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Separation of motile sperm for in vitro fertilization from frozen-thawed bull semen using progesterone induction on a microchip

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ABSTRACT

This study presents a novel method for the separation of motile sperm from non-progressive motile and immotile sperm and in vitro Fertilization (IVF). This separation of bull sperm was accomplished by inducing chemotaxis along a progesterone release agent in a 7.5-mm microchannel microchip composed of a biocompatible polydimethysiloxane layer and a glass gradient. The selected sperm was applied directly for IVF. In the first experiment, we tested the effect of different lengths of microchannnel (5 mm, 7.5 mm and 10 mm) on quality parameter of separated sperm. The results showed that separated sperm using 7.5-mm microchannel chip were improved in sperm motility, swimming velocity, and beat frequency compared with other groups. In the second experiment, a medium containing sperm from swim-up method and outlet reservoir of our 7.5-mm microchannel chip was collected and mitochondrial activity of the sperm was determined by fluorescence microscopy. The sperm from the microchip had higher mitochondria activity (47.6% ± 6.0%) than the sperm from the swim-up method (23.6% ± 4.7%) ($P < 0.05$). There were significant differences in rate of acrosome intactness between the swim-up method and the microchip (36.0% ± 4.1% vs. 66.8 ± 2.1%, respectively, $P < 0.05$). In the third experiment, we compared sperm penetration in the microchip-IVF system with a standard IVF method (droplet-IVF). The microchip-IVF group had the highest percentages of oocytes penetrated (82.2% ± 1.6% vs. 63.5% ± 2.4%) and monospermic oocytes (67.8% ± 3.4% vs. 42.4% ± 1.5%). In addition, early developmental competence of oocytes to the blastocyst stage was higher when the oocytes were inseminated in the microchip-IVF system compared with those inseminated in a standard droplet-IVF system. These results demonstrate that our microchip based on a sperm chemotaxis system is useful for motile sperm separation from frozen-thawed bull semen for IVF. Therefore, the optimized microchip system provides a good opportunity to sort motile bull sperm for IVF.

1. Introduction

Sperm selection is required for in vitro fertilization (IVF) and influences both the success rate and offspring health (Schultz and Williams, 2002; Said and Land, 2011). In IVF,

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an oocyte is incubated with an aliquot of 50,000 sperm taken from an original sample containing approximately 100 million sperm. Standard IVF protocols for common livestock species (e.g. cattle, sheep, swine, etc.) have been developed. Prior to IVF, it is important to select spermatozoa that are viable, motile, and of normal head size. Sperm motility is the most important selection criterion for fertility (Nosrati et al., 2014). Several motile sperm separation techniques are in use, such as migration-sedimentation, density gradient centrifugation, the swim-up method, and glass wool filtration (Lannou and Blanchard, 1988; Sakkas et al., 2000; Åkerlöf et al., 1987). However, these techniques have limitations including loss of motility and loss of sperm DNA integrity due to mechanical damage, as well as the production of reactive oxygen species (ROS) and endotoxins (Nosrati et al., 2014).

Microchips based on microfluidic technologies provide one of the most suitable methods for rapidly separating viable sperm while avoiding mechanical damage and maintaining sperm DNA integrity, compared with conventional semen processing techniques (Sano et al., 2010; Hyakutake et al., 2009). Shibata et al. (2007) and Li et al. (2016) described application of microchips to IVF and in vitro production of embryos, respectively. Microfluidic-based microchips have been applied to several other areas of biological research, such as separation or analysis of cells, DNA, and proteins (Hyakutake et al., 2009). In addition, some researchers have separated motile sperm from semen by microchip (Huang and Huang, 2014; Huang et al., 2015; Shirota et al., 2015). Many researchers are currently researching this technique because the microenvironment of the microchip can be formulated to more closely resemble in vivo conditions, such as those required for fertilization, than can a drop-IVF system. A wide variety of methods of motile sperm separation using microfluidic-based microchips has recently been reported (Denny, 2013; Fleming et al., 2008; Aitken et al., 2011). These microchip-based separation techniques generally use active pumping by external equipment such as a syringe pump or passive pumping by hydrostatic pressure. Stable fluid flow can be prepared by controlling the dimensions of the devices using microchip-based devices. However, these methods generate laminar flow. Non-progressive motile sperms are also constantly moving randomly and can enter into the laminar flow, which sorts motile sperms. Therefore, it is difficult to separate only progressive motile sperm. The complexities of using the microfluidic devices and the need for supporting infrastructure have been barriers to clinical implementation. In addition, the low sample volume characteristic of microfluidics is a disadvantage in the context of sperm selection because of the inherent milliliter-scale size of animal samples and sperm lifetime constraints (Nosrati et al., 2014).

