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Changes on membrane integrity and ultrastructure of human sperm after freeze-drying

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Objective *To observe changes on membrane integrity and ultrastructure of human sperm after freeze-drying.*

Methods *Semen samples from both normospermic donors (group A, n=15) and infertile men with abnormal sperm parameters (group B, n=15) were enrolled into this study. These samples were freeze-dried by using a freeze-drying method. The membrane integrity in the head and tail regions of individual spermatozoon was examined by using the combined hypo-osmotic swelling-eosin Y exclusion test. Sperm ultrastructure in groups A (n=3) and B (n=3) was observed by scanning electron microscopy (SEM).*

Results *After freeze-drying, all spermatozoa were types I (damaged both head and tail membranes) and III (damaged head membrane and intact tail membrane) membrane integrity in groups A and B. Type III of group B had lower value than that of group A ($P<0.01$). Under SEM, intact freeze-dried spermatozoa including abnormal morphology and normal-looking morphology were observed in both groups A and B. A few freeze-dried sperm heads had unsmooth or fuzzy surface. Isolated sperm heads, bent tails, broken sperm tails or fragmentary tails were more frequently seen in group B than those in group A.*

Conclusion *Freeze-dried human spermatozoa could have intact structural components. However, freeze-drying resulted in severe damage on membrane integrity and ultrastructure of sperm. Samples from infertile men would have less resistance to freeze-drying.*

Key words: human sperm, ultrastructure; membrane integrity; freeze-drying; lyophilization

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Sperm preservation has long been established to preserve male fertility, which has greatly facilitated human assisted reproductive programs. It is not only commonly used for the treatment of infertility in assisted conception clinics worldwide, but also has been successfully applied for banking of sperm prior to chemotherapy, radiotherapy or some surgical treatments liable to alter spermatogenesis[1-3].

Currently, the most common means for preserving sperm is cryopreservation. Freezing in liquid nitrogen (−196 ℃) is a fundamental method for long-term storage of sperm. However, cryopreservation induces damages on both structures and functions of sperm and yields several problems on practical uses such as high running costs, shipping of liquid nitrogen, requiring a special container, and occupying more room for storage, etc.[1-3] Obviously, from considerations on economy, practice, and safety, long-term storage of sperm without liquid nitrogen would be ideal.

Freeze-drying or lyophilization has been proposed as a potential alternative method for preserving male gametes, which allows sperm preservation without liquid nitrogen and transportation at room temperature[4-6]. Wakayama and Yanagimachi firstly demonstrated offspring derived from intracytoplasmic injection of freeze-dried mouse sperm[7]. Presently, freeze-drying has been applied in several mammalian species and humans[4-11]. However, it still needs more investigations for freeze-dried human sperm. In the present study, we observed changes of ultrastructure of freeze-dried human sperm with scanning electron microscopy (SEM). In addition, the membrane integrity in the head and tail regions of freeze-dried individual spermatozoon was examined by using the combined hypo-osmotic swelling-eosin Y exclusion test (HOS-EY test).

Materials & Methods

Semen samples

Fifteen semen samples from normospermic donors (group A) and 15 samples from infertile men with abnormal sperm parameters (group B) were enrolled into this study. These samples had semen volume >2 mL, and sperm concentation >50 × 10^6/mL. The ejaculates were obtained by masturbation after 3−7 d of sexual abstinence. Semen routine examination was carried out according to the World Health Organization (WHO) laboratory manual[12]. Sperm morphology was analyzed after slide staining with the modified Papanicolaou-staining method and using strict criteria.

Sperm freeze-drying

Semen samples were washed with DMEM medium (Gibco, USA) supplemented with 10% fetal calf serum (Every Green Co., China). The sperm suspension (0.1 mL) was put into a 1.5-mL polypropylene centrifugation tube. Then the tubes were plunged into liquid nitrogen for 1 min to freeze the sperm samples, and subsequently transferred to a cooled