Animal Reproduction Science 164 (2016) 72–81

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Enriching membrane cholesterol improves stability and cryosurvival of buffalo spermatozoa

J.S. Rajoriya[a], J.K. Prasad[b], S.S. Ramteke[b], P. Perumal[c], S.K. Ghosh[b], M. Singh[b], Megha Pande[d], N. Srivastava[d,*]

[a] Department of AR GO, College of Veterinary Science & Animal Husbandry, Rewa, MP, India
[b] Division of Animal Reproduction, ICAR-Indian Veterinary Research Institute, Izatnagar, UP, India
[c] Animal Reproduction Laboratory, ICAR-National Research Centre on Mithun, Jharnapani, Nagaland, India
[d] ICAR- Central Institute for Research on Cattle, Meerut, UP, India

ARTICLE INFO

Article history:
Received 22 June 2015
Received in revised form 4 November 2015
Accepted 14 November 2015
Available online 19 November 2015

Keywords:
Cholesterol-loaded-cyclodextrins
Phospholipids
Induced acrosome reaction
Membrane fluidity
Membrane integrity

ABSTRACT

Buffalo spermatozoa are comparatively more susceptible to freezing hazards than cattle spermatozoa. In recent times incubation of spermatozoa with cholesterol-loaded-cyclodextrins (CLC) has shown improvements in semen quality in several species. Therefore, this study was undertaken to evaluate the incubation level of CLC at which maximum benefit is derived for the buffalo spermatozoa. For the study, 120 million spermatozoa were incubated in 2, 3 and 4 mg/mL of CLC (Gr II, III and IV, respectively) and cholesterol and phospholipids content, their ratio, flow cytometric evaluation of plasma membrane integrity (PMI), plasma membrane fluidity and extent of cryoinjury (Chlortetracycline, CTC assay) were compared with an untreated control (Gr I). Additionally the ability of cholesterol-loaded-spermatozoa to undergo induced acrosome reaction (IAR) using ionophore calcium (A23187) was evaluated in frozen–thaw samples. Data show a significant and linear increase (CV = 0.88) in cholesterol content of spermatozoa in Gr II, III and IV and a significant decrease in phospholipids content at frozen–thaw stage in Gr IV than Gr III spermatozoa. The study revealed a significant improvement in PMI and significant reduction in plasma membrane fluidity and cryoinjury of CLC treated spermatozoa at progressive stages in three groups compared to control. Nevertheless, spermatozoa of Gr II, III and IV were significantly less responsive to ionophore calcium (A23187) than Gr I. This study shows for the first time that incubation of buffalo bull spermatozoa with CLC (3 mg/120 $\times$ 10^6) prior to processing permits greater numbers of sperm to survive cryopreservation while allowing spermatozoa to capacitate and the acrosome to react to AR inducer ionophore calcium (A23187).

1. Introduction

Buffaloes are considered a major livestock species in tropics providing draft power, milk and meat for human consumption, but breeding of buffaloes using frozen–thawed semen has been reported on a limited scale. This is important because of species' economic significance. The reasons for this vary from poor freezability and fertility of buffalo compared to cattle spermatozoa to the problems of sperm cell sensitivity to processing and ultra-low temperatures that can differ significantly among ejaculates of the same buffalo bull (Andrabi, 2009).

Processing of semen for cryopreservation induces many stresses on the cells including destabilization of the plasma membrane with loss of motility and function (Steponkus et al., 1983). Cell-cryodamage occurs due to intracellular

* Corresponding author. Tel.: +91 9412187937; fax: +91 5812310259.
E-mail addresses: sangnee15@gmail.com, sangnee15@yahoo.co.in (N. Srivastava).

http://dx.doi.org/10.1016/j.anireprosci.2015.11.014
0378-4320/

ice crystal formation (Mazur, 1984) and osmotic stresses due to addition or withdrawal of cryo-protectants from the buffering media (Morris et al., 2007). However, evidence indicates that intracellular ice formation does not cause significant cryo-damage for the reason of limited availability of free water in spermatozoa (Morris et al., 2007). On the other hand, several workers {buffalo, (Harshan et al., 2006); cattle, (Srivastava et al., 2012)} reported prolonged exposure of spermatozoa to detrimental proteins of seminal plasma during processing predispose sperm cells to increased incidence of cryoinjury and poor freezability.

