Animal Reproduction Science 149 (2014) 231–237

Contents lists available at ScienceDirect

Animal Reproduction Science

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

Effect of conventional and controlled freezing method on the post thaw characteristics of boar spermatozoa

S.K. Baishya[a,*,1], R.K. Biswas[b], G. Kadirvel[a], B.C. Deka[b], Suresh Kumar[a], S. Sinha[b], D.J. Dutta[b], G.K. Saikia[b]

[a] ICAR Research Complex for NEH Region, Umiam, Umroi Road, Meghalaya 793103, India
[b] College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati 781022, Assam, India

ARTICLE INFO

Article history:
Received 6 March 2014
Received in revised form 12 June 2014
Accepted 20 June 2014
Available online 28 June 2014

Keywords:
Boar semen
Cryopreservation
Freezing method

ABSTRACT

The objective of the present study was to evaluate the effectiveness of conventional, and controlled freezing method adopting three freezing rates 20 °C, 40 °C and 60 °C/min for cryopreservation of boar semen. Sixty sperm-rich fractions of ejaculates from six boars were utilized for freezing of semen with different freezing methods in lactose-egg yolk glycerol extender using 0.5 ml straws. Semen samples were evaluated for sperm motility, live sperm, acrosome integrity, plasma membrane integrity (PMI) by carboxyfluorescein diacetate plus propidium iodide (PI) staining, mitochondrial membrane potential (MMP) by combined JC-1 plus PI staining and lipid peroxidation (LPO) by BODIPY $^{581/591}$-C_{11} probe after equilibration and after freezing. The results revealed that the post thaw sperm motility, live sperm, live intact acrosome and plasma membrane integrity were significantly ($p < 0.05$) higher in all the three controlled freezing methods (20 °C, 40 °C and 60 °C/min) as compared to that in conventional method. In addition, the controlled freezing methods yielded higher ($p > 0.05$) mean values of live sperm with high MMP as compared to conventional freezing. However, the post thaw sperm LPO did not influence by difference in freezing methods. No significant difference on the post thaw sperm qualities was recorded among the three controlled freezing rates. All the sperm parameters assessed declined significantly ($p < 0.05$) after freezing as compared to that after equilibration irrespective of freezing method employed. In conclusion, cryopreservation of boar semen with controlled freezing methods conferred better post thaw sperm quality as compared to conventional method, and the freezing rates of either 20, 40 or 60 °C/min could provide better freezability of boar semen.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The application of artificial insemination (AI) with liquid stored-semen of improved breed of boar is of paramount importance for upgradation of indigenous pigs in most of the developing countries. The advantages of AI could be maximized if spermatozoa were cryopreserved, since it is the best alternative for long-range distribution of high quality genetic material (Roca et al., 2006). Frozen semen could also be used to establish genetic resource banks for conservation of genetic diversity and even for reserve supplies in response to sudden disease outbreak (Yoshida, 2000). Although frozen boar semen has been available since long, a very low proportion of the commercial artificial inseminations are carried out in the pig utilizing frozen

* Corresponding author. Tel.: +91 9436108526; fax: +91 364 2570011.
E-mail address: santosh.baishya@rediffmail.com (S.K. Baishya).
[1] This paper is a part of PhD research of the first author.

http://dx.doi.org/10.1016/j.anireprosci.2014.06.020
0378-4320/© 2014 Elsevier B.V. All rights reserved.

semen due to its lower survival post thaw that results in low farrowing rates and litter sizes (Roca et al., 2006; Hernandez et al., 2007). However, there is diversity of opinion regarding fertility status of frozen boar semen.

The low fertility of frozen thawed boar semen could result from the damage sustained by the sperm cells during cooling, freezing and thawing processes of cryopreservation (Holt, 2000; Watson, 2000) since boar spermatozoa are highly sensitive to cold shock when exposed to temperature below 15 °C (Gilmore et al., 1996). Boar sperm damage during freezing could be related to the lipid composition of sperm plasma membrane, oxidative stress and Reactive Oxygen Species (ROS) formation. An increased understanding of cryodamage is of utmost importance that could lead to improved cryopreservation methods rendering superior quality frozen semen and resultant higher fertility. The in vitro evaluation of sperm motility, sperm livability, membrane integrity, acrosome status, mitochondrial membrane potential (MMP) and lipid peroxidation (LPO) during cryopreservation process are good indicator of sperm quality and fertility status of spermatozoa.

