## It Is Time to Pay More Attention to Sperm Cryopreservation: Now More Than Ever!

Semen cryopreservation and sperm freezing has a long history of over 65 years. However, embryo freezing and oocyte cryopreservation in human infertility has a shorter history up to 40 years. Embryo and oocyte freezing had a tremendous progress and development in past four decades so it improved from slow freezing procedure with less than 10% survival rate at beginning to vitrification procedures with 100% survival rate through freezing/thawing cycle for oocyte and embryo at present. Relying on this improvement of the freezing techniques, fertility preservation for women due to medical or social reasons has been carried out as a secure and efficient service worldwide. Also, embryo freezing is a fixed part of IVF/ICSI infertility treatment cycle to increase the success rate of these cycles. The practice is done in a way that, at present, freezing of all embryos and subsequently transfer of frozen/thawed embryos in natural cycle without ovarian stimulation is possible. This current capacity is provided by the guarantee that no embryos are damaged through the freezing/thawing procedures. The situation in the case of semen/sperm is not similar even with longer history of freezing/thawing. Current procedures used for sperm freezing/thawing lead to a decrease of more than 50% in motility and survival rate. Sperm chromatin also damages following cryopreservation. For a healthy man the total count of sperm per ejaculate is more than hundred millions and the 50% reduction in quality and quantity of motile sperm will not be so problematic. Several millions of motile and intact sperm following thawing will be sufficient for fertility preservation through Assisted Reproductive Technologies (ARTs) (1).

However, the semen of oligoasthenteratozoospermic (OAT) men often contains a very limited number of spermatozoa and they are always candidates for sperm freezing and fertility preservation in future. Unfortunately, current methods of cryopreservation have little efficiency to preserve limited number of sperm in total semen precipitate. During the past decade, many researchers attempted to invent new technologies in particular for freezing of individual or limited numbers of human spermatozoa in men with severe male factor infertility. They applied several biological and non-biological carriers including human, mouse and hamster zona pellucida, agarose and alginate microspheres, ICSI pipettes, cryoloops, mini-straws, microdroplets and many other carriers for cryopreservation of several small aliquots or even small numbers of sperm to preserve fertility especially for men with transient azoospermia. They failed to present an ideal and efficient carrier or vehicle and also freezing method with acceptable clinical outcomes for widespread easy and safe application (2).

However, vitrification procedure provided excellent results on oocyte/embryo cryopreservation but its early try for sperm freezing using different cryoprotectants failed to provide acceptable results in comparison with vapor phase freezing method and therefore, it did not attract the scholars' interest. More recent studies on vitrification of processed and washed spermatozoa, free of seminal plasma and without any cryoprotectant, provide better outcomes in comparison with routine method of sperm freezing. So, this preliminary finding provides new opportunity for cryostorage of sperm in infertile men that surely has a limited number of sperm (3).

On the other hand, researches on freeze-drying of human and animals' sperm continue. Although the results on human sperm was not satisfactory but early attempts reported encouraging results on mouse, fowl, rabbit, and bull. The ability of storage of sperm at ambient temperature or refrigerator  $(4-8 \,^\circ C)$  significantly reduces the expenses of handling, transport and storage of semen samples (4). This opens a bright prospect in the field of fertility preservation for men.

Now, with regard to the harmful environmental factors and the issue of declining of male fertility and semen quality over the time, the need for paying more attention to sperm cryopreservation is the hot topic. The future cryopreservation technologies should be more investigated at the level of clinical outcomes. The recent evidence is not enough for distinguishing the superiority of one technique over the others. However, all of available techniques have degrees of partial failures but they meet daily needs of infertility clinics and need serious improvement in future.

## References

- Konc J, Kanyo K, Kriston R, Somoskoi B, Cseh S. Cryopreservation of embryos and oocytes in human assisted reproduction. Biomed Res Int. 2014;2014:307268.
- AbdelHafez F, Bedaiwy M, El-Nashar SA, Sabanegh E, Desai N. Techniques for cryopreservation of individual or small numbers of human spermatozoa: a systematic review. Hum Reprod Update. 2009;15(2):153-64.
- 3. Slabbert M, du Plessis SS, Huyser C. Large volume cryoprotectant-free vitrification: an alternative to conventional cryopreservation for human spermatozoa. Andrologia. 2015;47(5):594-9.
- 4. Keskintepe L, Eroglu A. Freeze-drying of mammalian sperm. Methods Mol Biol. 2015;1257:489-97.

Mohammad Reza Sadeghi Editor-in-chief