Theriogenology 83 (2015) 307–313

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Review

Fertility of boar semen cryopreserved in extender supplemented with butylated hydroxytoluene

Monika Trzcińska*, Magdalena Bryła, Barbara Gajda, Piotr Gogol

National Research Institute of Animal Production, Department of Animal Reproduction Biotechnology, Balice, Kraków, Poland

A R T I C L E I N F O

Article history:
Received 6 June 2014
Received in revised form 25 July 2014
Accepted 26 July 2014

Keywords:
Boar
Frozen-thawed semen
Butylated hydroxytoluene
Intrauterine artificial insemination
Reproductive performance

A B S T R A C T

The present study was to determine the effect of butylated hydroxytoluene (BHT) on quality and fertilizing ability of frozen-thawed boar semen. In the first experiment, five crossbreds of Polish Landrace and Large White boars (five ejaculates per boar) were frozen in 0.5 mL straws after dilution with lactose-egg yolk-glycerol extender supplemented with 0 (control), 0.5, 1.0, and 2.0 mM BHT. The sperm quality was verified based on the motility (computer-assisted sperm analysis; total motility, %; progressive motility, %), membrane integrity (YO-PRO-1/propidium iodide [PI] assay), acrosome integrity (fluorescein isothiocyanate–conjugated with peanut agglutinin/PI), and lipid peroxidation (chemiluminescence method) at 15 minutes postthaw. In the second experiment, the semen cryopreserved in extender supplemented with 1.0 and 2.0 mM BHT were selected for intrauterine artificial insemination of synchronized gilts. An intrauterine artificial insemination with low numbers of spermatozoa (500×10^6) was surgically infused into each uterine horn. The highest ($P < 0.001$) progressive motility (%), membrane integrity, and acrosomal integrity were noted by the addition of 1.0 and 2.0 mM BHT to the freezing extender. Moreover, the various concentrations (0.5–2.0 mM) of BHT caused a considerable decrease in lipid peroxidation in relation to the control extender ($P < 0.001$). The highest reproductive performance of inseminated gilts (farrowing rate, 86.7%; litter size, 10.8 ± 1.6) was observed when semen was cryopreserved in extender supplemented with 1.0 mM BHT. These findings demonstrate that the addition of 1.0 mM BHT to the freezing extender efficiently improves the fertilizing ability of postthaw boar spermatozoa.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Frozen-thawed (FT) boar semen for artificial insemination (AI) in commercial pig production is still only used in exceptional cases, mostly to improve the genetic progress [1]. This semen has not been used under production conditions as efficiently as liquid-preserved semen, because of the high susceptibility of boar spermatozoa to damage during cryopreservation and a complicated process of deep freezing. The processes of cooling, freezing, and thawing produce physical and chemical stress on the sperm membrane [2]. Moreover, a cryopreservation protocol produced cold shock and oxidative attack on the sperm membrane, which decreased sperm survival and freezing ability leading to the death of the sperm. The relatively low fertility of FT boar semen is associated with many factors. It has been reported that reactive oxygen species (ROS) generation, induced by the cryopreservation process, can be responsible for mammalian sperm damage [3]. An important indicator of the membrane damage is a change in membrane permeability, such as increased permeability to stains and a release of intracellular substances. It is proposed that this could be caused by lipid peroxidation because of the relatively high content of unsaturated fatty acids in phospholipids of the boar sperm membrane [4]. Spermatozoa are particularly vulnerable to oxidative

* Corresponding author. Tel.: +48666081381; fax: +48122856733.
E-mail address: monika.trzcinska@izoo.krakow.pl (M. Trzcińska).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.07.045

damage during *in vitro* storage when the production of free radicals could be considerably enhanced as a result of metabolic changes [5]. The attack of free radicals on the unsaturated fatty acid rich lipids of sperm cell membranes leads to irreversible reduction in membrane fluidity and damage of cell membrane related to ATPases, which are responsible for regulating the intracellular level of ions necessary to maintain normal sperm motility [6]. Sperm isolated from fresh [4], hypothermic liquid stored [7], and FT boar semen are susceptible to $FeSO_4$ and ascorbate catalyzed lipid peroxidation, as measured by malondialdehyde (MDA) formation. Measurement of MDA is used as an assay for lipid peroxidation in spermatozoa, but provides only an indirect measure of lipid peroxidation [8]. Moreover, MDA only accounts for around 5% of the products generated during lipid peroxidation. The lipid peroxidation process in spermatozoa leads to the creation of substances having cytotoxic properties, such as MDA and 4-hydroxynonenal [9], which are not accounted for in the MDA assay [10]. Chemiluminescence is considered to be a sensitive method to assess the oxidation or autoxidation of lipids [11]. The results of the previous study [10] revealed that induced luminescence is strictly related to lipid peroxidation in spermatozoa.