Now-a-days cryopreservation method for boar semen has emphasized on various freezing strategies to improve the quality of post thawed spermatozoa, increase the number of spermatozoa for oocytes fertilization and reduced the sperm damage. It has been documented that sperm damages emanated during cryopreservation procedures could be mitigated to some extent through improvement in freezing strategies (Fiser and Fairfull, 1990; Woelders and Besten, 1993; Roca et al., 2006). Adoption of suitable freezing protocol has resulted in improvement of cryopreservation technique in the boar (Eriksson and Rodriguez-Martinez, 2000). It has been reported that controlled rate of freezing method yielded superior post thaw sperm quality as compared to conventional freezing method (Verheyen et al., 1993; Petyim et al., 2007; Kaeoket et al., 2008). A comparative study of different freezing methods helps find out a superior freezing method for production of frozen boar semen; moreover, few studies have been conducted in this aspect earlier. Therefore, a study was designed to assess the effect of conventional, and controlled freezing methods adopting freezing rates either of 20, 40 or 60 °C/min on sperm motility, live sperm, live intact acrosome, plasma membrane integrity, mitochondrial membrane potential and lipid peroxidation in order to find out a suitable freezing method for cryopreservation of boar semen.

2. Materials and methods

The present study was conducted at Artificial Insemination Laboratory of Livestock Production Division, ICAR Research Complex for NEH Region, Umiam, Meghalaya, India and Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, AAU, Khanapara, Guwahati, Assam, India. All the six boars used for semen collection were housed in individual, well-ventilated and uniform pens with open paddock. They were fed with concentrate feed (consisting of 40% maize, 30% wheat bran, 16% ground nut cake, 12% soyabean meal with 1.5% mineral supplements and 0.5% salt) two times a day. The animals were humanely treated and the study was designed as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Govt. of India and Institutional Animal Ethics Committee (IAEC).

2.1. Semen collection, processing and freezing

A total of 60 ejaculates from six healthy and fertile boars consisting of two boars each of Hampshire (HS), Hampshire × Khasi local (HS × KL) with 87.5% exotic inheritance and HS × KL with 75% exotic inherit were collected by gloved hand technique using dummy sow. Immediately after collection semen was brought to the laboratory at 35 °C and evaluated for semen volume, sperm motility, sperm concentration and live sperm using standard laboratory procedure. Semen ejaculate showing more than 70% progressive motility, 80% sperm with normal morphology were selected for freezing. Semen samples were processed and frozen following straw freezing procedure as described below. The sperm-rich fraction was collected in an insulated thermos flask, separating the gel fraction through gauze during collection. Semen was transferred to measuring glass cylinder and diluted with Beltville thawing solution (BTS) (Johnson et al., 1988) at 1:1 ratio, transferred to 50 ml centrifuge tube, held at 24 °C for 2 h and then at 18 °C for 1 h in a BOD incubator (NSW-152, Narang Scientific Works Pvt. Ltd., Mayapuri Ind. Area, New Delhi, India). After a total of 3 h of holding semen in 50 ml tubes was centrifuged (REMI C-24, Mumbai, India) for 10 min at 600 × *g* at 18 °C. The supernatant was discarded and sperm pellet was re-suspended with fraction I of lactose-egg yolk extender (lactose 11% – 80 ml and egg yolk 20 ml) to a concentration of 1.5×10^9 sperm/ml. The extended semen was cooled to 5 °C for 90 min in the BOD incubator. The extended semen cooled to 5 °C was then transferred to cold handling cabinet (IMV Technologies, France) maintained at 5 °C and mixed with fraction II of lactose-egg yolk-glycerol extender (lactose 11% – 74 ml, egg yolk 20 ml and 6 ml glycerol) to make the final concentration of semen to 1.0×10^9 sperm/ml. Then, semen was equilibrated for 60 min at 5 °C at cold handling cabinet and during this period filling and sealing of straws were carried out. Medium straws (0.5 ml, Genuine Cassou straw, IMV Technologies, France) were used for loading of semen. All the equipments required i.e., straws, sealing powder, towel, filling comb and freezing rack were kept in a cold handling cabinet at 5 °C. After 30 min of equilibration, the straws were filled with semen by filling comb and open ends were sealed with PVC powder (IMV Technologies, France). At the end of 60 min of equilibration period, the straws were placed on a pre-cooled (5 °C) towel and were dried by rolling between the folds of the towel. The straws were put horizontally on the freezing rack for freezing. For conventional freezing method (freezing method 1) the straws were arranged horizontally 3 cm above the liquid nitrogen level in a thermocole box and exposed to liquid nitrogen vapours for 15 min. Immediately after vapour freezing, the straws were transferred into a goblet containing liquid nitrogen and placed in liquid nitrogen container for storage. The controlled rate of freezing was done in a programmable freezing machine

Download English Version:

https://daneshyari.com/en/article/8404614

Download Persian Version:

https://daneshyari.com/article/8404614

Daneshyari.com