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Coulter counter-based evaluation of sperm volume to assess sperm viability of bull semen and application to X/Y sperm sorting

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Abstract

The Coulter Counter Hypo-Osmotic Swelling test (CC-HOS) was developed to provide insight into the membrane integrity (relative volume shift V_r) of sperm necessary for fertilization, and to identify the optimum buffer needed for the X/Y chromosome sorting process. Using the CC-HOS test on neat bovine semen, the mean relative volume shift V_r for July and August was 1.20 and 1.14, respectively, whereas mean V_r values ranged from 1.32 to 1.41 during September to November. There was an inverse relationship between V_r magnitude and environmental temperature; we inferred that this enhanced sperm viability during autumn relative to summer. A method was developed to measure the dynamics of volume change of sperm in the buffer (pH 6.5) used for the X/Y chromosome sorting process. When exposed to the buffer (4 mM K^+, 153 mM Na^+, 140 mM Cl^-), sperm from Bull C had a mean modal volume of 22.8 ± 0.2 fL during a 0–300 s time interval, which did not significantly vary from sperm volumes (21.88 ± 0.66 fL for Bull A and 22.46 ± 0.38 fL for Bull B)) noted in isotonic Isoton® II solution. However, when exposed to lower ionic concentrations (2 mM K^+, 62 mM Na^+, 47 mM Cl^-), the mean volume of Bull C sperm increased to 29.2 ± 1.5 fL and exhibited slower rates toward stabilized volumes relative to higher ionic concentration buffers. Utilization of volume swelling measurements for measuring the impact of ion concentrations in X/Y chromosome sorting process buffers illustrated the importance of its application for emerging sperm-based biotechnologies.
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1. Introduction

Volume regulation [1–6] is an essential property that sperm must possess to maintain sperm viability necessary for fertilization. Empirical relationships between the ability of sperm to swell under hypotonic conditions and the potential for fertilizing an oocyte have been reported [7,8]. Several investigators have also established a microscope-based qualitative test (Hypo-Osmotic Swelling test or HOS test) for measuring sperm swelling under hypotonic conditions. In a frequently cited paper [9], human sperm was exposed to solutions at various osmolalities (0–300 mOsm/kg). The maximum percentage of swollen sperm occurred in 150 mOsm/kg solutions; there was an excellent correlation between the percentage of swollen sperm and the percentage of denuded hamster oocytes penetrated by the sperm. From this work and the works of Liu and Foote [10], Correa and Zavos [11], Wang and Suh [12] and Hossain et al. [13], the microscopic-based HOS test was firmly established as a method for

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qualitatively evaluating human and bull sperm by measuring both the percentage of swollen sperm (after exposure to hypotonic conditions), as well as the degree of swelling.

A major drawback to the microscopic-based HOS test is the subjectivity inherent in all qualitative tests. Categorizing the sperm as swollen requires the tails to be curled and it is difficult to determine whether the tails are curled as a result of marginal swelling. Another drawback is the extremely low number of sperm that are evaluated as representative of the total. An alternative approach to the microscope-based HOS test was pursued by Petzoldt and Engel [14] and Petrunkina et al. [8,15–17] in which ejaculates from the boar, dog, bull and human were analyzed using a Coulter counter and particle size analyzer (CASY1 cell counter), respectively (Coulter Counter Hypo-Osmotic Swelling test (CC-HOS)). For a given ejaculate, this method provided the volume distribution of the sperm, which, in turn, provided identification of subpopulations. Each subpopulation exhibited distinct modal or peak volumes, resulting in observable shifts in peak volumes when exposed to hypotonic and hypertonic conditions. This approach to the HOS measurement was also used to study boar sperm volume changes due to capacitation conditions [18] and the cryopreservation of semen [16,19–21]. Another approach to HOS measurement was pursued by Yeung et al. [22] using light scattering from a flow cytometer.

Although the amount of scientific literature describing the advances in the development and application of the CC-HOS is extensive, the challenge was to apply this methodology to the process of separating X chromosome-bearing sperm from their Y chromosome counterparts [23–26] by flow cytometry-based sorting. Discussed in this paper are: (1) the development of the CC-HOS test for neat semen and AI frozen straws, using literature precedent as a guide; (2) a statistical study to evaluate the impact of test variability associated with two different analysts; (3) application of the CC-HOS test in evaluating neat semen for sperm viability from two bulls over a 6-mo interval; (4) extension of the CC-HOS test methodology for measuring sperm volume in process buffer (X/Y sperm sorting) of varying ionic composition.

2. Materials and methods

2.1. Chemical reagents and preparation of Isoton II® solutions of varying osmolality

The citric acid monohydrate, fructose, pyruvic acid and Trizma® base were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). The GIBCO Ultrapure™ water used for all experiments was obtained from Invitrogen Corporation (Carlsbad, CA, USA). Sperm volume measurements were performed on the Beckman Coulter Multisizer™ 3 Coulter Counter (Beckman Coulter, Inc., Fullerton, CA, USA). Daily instrument check was performed using low (18×10^6/mL) concentration and high (35×10^6/mL) concentration AccuBeads® (Hamilton Thorne Biosciences, Beverly, MA, USA). The standard 3-μm diameter L3 latex beads used for determining the volume calibration factors and the Isoton® II solution (pH 7.35–7.65) were obtained from Beckman Coulter, Inc. (Fullerton, CA, USA). Composition of the Isoton® II solution was sodium (174 ± 7 mequiv/L), potassium (5.4 ± 0.3 mequiv/L), phosphorus (46 ± 5 mg/dL), chloride (141 ± 5 mequiv/L), fluoride (0.03 ± 0.01 g/dL) and phenoxyethanol (0.3% (w/v) $\pm$ 0.05%). The Dulbecco's PBS buffer was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

The osmolality of all solutions used was verified with the Advanced™ Micro Osmometer Model 3300 (Advanced Instruments, Inc., Norwood, MA, USA), and all solutions were filtered using a Corning 0.22 μm cellulose acetate filter (VWR International, Batavia, IL, USA) before use. The isotonic solution (300 mOsm/kg) and hypotonic solution (150 mOsm/kg) were prepared by diluting the Isoton® II stock solution (340 mOsm/kg) with GIBCO Ultrapure™ water. The hypertonic solution (450 mOsm/kg) was prepared by the addition of sodium chloride to the Isoton® II stock solution.

2.2. Semen collection and analysis

Neat semen from three mature Holstein bulls (Bulls A–C) was obtained from the Plunkett-Mason Farms (Bonne Terre, MO, USA). Bulls were fed a commercial cattle ration and had access to grass hay ad libitum. Semen was collected in an artificial vagina; thereafter, the artificial vagina was taken to a heated laboratory and the collection tube was removed from the collection cone and placed in a water bath (approximately 23 °C). The collection tube lid was screwed into place and covered with parafilm. The sample was transported to the laboratory in an insulated thermos using water from the heated bath. The neat semen was analyzed for total sperm concentration, percentage of total motility and percentage of progressive motility by IVOS (Integrated Visual Optical System; Hamilton Thorne Biosciences, Beverly, MA, USA) [27] measurements. Samples for the IVOS assay were prepared by slowly adding a TCA-based buffer (980 μL) to 15–20 μL of neat semen

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