Sperm damage through ROS during the cryopreservation process can be successfully minimized with the addition of antioxidants to the cooling and freezing media. Good results concerning postthaw sperm quality have been obtained with, e.g., vitamin E, alpha-tocopherol, glutathione, superoxidase dismutase, catalase, and butylated hydroxytoluene (BHT) [12].

Butylated hydroxytoluene is a synthetic analog of vitamin E that checks the autooxidation reaction by converting peroxy radicals to hydroxyperoxides. Butylated hydroxytoluene has been tested in our laboratory successfully to preserve liquid semen in boar [13,14]. This antioxidant has been tested successfully to minimize cold shock damage in goat [15], bull [16], buffalo [17], dog [18], and boar [4]. Moreover Roca et al. [4] evaluated the postthaw sperm ability to produce embryos *in vitro* and develop to the blastocyst stage. No study has been conducted to evaluate the actual impact of supplementing freezing extender with BHT on *in vivo* fertilizing ability of boar spermatozoa.

Another fact is that AI with FT boar semen still results in low conception rate and small litter sizes [3,19]. The lifespan of FT boar semen in the female reproductive tract is shorter (2–8 hours) than that of its liquid-preserved counterpart [20]. The preovulatory AI is recommended to establish a sperm reservoir in the oviduct, and thus sufficient numbers of spermatozoa are available to fertilize the ovulated oocytes. The AI must be done within 4 to 6 hours before ovulation. Fertilizing ability of the ovulated oocytes is also short, 6 to 8 hours, so the timing of sperm deposition in relation to the moment of ovulation is very important [21].

The main aim of the present study was to evaluate *in vivo* fertilizing ability of boar semen cryopreserved in extender supplemented with BHT after preovulatory intrauterine AI. Moreover, the effect of adding BHT to the freezing media on postthaw sperm quality was assessed based on the motility, membrane and acrosome integrity, and lipid peroxidation.

2. Material and methods

Procedures involving animals were approved by the Local Ethics Board for Animal Experiments in Kraków (Poland).

2.1. Experiment 1: Effect of BHT supplementation on postthaw sperm quality

2.1.1. Animals

Five crossbreds of Polish Landrace and Large White boars aged 2 to 4 years and selected according to normal semen quality and proven fertility were used in this study. Preselection for semen freezability was performed. Sperm cryosurvivability was determined as a percentage of motile spermatozoa (total motility [TM]%) that survived the freezing process: percentage of FT motile sperm/percentage of fresh motile sperm $\times$ 100. In the experiment, good freezability semen with high cryosurvival rate of 60% or greater was used.

Boars were kept at the Boar AI Station in Klecza Dolna. All boars were housed in buildings with stable conditions of controlled temperature and humidity and were fed an adjusted commercial diet.

2.1.2. Semen cryopreservation

The sperm rich–fraction was collected by hand manipulation into water-jacketed vessels. The volume, concentration, and motility of the sperm were estimated immediately after collection. Only ejaculates with greater than 70% progressively motile sperm and 80% morphologically normal spermatozoa were used for cryopreservation. Immediately after collection, the semen was diluted (1:1) in *Biosolwens Plus* (BP) extender (Biochefa, Sosnowiec, Polska). Spermatozoa were cryopreserved using the method described by Westendorf et al. [22] with modifications described subsequently. The diluted semen was transferred to 50 mL centrifuge tubes, equilibrated at 15 °C for 60 minutes, and centrifuged at 800$\times$ *g* for 25 minutes. The supernatant was discarded and the sperm pellet was resuspended with extender A (80 mL of 11% lactose solution and 20 mL egg yolk) to a concentration of 1.5×10^9 spermatozoa/mL. The diluted semen was cooled to 5 °C for 120 minutes. Two parts of semen were mixed with one part of extender B (89.5% extender A with 9% glycerol and 1.5% Equex-STM paste whose active ingredient is sodium dodecyl sulphate, Nova Chemical Sales, Scituate Inc, MA, USA). The final concentration of semen was 1.0×10^9 spermatozoa/mL and 3% glycerol. The diluted and cooled semen was loaded into 0.5 mL straws (Minitüb). The straws were sealed with polyvinyl chloride powder before being placed in contact with nitrogen vapor for 15 minutes in a polystyrene box. After that, the straws were plunged into liquid nitrogen (−196 °C) for storage. After 2 weeks of storage, samples were removed from the liquid nitrogen and thawed. Thawing was carried out by immersing the straws in a circulating water bath at 37 °C for 40 seconds. Immediately after thawing, the semen was diluted in BP extender at 37 °C.

For maximum solubility in aqueous buffers, BHT was first dissolved in absolute ethanol, dried down under air in tubes, and diluted in BP extender.

Download English Version:

https://daneshyari.com/en/article/2095030

Download Persian Version:

https://daneshyari.com/article/2095030

Daneshyari.com