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Fertilization rates and *in vitro* embryo production using sexed or non-sexed semen selected with a silane-coated silica colloid or Percoll

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Abstract

The aim of this study was to evaluate sperm fertilization rates and *in vitro* embryo development rates for sexed and non-sexed semen selected using a silane-coated silica colloid method (Isolate) or Percoll. Frozen/thawed, sexed and unsexed semen samples from four Holstein bulls were randomly allocated to one of two different density gradient selection methods. Sperm quality (motility, concentration, morphology and membrane integrity) were evaluated and compared before and after sperm selection. Sperm motility and morphology improved ($P < 0.005$) after the sperm selection process with no differences between the two methods. For non-sexed semen, Percoll gradient increased the mean (± SEM) percentage of sperm recovered (57.3 ± 2.8) compared to Isolate (46.0 ± 1.8; $P < 0.01$). However, membrane integrity was higher after Isolate than Percoll (sexed semen: 41.0 ± 0.6 vs. 38.8 ± 0.8 and non-sexed semen 60.8 ± 1.6 vs. 58.8 ± 0.5; $P < 0.05$). The percentage of blastocysts produced was higher when either sexed or non-sexed semen was selected by Isolate (14.0 ± 1.0; 22.0 ± 1.1) than by Percoll (10.5 ± 1.5; 17.0 ± 2.1, respectively; $P < 0.05$). In summary, Isolate was a more effective method for the recovery of high quality sperm for *in vitro* fertilization embryo production.

Keywords: Sperm selection; Sexed semen; Non-sexed semen; Isolate; Percoll

1. Introduction

Density gradient selection methods have the capability to accumulate the largest number of morphologic normal and progressive motile spermatozoa. Therefore, sperm separation methods have a very important role in *in vitro* embryo production (IVP). Since sexed semen became commercially available, there have been conflicting opinions on the performance of sexed semen for IVP, because of the lower sperm quality parameters before and after selection [1,2]. A more efficient selection system may be necessary because of the low sperm concentration in commercial doses (2×10^6sperm/straw) and the low post-thaw motility after the sex-sorting process [3]. Percoll is the most widely density-gradient medium used for sperm selection in bovine IVP [4]. However, it is known that some batches of Percoll have endotoxins that could affect cleavage and embryo development rates [5,6]. Non-toxic silane-coated silica colloids have been used for sperm selection and may be a good substitute for Percoll. Several

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species-specific commercial preparations of silane-coated colloids, such as BoviPure [4], EquiPure [7] and PorciPure have been developed [8]. Previous studies have demonstrated an increase of bovine IVP rates when the silane-coated colloid Bovipure was used for sperm selection compared with swim-up or Percoll [4,9]. Two others, PureSperm [5,10] and Isolate [11] have been used with human spermatozoa, but there are no reports about the performance of Isolate on IVP with non-sexed or sexed bovine semen. The aim of this study was to compare Percoll with the silane-coated silica colloid, Isolate for selection of sexed and non-sexed bovine sperm on sperm quality parameters after selection, fertilization and IVP.

2. Materials and methods

2.1. Chemicals

All chemical reagents used for this experiment were purchased from Sigma Chemical Company (St. Louis, MO, USA) except for fetal calf serum (FCS), which was obtained from Natocor (Carlos Paz, Argentina) and the density gradients Isolate and Percoll 90%, which were supplied by Irvine Scientific (Santa Ana, CA, USA) and Nutricell (Campinas, SP, Brazil), respectively.

2.2. Sperm samples

For this experiment straws of non-sexed frozen semen (n = 10) from four different bulls of proven fertility and 18 straws of sexed semen from the same bulls were used. The sexed and non-sexed semen from all bulls was processed and frozen in the same laboratory. Each straw was thawed at 37 °C for 60 s and divided into two equal aliquots to be treated by the two different sperm selection procedures.

2.3. Sperm selection procedures

2.3.1. Percoll gradient

Percoll discontinuous gradient (45 and 90%) was performed as described [12]. The 45% Percoll solution was obtained by diluting a Percoll 90% solution (Nutricell, SP, Brazil) at a 1:1 ratio with sperm-TALP medium. One mL of the 90% Percoll solution was placed in a 15 mL Falcon centrifuge tube, and 1 mL of 45% Percoll was smoothly layered over this. An aliquot of 250 μL of thawed semen was layered on top of gradients and then tubes were centrifuged for 15 min at 700*g*. The pellets were re-suspended in the same amount of sperm-TALP medium and centrifuged for 5 min at 700*g*. The supernatant was removed and the final concentration of the sperm pellet was adjusted with Fert-TALP to 1×10^6 sperm/mL for non-sexed semen and 2×10^6 sperm/mL for sexed semen.

2.3.2. Isolate gradient

The Isolate gradient for sperm selection was prepared by placing 1 mL of the commercially supplied "Lower layer" Isolate solution into the bottom of a 15 mL centrifuge tube and layering 1 mL of supplied "Upper layer" Isolate carefully on top of the lower layer. An aliquot 250 μL of thawed semen was layered on the top and then tubes were centrifuged for 15 min at 700*g*. The pellets were re-suspended in the same amount of sperm-TALP medium and centrifuged for 5 min at 700*g*. The sperm pellets were then re-suspended in Fert-TALP and the final concentration was adjusted to 1×10^6 sperm/mL for non-sexed semen and 2×10^6 sperm/mL for sexed semen.

2.4. Sperm quality parameters assessment

All sperm samples were evaluated, before and after gradient density treatments, for concentration, total motility, morphology, and integrity of the plasma membrane.

2.4.1. Assessment of motility, morphology and sperm concentration

The determination of progressively motile sperm was performed with 10 μL of diluted semen placed on a clean microscope slide, and covered with a cover slip. The percentage of progressively motile spermatozoa was determined by counting a minimum of 200 sperm microscopically at 400× magnification with phase-contrast lens. For sperm morphology analysis, samples were stained with eosin-nigrosin according to Barth and Oko [13]. A total of 200 cells were counted under bright field microscope at a 1000× magnification. The percentage of head defects, nuclear vacuoles, acrosome defects, midpiece defects, proximal droplets, and tail defects were determined. Sperm concentration was determined with a hemocytometer at a 1:200 dilution. The results are presented as sperm/mL, and the recovery rates were calculated according to the formula describe by Machado [14] that recovered sperm (%) = [(Final concentration × final Volume)/(Initial concentration × initial volume)] × 100.

2.4.2. Assessment of plasma membrane integrity

The integrity of the sperm membrane was evaluated using the hypo-osmotic swelling Test (HOS) as described by [15]. The hypo-osmotic solution consisted of sodium citrate (4.9 g/L; Sigma, Chemical Co., St.