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Desalted and lyophilized bovine seminal plasma delays induction of the acrosome reaction in frozen-thawed bovine spermatozoa in response to calcium ionophore

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ABSTRACT

Cryopreservation is partially damaging and induces capacitation-like changes in spermatozoa. Seminal plasma (SP) contains a variety of biochemical components, such as protein and lipids, which are specific for the regulation of sperm cell function including those effective for decapacitation of spermatozoa. Therefore, this study tested the hypothesis that desalted and lyophilized SP could prevent premature capacitation (cryocapacitation) of Japanese Black bull spermatozoa. Seminal plasma was desalted by using Sephadex G-25 desalting column and lyophilized before added to semen extender at final concentrations 0, 2.5, 12.5, and 25 mg/mL. Frozen-thawed sperm progressive motility, acrosomal integrity, abnormal morphology, and the calcium ionophore A23187–induced acrosome reaction were assessed. Protein and lipid compositions in SP were analyzed by SDS-PAGE and thin-layer chromatography, respectively. The results revealed that progressive motility, intact acrosome, and abnormal morphology were not substantially modified by addition of SP. Stimulation of spermatozoa with calcium ionophore A23187 resulted in a time-dependent induction of the acrosome reaction, which was delayed by the desalted and lyophilized SP. There was no difference in the protein profile of SP before and after gel filtration. In total, 19 protein bands with molecular masses ranging from 5.2 to 185.8 kDa were detected and those of 185.8, 80, 34, 20.8, 18.8, 17.5, and 10 kDa were considered as novel proteins. Neutral lipids and phospholipids before and after gel filtration were the same, and the detected neutral lipid spots were monoacylglycerol, cholesterol, 1,2- and 1,3-disaturated diacylglycerol, 1,2- and 1,3-saturated, unsaturated diacylglycerol, whereas the detected phospholipid spots were sphingomyelin, phosphatidylcholine, phosphatidylserine, and three species of phosphatidylinositol, phosphatidylethanolamine, cerebroside, and polyglycerol phosphatide. The results suggest that premature capacitation during freeze-thaw processes could be reduced by adding desalted and lyophilized SP.

1. Introduction

Cryopreservation is an important assisted reproductive technology available to animal industries, but it is partially

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damaging for gametes and induces capacitation-like changes in bovine [1], porcine [2], and equine [3] spermatozoa, which is known as cryocapacitation. It has been suggested that cryopreservation induces modifications in sperm membranes and makes them more reactive to their environment after thawing [4] and undergo capacitation more quickly than fresh spermatozoa [5]. It has also been suggested that premature capacitation status observed in frozen-thawed bull spermatozoa was related to subfertility [6].

Seminal plasma (SP) has been shown to stabilize the membrane of ram spermatozoa during *in vitro* processing, partly alleviating damage induced by high dilution, freezing, and oxidative stress [7]. Seminal plasma has also been reported to reverse freeze-thaw damage of ram spermatozoa frozen with cryoprotectants [8,9] and improving their *in vitro* [8] and *in vivo* fertility [10]. In contrast, in bulls, protective effects of SP have not been recognized widely [11]. Also, SP contains decapacitation factors [12–14], which might prevent, at least partially, cryocapacitation or premature capacitation.

Lipids are a basic component of semen, contributing to the membrane structure, metabolism of spermatozoa, and their ability to capacitate and fertilize the female gamete [15] and are not only of relevance regarding the storage of energy, but are also massively involved in signal transduction processes [16]. Among them, cholesterol is a major free sterol in ejaculated bovine semen [17], and the efflux of membrane cholesterol leads to capacitation [18]. Supplementation of bison [19] and bull [20,21] semen extender with cholesterol–methyl-β-cyclodextrin has been shown to be protective against freeze-thaw damage.

