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ORIGINAL ARTICLE

Preservation of human spermatozoa in a simple medium

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Abstract The objective of this study was to estimate the best conditions for sperm preservation in a simple medium and to examine the changes in sperm morphology and vitality due to preservation.

Swim-up technique was carried out using KSOMaa medium (potassium simplex optimized medium with amino acid) then, such sperms were preserved without/with supplementation of BSA to the medium in two different osmolarities (271 and 800 mOsmal). Sperms were preserved for 2 weeks in three different temperatures (37 °C, 4 °C and −20 °C). Our results demonstrated that: (1) KSOMaa medium is a good medium to obtain progressive motile sperms with a good morphology. (2) The best conditions for preserving human spermatozoa were 800 mOsmol KSOM-BSA and a holding temperature of −20 °C. (3) Light and electron microscopy showed that cryodamage has been induced in some human spermatozoa due to preservation. Collectively, our data indicate that this new simple procedure could be the method of choice for selecting motile and morphologically normal spermatozoa. This new preservation method may help *in vitro* fertilization centers but should be tested to check the embryonic development after intracytoplasmic sperm injection.

1. Introduction

Preservation of human spermatozoa is one of the most important issues for the possibility of pregnancy following intrauterine insemination (Bunge and Sherman, 1953). Since about half a century and a lot of methods concerned with freezing of human sperms have been improved (Bunge and Sherman, 1953; Royere et al., 1996).

Recently, the cryopreservation of mammalian spermatozoa is widely spread in many laboratories where it can help the sperm genetic material unaffected so that increases the success rate of the assisted reproduction (Hori et al., 2004; Suppawiwat et al., 2006).

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Spermatozoa from some animals have been successfully cryopreserved (Gabriel et al., 2000; Eriksson et al., 2002; Cormier and Bailey, 2003) but, the cryopreservation process affected sperm activity and morphology (Yoshida et al., 1990; Hammadeh et al., 2001).

Preservation of human spermatozoa in liquid nitrogen is the most successful method. Since liquid nitrogen can cause some problems and it is not available in all laboratories so, investigators tried to freeze sperms without liquid nitrogen but this procedure have been succeeded only in mice (An et al., 1999; Nguyen et al., 2005; Kishikawa et al., 1999) but not in human.

The study aimed to apply such a method reported in mice (Nguyen et al., 2005) using human spermatozoa, and to investigate the effects of the preservation method on human spermatozoa in a simple medium with/without supplementation of BSA in different osmolarities and different temperature. This new method is rapid, simple, and inexpensive and recovers most of motile sperms in the specimen.

2. Materials and methods

2.1. Collection and evaluation of semen samples

Twenty healthy donors were used in our study. The semen samples were obtained by masturbation directly into sterile plastic containers after at least 2 days of sexual abstinence. Samples were allowed to liquefy for 30 min at 37 °C (WHO, 1999). Each specimen was evaluated according to standard procedures recommended by the World Health Organization (WHO) manual with a phase-contrast microscope (WHO, 1999). Semen parameters assessed included sperm volume, count, motility, morphology, vitality and viability. Donors specimen were included if they had sperm parameters within the normal range defined by the WHO (WHO, 1999). All studies were approved by the Human Investigation Committee of Libyan health organization.

2.2. Testing of the KSOMaa medium efficiency

One milliliter of each seminal sample was diluted 1:2 with KSOMaa media prepared as previously mentioned (Suppawiwat et al., 2006). Samples were centrifuged for 10 min at 300*g*, then the supernatant was removed and an aliquot of 0.5 ml of KSOMaa media was added. Specimens were incubated for 18 h in 5% CO_2 incubator (RS Biotech Laboratory, UK) (Suppawiwat et al., 2006). Sperm motility was observed and compared to that in fresh semen.

2.3. Usage of KSOMaa medium in swim-up procedure

The used medium in the separation process was KSOMaa medium. Progressively motile sperms have been separated by swim-up technique as mentioned previously (Younglai et al., 2001). After swim-up sperm assessed parameters have been evaluated. To check the efficiency of the KSOMaa medium, the same procedure have been done using the commonly used medium in laboratories FertiCult™ IVF medium–0.4% human serum albumin (FetriCult, Beemen, Belgium) then the results were compared.

2.4. Preservation of spermatozoa

Sperms were collected from swim-up were stored in KSOMaa media with or without supplementation of 4 mg/ml bovine serum albumin (BSA) at different osmolarities (271 and 800 mOsmal) and different temperatures (−20 °C, 4 °C, 37 °C) for 2 weeks. Osmolarity was adjusted as mentioned previously (Nguyen et al., 2005) using Micro-Osmometer 3320 (Advanced® Instruments, USA). Spermatozoa were stored in 1.5 ml sampling tubes at a concentration of $5–8 \times 10^6$ spermatozoa/ml.

2.5. Thawing and analysis of the preserved spermatozoa

After 2 weeks storage in KSOMaa medium at −20 °C specimen were thawed in a water bath at 37 °C for 10–15 min. Estimation of sperm count, motility, viability staining, hypoosmotic swelling (HOS) testing have been carried out according to WHO (1999).

2.6. Sperm function testing

Sperm motility was assessed before and after swim-up according to the methods described by the WHO (1999). In brief, a motile sperm was defined as a cell having a progressive or nonprogressive motion, with nonprogressive sperms showing clear flagellar movement but no change in position. Immotile sperms included all nonmoving cells without flagellar motion and sperm heads without a flagellum.

Viability of spermatozoa was determined by mixing 10 μl of an aliquot of spermatozoa with one drop of a supravital stain (0.5% eosin Y in aqueous solution of 0.9% NaCl) (WHO, 1999). Counting of living sperms (unstained) and dead ones (stained) were observed at ×400.

To assess the sperm membrane function, HOS test were carried out as mentioned previously (Jeyendran et al., 1984).

2.7. Evaluation of sperm morphology

Sperm morphology was assessed before and after swim-up and also after preservation and thawing process. The sample (5 μl) was spread along the length of a microscope slide. The resulting thin smear was allowed to air dry for 20 min before staining with Giemsa stain (WHO, 1999). Sperm morphology has been estimated at ×1000 magnification under oil emersion and at least 100 spermatozoa were counted on each slide according to Kruger et al. (1987).

2.8. Transmission electron microscopy

After swim-up, the preserved sperms in KSOM-BSA (of osmolarites 271 and 800 mOsml at −20 °C for 2 weeks) were fixed for 2 h 2.5% glutaraldehyde. The fixed sperms were centrifuged at 1200*g* for 15 min. The pellet was washed in phosphate buffer then postfixed in 1% buffered osmium tetroxide, dehydrated in ethanol and then, embedded in Epon resin. Semi-thin sections were stained with methylene-blue and examined under the light microscope, and then ultra-thin sections were collected in copper grids contrasted with uranyl acetate and lead citrate and observed with electron microscope (Joel 1200 EXII).