Sperm chemotaxis is a cell transport mechanism that guides spermatozoa up an attractant concentration gradient. The occurrence of mammalian sperm chemotaxis to follicular fluid (e.g. progesterone, atrial natriuretic peptide (ANP)) has been demonstrated in vitro in humans (Ralt et al., 1994), mice (Oliveira et al., 1999), rabbits (Fabro et al., 2002) and cattle (Teves et al., 2007; Iqbal et al., 1980). Therefore, sperm chemotaxis could potentially be used as a tool to select motile spermatozoa. Progesterone released by the cumulus cells surrounding the egg is a potent stimulator of mammalian spermatozoa. It attracts spermatozoa towards the egg and helps them penetrate the egg's protective vestments. Progesterone induces Ca^{2+} influx into the spermatozoa (Lishko et al., 2011; Smith et al., 2013) and triggers multiple Ca^{2+}-dependent physiological responses essential for successful fertilization, such as sperm hyperactivation, acrosome reaction, and chemotaxis toward the egg. Ko et al. (2012) have reported a novel microchip system that separated progressive motile sperm using chemotaxis induced by a chemical gradient of chemoattractants in microchannels. In addition, Xie et al. (2010) confirmed the first efficient chemotaxis screening with a microchannel using in vitro cultured cumulus cells.

Based on this background, we present a novel microchip system that separates progressive motile sperm using chemotaxis induced by progesterone. We determined the optimal conditions of different length microchannels by progesterone induction for the separation of motile sperm from the frozen-thawed bull semen. We examined whether the application of the device to co-culture cattle oocytes isolated with highly motile spermatozoa improved the efficiency of in vitro fertilization.

2. Materials and methods

2.1. Chemicals and culture media

All chemicals were purchased from Sigma-Aldrich [China (Shanghai)] unless otherwise noted.

The medium used for collecting and washing cumulus-oocyte complexes (COCs) was modified M199 medium (Li et al., 2016), which contained M199 (Earl's salts; Gibco, Grand Island, USA, lot No. 11146397) supplemented with 2 mM $NaHCO_3$ (Sigma, USA, lot No. 011M01472 V), 10 mM HEPES (Sigma, USA, lot No. 81M5435 V), 0.1% (w/v) polyvinyl alcohol (Sigma, USA, lot No. BCBG1529 V), 25 μg/mL gentamicin, and 65 μg/mL potassium penicillin G.

The in vitro maturation (IVM) medium (Li et al., 2016) contained M199 supplemented with 2 mM $NaHCO_3$, 0.01 IU/mL FSH (Ningbo Sansheng Pharmaceutical Co., Ltd, lot No. 120216), 0.01 IU/mL LH (Ningbo Sansheng Pharmaceutical Co., Ltd, lot No. 120510), 1 μg/mL E_2 (Sigma, Lot No.021M8707 V), 10% FBS (Zhejiang Tianhang Biological Technology Co., Ltd. lot No. 120104), 25 μg/mL gentamicin, and 65 μg/mL potassium penicillin G.

BO medium was used for IVF, as described by Brackett et al. (1982) for the fertilization of cow oocytes in vitro.

Embryo culture medium contained M199 supplemented with 2 mM $NaHCO_3$, 5 mg/mL BSA, 25 μg/mL gentamicin, and 65 μg/mL potassium penicillin G.

All media were equilibrated at 39 °C in an atmosphere of 5% CO_2 in air overnight prior to use.

2.2. Device fabrication

The microchip was made of Polydimethylsiloxane (PDMS) and glass. Both substrates are biocompatible with spermatozoa cells. Polydimethylsiloxane is widely used in