FERTILITY PRESERVATION FOR MEN: RECENT DEVELOPED TECHNIQUES FOR CRYOPRESERVATION OF A SMALL NUMBER OF SPERM CELLS

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INTRODUCTION

Subfertility affects about 10% to 15% of couples trying to conceive. There are many factors that may impact a couple´s ability to conceive. Diseases such as cancer and/or autoimmune diseases can affects fertility directly or after treatment. Assisted Reproductive Techniques (ART) has improved gradually so that men and women find the opportunity to preserve their gametes and possibly realize their dreams of having a child in the future. For men, semen cryopreservation is relatively easy when compared to methods applied for women. However, due to illness and/or treatment, the semen quality may be compromised at the time of collection. Cryopreservation of few spermatozoa is still a major challenge for fertility preservation in men, but methods have been developed for cryopreservation of a small number of sperm cells.

OBJECTIVE

Considering the importance of male fertility preservation, the aim of this study was to discuss current issues related to semen cryopreservation techniques and the recent advances on the methods for cryopreservation of few sperm cells, which can be applied both for patients with cancer or autoimmune diseases and for patients with low sperm quality.

MATERIAL AND METHODS

The literature on cryopreservation of a small number of sperm cells was searched using PubMed database. The scientific background, current developments and potential future applications of these methods were reviewed.

CURRENT RESULTS

Cryopreservation of few spermatozoa was studied by Cohen et al. (1997) and Desai et al. (1998) who used the zona pellucida as freezing carrier to store few sperm. Desai et al. (2004) adopted the cryoloop to cryopreserve testicular and epididymal sperm. Subsequently, Huang Weihua et al. (2013) reported cryopreservation of few spermatozoa using vitrification method without adding cryoprotectants. However, these studies have their advantages and disadvantages. The existence of foreign proteins in zona pellucida may cause biological contamination, use of cryoloop costs too much and open freezing straws without adding cryoprotectants may lead to contamination from liquid nitrogen.

The use of micro-straws has been reported showing better sperm motility and acrosomal integrity than traditional 0.25 ml and 0.5 ml straws, possibility related to the thinner and very small volume, causing a faster freezing rate of micro-straws. The inner tube with a fine end is made of transparent polymerized resin. Besides that, the outer metal shell contributes to accelerating low-temperature conduction and protecting the inner tube during freezing. Hollow-core agarose capsules (Figure 1) also has been tested to the cryopreservation of one single sperm cell, method in which it is used the conventional intracytoplasmic sperm injection (ICSI) to insert the spermatozoa into the capsule and then it is cryopreserved on polycarbonate or nylon mesh sheets using nitrogen vapor. The method showed good results and is expected to be useful in ICSI treatment in patients with few spermatozoa. Another study freezed cells in a nitrogen-free equipment and no observed differences in viability and motility of spermatozoa cryopreserved compared with the conventional vitrification technique. Therefore, this can also be a useful technique for preserving small number of sperm cells.

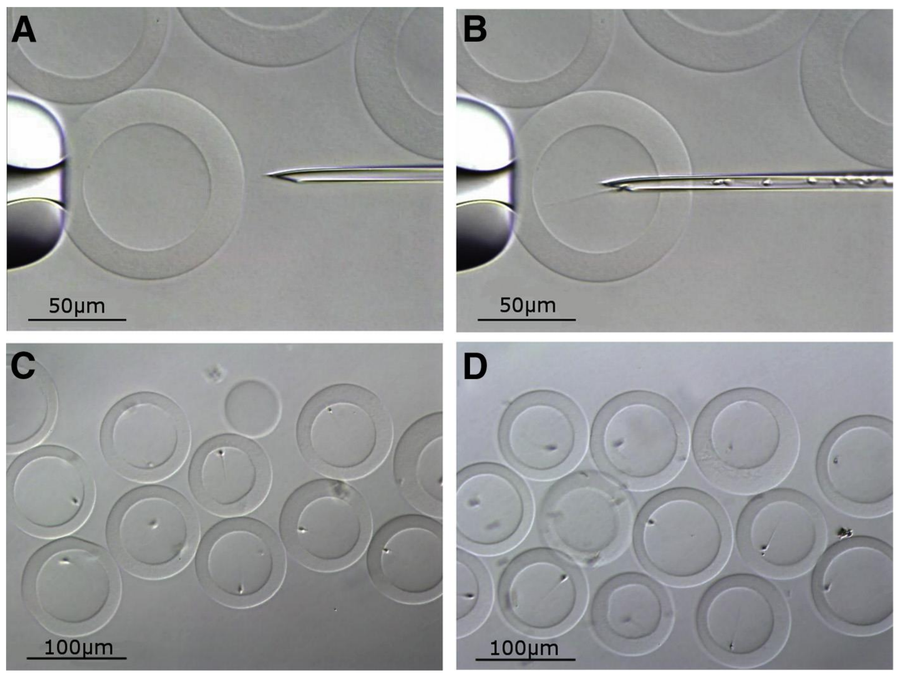

Figura 1: Sperm injection into an agarose capsule. (A) Agarose capsule held by a holding pipette before injection. (B) Injection pipette inserted into an agarose capsule. A single sperm was injected into the capsule. (C) Agarose capsules containing a sperm before freezing. (D) Agarose capsules after thawing.
Reference: Araki Y, *et al*. 2015.

CONCLUSION

Assisted Reproductive Techniques and methods for fertility preservation allowed the realization of dreams for many families. Therefore, the continuous improvement of those methods and freezing protocols is essential. The common techniques and protocols used for semen cryopreservation still have certain limitations and there is still no effective method for clinical cryopreservation of few human spermatozoa.

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