Various protocols have been attempted to improve freezability of spermatozoa. Srivastava and co-workers (2012) reported sequestration of BSP-A1/-A2 protein from semen ejaculates by formation of protein–antibody complexes minimized cryoinjury to bull spermatozoa. On the other hand, Moussa et al. (2002) suggested that the active principle of hen's egg yolk (EY) viz. low-density lipoprotein, LDL, instead of whole EY, is responsible for protection of spermatozoa against freezing damages by preventing the loss of membrane lipids (Singh et al., 2007). Yet another protocol to reduce cryoinjury is to expose spermatozoa to exogenous cholesterol before processing for cryopreservation bull, (Amorim et al., 2009), ram, (Moce et al., 2010), equine, (Murphy et al., 2014; Hartwig et al., 2014). In buffaloes however, neither effect of addition of antibodies against detrimental seminal proteins nor exposure of spermatozoa to exogenous cholesterol on sperm cell survival following cryopreservation have been reported.

When cyclodextrins, cyclic oligosaccharides of glucose that contain a hydrophobic centre capable of incorporating cholesterol (CLC, Klein et al., 1995), were incubated with bull sperm before cryopreservation, higher percentage of viable and motile cells as compared with control were recovered (Purdy and Graham, 2004). CLC can be used to incorporate cholesterol into sperm cell membranes (Cross, 2003) and this added cholesterol probably benefits sperm cells by lowering the temperature at which the sperm plasma membrane undergo the lipid phase transition from the fluid to the gel state as the cells are cooled (Ladbroke et al., 1968). Conversely, it has been shown that cholesterol incorporated spermatozoa was less fertile than controls in mares (Zahn et al., 2002). This fact has been attributed to anti-fertility action of cholesterol, because it inhibits acrosome reaction to occur (Davis, 1980). In this investigation we have evaluated effect of incubation of buffalo bull spermatozoa with cholesterol-loaded-methyl-β-cyclodextrins before processing for cryopreservation on the semen quality parameters (SQP) with the hypothesis that more cholesterol content of sperm at fresh stage will resist membrane damage and hence effect of cryoinjury would be less evident at progressive stages of cryopreservation protocol. A previous study in our laboratory (Kumar, 2013) we found that of the two groups with incubation with CLC of 1 and 2 mg, second group showed improved SQP. Thus in the present study we decided to evaluate effect of CLC at higher than 2 mg on SQP of buffalo spermatozoa. To optimize the inclusion level of cholesterol in buffalo bull semen and to evaluate influence of cholesterol incorporation on ability of spermatozoa to capacitate and acrosome react, induced AR (IAR) in frozen-thawed sperm cells was carried out.

2. Materials and methods

2.1. Experimental design

To know the stated beneficial effect of incorporation of cholesterol in fresh spermatozoa following cryopreservation, an experiment was designed as follows: Ejaculates from buffalo bulls were selected based on mass activity, individual progressive motility and cholesterol content of spermatozoa. Semen quality variables were determined for fresh semen and the rest of the ejaculates were incubated with CLC at varying concentrations. After determining cholesterol and phospholipids contents of incubated spermatozoa (InC and InP, respectively), samples were processed for cryopreservation and evaluated for viability, cholesterol and phospholipids content of spermatozoa, plasma membrane integrity (PMI, Carboxy Fluorescent Diacetate/Propidium Iodide, CFDA/PI, Sigma–Aldrich, Missouri, United States), plasma membrane fluidity (Merocynine 540/Yo-Pro-1 assay, Sigma–Aldrich, Missouri, United States) and level of cryoinjury as indicated by distribution of capacitated or acrosome reacted spermatozoa (Chlortetracycline, CTC assay) at fresh, pre freeze (after equilibration period) and post thaw stages in each treatment groups as well as ability of frozen-thaw spermatozoa to acrosome react to AR inducer (Ionophore calcium, A23187, Sigma–Aldrich, Missouri, United States). Results were analyzed statistically and presented point-wise.

The present investigation was conducted at the Germ-Plasm Centre, Division of Animal Reproduction, Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P.). The institute is located at an altitude of 564 feet above the mean sea level at latitude of 28° North and a longitude of 79° East. This place has a subtropical climate and experiences both the extremes of hot and cold weather conditions with the relative humidity ranging from 15 to 85% in different months of the year. Four healthy breeding Murrah buffalo bulls maintained under identical management condition (aged between 4 to 6 years) were used for the collection of the semen. Semen was collected during morning hours, between 8 to 9 AM, using an artificial vagina as per the standard practice. For uniformity, twice a day biweekly collected ejaculates having mass activity of 3 and above (on a scale of 0–5) and individual motility of 70% and above were selected for processing. From each experimental bull, two consecutive ejaculates on collection days were mixed and taken as a single sample. Fresh samples with cholesterol content of spermatozoa varying more than ± 1.5 μg from the average of the study were discarded retrospectively. Out of 98 ejaculates collected initially, 64 ejaculates i.e. 32 samples qualified for the study (8 samples each from four bulls). Each sample was divided into five parts; one aliquot was used for fresh semen studies while the others were incubated for incorporation of cholesterol followed by processing for cryopreservation at −196 °C. For the present investigation all the chemicals were of