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Animal Reproduction Science 91 (2006) 237–247

ANIMAL REPRODUCTION SCIENCE

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A comparison of BoviPure® and Percoll® on bull sperm separation protocols for IVF

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Received 18 February 2005; received in revised form 19 April 2005; accepted 29 April 2005

Available online 8 June 2005

Abstract

In the present study, we have examined the effect of density gradient preparations BoviPure® and Percoll® on bull sperm separation and the in vitro fertilization (IVF) and culture (IVC) results. Frozen/thawed semen from five simmental bulls were pooled. Sperm quality parameters such as sperm motility, concentration, membrane activity (HOS assay), membrane integrity (SYBR-14/PI assay) and acrosomal status (EthD-1/FITC-PSA assay) were evaluated before and after sperm processing for IVF using BoviPure® and Percoll® density gradient separations. The results of the evaluated parameters before sperm processing were: motility 50%, concentration 82.33×10^6 spz/mL, membrane activity 39.05%, membrane integrity 42.97% and the acrosomal status 46.90% of the live spermatozoa with intact acrosomes. After sperm processing with BoviPure® and Percoll® the motility was 66.67 and 64.17%, the concentration was 25.50×10^6 and 27.67×10^6 spz/mL, the membrane activity was 53.78 and 56.58%, the membrane integrity was 70.85 and 68.76% of and the acrosomal status was 74.16 and 67.46% of the live spermatozoa with intact acrosomes, respectively. Percentages were referred to the total number of spermatozoa. There were significant differences ($P < 0.05$) between the evaluated parameters before and after sperm processing for both separation protocols. We found no significant differences ($P > 0.05$) regarding sperm evaluation parameters between the protocols. A total of 492 oocytes were matured and fertilized in vitro and cultured in SOFaaBSA in six replicates. The cleavage (D2) and blastocysts (D7) rate were significantly higher ($P < 0.05$) for the BoviPure® group compared

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0378-4320/$ – see front matter

doi:10.1016/j.anireprosci.2005.04.005

to the Percoll® group: 75.80 and 28.21%; 61.58 and 20.83%, respectively. However, the number of hatched blastocysts (D10) did not differ significantly between sperm separation protocols ($P > 0.05$). Our results indicate that both protocols gave suitable sperm for IVF. This finding and the similarity between those two density gradient preparations suggests that BoviPure® is a good alternative for sperm separation in bovine IVF.

Keywords: Bull; Sperm; BoviPure®; Percoll®; IVM; IVF; IVC

1. Introduction

Sperm separation procedures are able to significantly improve the sperm quality enhancing progressive motility and morphological normal spermatozoa. For this reason in the IVF, sperm separation methods have a very important role. Such selection of spermatozoa separates motile sperm from nonmotile, remove seminal plasma, cryoprotective agents, other background materials and debris (Zavos, 1992), and also at the same time initiates the capacitation of the sperm (Centola et al., 1998). One method of sperm separation is selective fractionation by density-gradient centrifugation. Percoll is one commercial medium for the density-gradient centrifugation of cells, viruses and subcellular particles also widely used in IVF. It is composed of colloidal silica particles (15–30 nm in diameter) coated with nondialysable polyvinylpyrrolidone (PVP). Percoll density-gradient fractionation clearly separates spermatozoa from foreign material such as extender particles, cells and bacteria. The morphological selection of spermatozoa in the prepared population varies, with most tail, and midpiece defects being primarily excluded (Rodriguez-Martinez et al., 1997). The problem is that some batches of Percoll® have endotoxic effect so it was discarded for use in assisted reproduction technics in human medicine (Chen and Bongso, 1999). There have been reports that batches of Percoll® differ in composition and this variation may affect cleavage rates and embryo development (Mendes et al., 2003). As a result of Percoll® endotoxcity many pharmaceutical companies researched for a good quality substitute for Percoll®. Recently, BoviPure®, a sperm separation and purification product formulated specifically for use with bull sperm became available. BoviPure® is an iso-osmotic salt solution containing colloidal silica particles coated with silane. At this time, very few studies have been conducted to evaluate BoviPure® for in vitro production of bovine embryos (Sieren and Youngs, 2001).

The control of sperm quality after commercial freezing/thawing of bull semen is still restricted to the subjective assessment of sperm motility, despite its low correlation with fertility (Söderquist et al., 1991; Kjaestad et al., 1993). The integrity of the plasma membrane reflects the viability of spermatozoa and can be assessed by dual staining of the membrane with permanent nucleic acid stain, SYBR-14, combined with propidium iodide. Correa and Zavos (1994, 1996) and Rota et al. (2000) have used hypoosmotic swelling (HOS) test for the evaluation of the functional integrity of the frozen/thawed bovine sperm membrane. Capacitation is a sequence of biochemical changes leading to destabilization of the sperm membranes which enables the sperm to become able for fertilization (Van