46 and 67.99 ± 0.45. Comparison between two extenders did not reveal any significant difference. The present finding of hypo osmotic swollen sperm percentage was supported by Gil *et al.*, (2003). The overall percent PIA was 69.53 ± 0.52 and 69.55 ± 0.52 in TEYG and Bioxcell® extender respectively. There was no significant difference between the values in two different groups. The present finding supports the finding of Giri (2010) while higher percent in Bioxcell® have been reported by (Andrade *et al.*, 2010). Lower PIA in TEYG might be due to presence of Ca^{++} ions in egg yolk which is responsible for acrosomal damage.

The C.R. following artificial insemination was 53.20 % and 52.67 % with TEYG and Bioxcell® extended semen, respectively. Comparison of the conception rates showed no significant difference between semen extended with TEYG and Bioxcell®. The present finding disagrees with the finding of Wolf *et al.* (1996) while Buckowinski *et al.* (1989) who observed a higher fertility rate of 74.5% with Bioxcell® semen.

In the present study similar post thaw sperm parameters and conception rates were recorded in bull semen cryopreserved in TEYG and Bioxcell® extender. Although Bioxcell® produced fertility results equivalent to routinely use *TEYG* extender but it has got the advantage that it is chemically defined, ready to use and avoids disease transmission. So it is concluded that commercially available Bioxcell may be used for the cryopreservation of bull semen with an equal efficiency to TEYG extender.

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