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Effect of semen dilution to low-sperm number per dose on motility and functionality of cryopreserved bovine spermatozoa using low-density lipoproteins (LDL) extender: Comparison to Triladyl® and Bioxcell®

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Abstract

Artificial insemination with doses containing low-sperm numbers has been utilized to optimize the use of elite bulls. Hen egg yolk is widely used as a cryoprotective agent in semen freezing extender protecting the spermatozoa. Its action is due to the presence of low-density lipoproteins (LDL) in the hen egg yolk. The objectives of the present study were to evaluate the effects of the semen dilution to low-sperm number/dose on sperm motility and integrity of sperm plasma membrane in the cryopreservation process, using two commercial extenders (Triladyl®, Bioxcell®) and LDL extender prepared in our laboratory, 97% purity. Fifteen ejaculates were collected from five fertile crossbred bulls (*Bos taurus* × *Bos indicus*). After collection, sperm motility was examined by Computer-Assisted Semen Analysis (Hamilton Thorne), morphological sperm characteristics were evaluated by differential interference microscopy and the integrity of plasma membranes was determined using the hypo-osmotic swelling test. The semen was subsequently divided into three aliquots and diluted with the three extenders into 120×10^6, 60×10^6 and 20×10^6 sperm/mL, corresponding to 30×10^6, 15×10^6 and 5×10^6 sperm/dose, respectively. This study revealed that LDL extender was more effective in preservation of motility and integrity of the plasma membrane of spermatozoa than Bioxcell® and Triladyl® ($p < 0.05$), but no significant difference was observed between Triladyl® and Bioxcell®. Therefore we can conclude that LDL extender could be used instead of Triladyl® or Bioxcell® at low semen concentration per dose for elite bulls, it also could be envisaged for the industry of sex-stored semen.

Keywords: Bull; Semen dilution; LDL extender; Cryopreservation; Motility

1. Introduction

Artificial insemination (AI) is the most widely used biotechnology in developed and developing nations in livestock farming. Its notable benefits include extending

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the use of males with proven genetic merit in desired traits to a vast number of females, in comparison with natural service. Frozen semen allows genetic progress to be disseminated worldwide [1]. However, the genetic impact of individual bulls in the AI industry is limited by the efficiencies of semen production, a high percentage of spermatozoa lose their integrity or functionality when subjected to cryopreservation [2,3]. Significant improvement can be obtained through the use of different extenders, which should interact with semen and provide protection to the spermatozoa during the cooling, freezing and thawing processes [4]. Pace and Graham [5] purified egg yolk and observed that the low-density lipoproteins (LDL) fraction displayed a cryoprotective property. Many other studies have confirmed that the LDL fraction has the same cryoprotective action [6,7,8]. The advantage of the LDL extender over to standard egg yolk extender is that it has a less complex chemical composition and does not interfere with microscopic observations [8]. On the other hand, use of AI-doses containing low-sperm numbers are increasingly widespread to optimize the benefit of elite bulls, as well as to accommodate an eventual wider application of sex-sorted semen [9]. Numerous studies have confirmed that acceptable fertility can be maintained at 10×10^6 sperm/dose [10], however AI-doses containing low-sperm numbers result in reduced post-thaw viability [11]. The magnitude of these reductions in viability appears to be related to the volume and sperm concentration of the undiluted semen and seminal plasma concentration at the final dilution [12]. The aim of the present study was to evaluate the effects of cryopreservation at high dilution rates on sperm motility and, plasma membrane integrity, using LDL extender in comparison with Bioxcell® (soy bean extract extender) and Triladyl® (standard egg yolk extender).

2. Materials and methods

2.1. Semen collection and pre-cryopreservation analysis

Semen samples were collected with an artificial vagina ($n = 15$ ejaculates) from five crossbred bulls (*Bos Taurus* × *Bos indicus*), of proven fertility, aged 6–8-year old, belonging to the Central University of Venezuela. Following collection and before freezing, the semen was analyzed for volume, motility, morphology, concentration and plasma membrane integrity.

2.2. Computer-assisted analysis of fresh sperm motility

The sperm motility of each ejaculate was analyzed using the Computer-Assisted Sperm Analysis System (CASA) system (HTM-IVOS-Ultimate; Hamilton Thorn Biosciences, Beverly, MA, USA), the CASA had been configured for bovine sperm analyses. To assess the motility of fresh ejaculate the semen sample was diluted to a final concentration of 25×10^6 sper- spermatozoa/mL in Tris buffer (2.45 g, free of egg yolk and glycerol) supplemented with citric acid (1.48 g), fructose (1 g), penicillin (50 000 IU), gentamicin (25 mg) and 100 mL sterile water, pre-warmed to 37 °C, for 10 min. Two microlitres of that sample were placed in a 20-μm standard counting chamber (SC20.01.FA; Leja, Nieuw-Vennep, The Netherlands). A minimum of 20 fields were selected for each analysis.

2.3. Assessment of fresh spermatozoa morphology

Morphological sperm characteristics were determined using differential interference microscopy (Olympus CK2, ULWCD 0.30). A microscope slide was prepared from single extended sperm samples prior to freezing. At least 200 spermatozoa were analyzed for each ejaculate. The morphology was expressed as a percentage of normal sperm.

2.4. Assessment of fresh spermatozoa membrane integrity

The plasma membrane integrity was determined using the hypo-osmotic test (HOS) [13]. Hundred microlitres of each ejaculate were mixed with 1 mL of an hypotonic solution (100 mOsm/kg H_2O) prepared with 75 mM fructose and 25 mM tri sodium citrate in distilled water. The mixture was incubated at 37 °C for 60 min. Following incubation, 15 μl were placed on a slide, covered and observed under the microscope (Olympus CK2, ULWCD 0.30) at 400×. The spermatozoa were classified according to the presence or absence of swollen tail [9,10]. At least 200 spermatozoa were observed and recorder to express the results in percentages. Normal spermatozoa, when exposed to hypo-osmotic stress due to the influx of water, undergo swelling and subsequent increase in volume. The sperm plasma membrane surrounding the tail fibers appears to be more loosely attached than the membrane surrounding the head, so, the tail regions shows the swelling effect more clearly [9,10].