During capacitation process, external bicarbonate ion (HCO_3^-) activates sperm adenylate cyclase to produce intracellular cAMP, and Ca^{2+} internalizes into spermatozoa [22]. The subsequent acrosome reaction (AR) is an irreversible exocytotic process leading to the release of hydrolytic enzymes and enabling the sperm to penetrate the zona pellucida. Acrosomal exocytosis is induced in response to the oocyte-derived agonists, progesterone, and zona pellucida [23], but can also be triggered by Ca^{2+} and Ca^{2+} ionophore A23187 [24,25] and enhanced by cAMP [26]. Therefore, it was expected that the AR induced by Ca^{2+} and A23187 may mirror the capacitated status; rapid induction of AR is expected in spermatozoa with at least high intracellular [Ca^{2+}] and [cAMP] in which capacitated status is thought to be advanced.

Desalted and lyophilized SP would be of the most commercial benefit if they could be added to the spermatozoa as a supplement before freezing, possibly as an ingredient in the cryodiluent to minimize premature capacitation of bull spermatozoa during freezing and thawing. For this, gel filtration (desalting) was used to remove electrolytes including Ca^{2+} and HCO_3^-, which are responsible for capacitation of spermatozoa. Therefore, the present study aimed to

(1) investigate the optimal conditions for desalting bovine SP by gel filtration to be added to cryoprotective diluents before cryopreservation as a lyophilized powder.
(2) Investigate the effect of desalted and lyophilized SP on routine semen parameters and the AR induced by Ca^{2+} and A23187 as an indicator to examine sperm capacitated status in frozen-thawed Japanese Black bull spermatozoa.
(3) Identify new protein and lipid species in SP of Japanese Black bulls.

2. Materials and methods

2.1. Collection of samples

Semen was collected by an artificial vagina from a total of nine mature healthy Japanese Black bulls (bull nos. 1–9; 1.5–6.5 years old at the time of semen collection) kept at Hida Beef Cattle Research Department, Gifu Prefectural Livestock Research Institute, Japan (altitude 709 m, humidity 77%, minimal daily ambient temperature 6.5 °C, maximal daily ambient temperature 17.0 °C, and average ambient temperature 11.0 °C).

The nine bulls were of proven fertility and had conception rates higher than 50% after artificial insemination with frozen-thawed semen. For experiments using gel-filtrated, fractionated SP, semen was collected from three of the nine bulls (bull nos. 1, 2, and 3; two times per bull) and the two samples were pooled per bull. To obtain gel-filtrated and lyophilized SP (SP powder) to be added to frozen semen, one or two ejaculates were collected from four of the nine bulls (bull nos. 1, 2, 4, and 5) and pooled. This collection was carried out four times and thus four different batches of SP powder were obtained. For experiments in which frozen semen was supplemented with SP powder, one ejaculate was collected per bull from four of the nine bulls (bull nos. 6, 7, 8, and 9) and was supplemented with four concentrations of SP powder (0, 2.5, 12.5, and 25 mg/mL as described subsequently) and kept frozen in liquid nitrogen as described subsequently.

2.2. Source of chemicals and reagents

All chemicals and reagents used were of high purity or analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Wako Pure Chemicals Industries (Osaka, Japan) unless otherwise stated.

2.3. Media

Tris-based diluent, composed of 130 mM of Tris, 29.8 mM of sodium citrate, 41.3 mM of lactose, 50.4 mM of raffinose, 20% of egg yolk (v:v), 0.6 mg/mL of streptomycin, and 600 IU/mL of penicillin G potassium, and the same diluent including 7% glycerol was used for semen dilution and freezing [27]. The media used for sperm washing and incubation were as previously described [28]. Briefly, the saline medium used for dilution and incubation of spermatozoa consisted of 142 mM of NaCl, 2.5 mM of KOH, 10 mM of glucose, and 20 mM of HEPES adjusted to pH 7.55 at 25 °C with NaOH [29]. Saline medium containing 275 mM of sucrose in place of NaCl was used for washing spermatozoa and designated as sucrose medium [29]. Both media also contained 0.1 wt/vol% polyvinyl alcohol