Editorial comment on: Vitrification of Neat Semen Alters Sperm Parameters and DNA Integrity

Vitrification has brought about important changes in cryopreservation and human fertility preservation. Easiness and speed and no need for costly freezing technologies are reasons for its rapid development. Vitrification is the solidification of a liquid without crystallization. As cooling continues, however, the molecular waves in the liquid permeating the tissue decline. Finally, an "arrested liquid" state known as a glass is attained. Vitrification has been demonstrated to afford higher preservation for a number of cells, including monocytes, ova and early embryos and pancreatic islets.[1]

There are a number of major contests for performing of vitrification for tissue engineered medical products. Without adhering to these standards, certainly the process of vitrification will fail. The first one is vitreous state. There is no explanation about vitreous state in this study. Stability of the vitreous state is critical for the maintenance of vitrified tissue integrity and viability. In present study the method of vitrification has not been explained in details and it seems most of standards for vitrification have not been considered. Vitrification methods to preservation have some of the limitations associated with conventional freezing methods.[2] First, both methods entail low temperature storage and transportation conditions. Neither can be stored above their glass transition temperature for long without significant risk of product damage due to inherent instabilities resulting to ice formation and growth. Both methods use cryoprotectants with their associated problems and necessitate experienced technical support during rewarming and cryoprotectant elution phases. The very high concentrations of cryoprotectants needed to facilitate vitrification are potentially toxic since the cells may be exposed to these high concentrations at higher temperatures than in freezing methods of cryopreservation. Cryoprotectants can kill cells by direct chemical toxicity, or indirectly by osmotically-induced stresses during suboptimal addition or removal.[3] Upon complete achievement of warming, the cells should not be exposed to temperatures above $0^{o}C$ for more than a few minutes before the glass-forming cryoprotectants are removed. It is possible to employ vitrified products in highly controlled environments, such as a commercial manufacturing facility or an operating theater, but not in an outpatient office. There isn't any data about above mentioned points in this study.[4] Another issue is heat transfer. Heat transfer issues are the primary problem for scaling up the successes in somewhat small tissue specimens to larger tissues and organs. The limits of heat and mass transfer in bulky systems result in non-uniform cooling and leads to stresses that might begin cracking. In fact, the higher cooling rates that facilitate vitrification will typically lead to higher mechanical stresses.[5] In present study there is no information on the used material properties of vitreous aqueous solutions. Material properties such as thermal conductivity and fracture strength of vitreous aqueous solutions have many connections with their inorganic analogues that happen at normal temperatures. Any material that is unrestricted will undergo a change in size (thermal strain) when subjected to a change in temperature. Additional important issue that has not been addressed, is the stresses that arise to billet the differential shrinkage. Thermal stress can definitely reach the produced strength of the frozen tissue resulting in plastic deformations or fractures.[6] One more major obstacle for performing

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of vitrification is the technique used for warming. This issue also has been ignored in present study. The warming technique should be highly effective to prevent devitrification and ice growth by recrystallization.

The rational for vitrification of neat semen has not been mentioned. What are the advantages of vitrification of semen instead of sperm? Is there any scientific background for this procedure? For vitrification, it is recommended that, even the plasma of sperm should be removed. For vitrification the sperm plasma is removed, it means that by using this technique many infecting agents such as HIV, hepatitis and other viruses will be removed from the sperm, and therefore these infectious microorganism cannot be transmitted via sperm. Hence HIV+ men will have the chance to father children without the risk of passing infectious organisms to baby and mother. After separation of plasma from the sperm, the vitrified sperm should be stored in an ultra-cold deep freeze at -86ºC environment. This method has several advantages compared to other methods, first the motility of rethawed sperm increases significantly (75% using this method vs. 31% using conventional methods) second a higher number of viable sperm can be achieved and this can result in higher chance of fertilization in ARTs, such as IVF and ICSI.[7]

However, two decades past the first live-birth from vitrified embryos, there are still some uncertainties on the safety of these techniques and its possible toxic effects on the health of children born from vitrified embryos or oocytes. There is fear that use of high concentrations of cryoprotectants may result in genetic or epigenetic abnormalities with ensuing inborn malformations. Therefore, there is no agreement or scientific recommendations for the replacement of slow freezing method with vitrification universally.

The techniques for performing vitrification are evolving. Recently vitrification of metaphase II oocytes has been described to hold ability for oocyte preservation, which can be vital in countries where a limited number of oocytes can be inseminated and embryo cryopreservation is illegal, as well as in oocyte donation and fertility preservation prior to cancer treatment.[8]

The two most commonly used tests to determine sperm DNA damage are the TUNEL assay and the sperm chromatin structure assay (SCSA).[9] the TUNEL assay has never been adjusted for use with human spermatozoa and lower normal threshold values have not been obviously recognized. DNA testing by SCSA has been widely standardized. TUNEL test has not been standardized to the same level as SCSA. TUNEL assay cannot selectively differentiate clinically significant DNA fragmentation from clinically insignificant fragmentation. The assay also cannot differentiate normal DNA grooves from pathologic grooves. Moreover, the TUNEL test does not give any information concerning the particular genes that may be affected by DNA fragmentation. This assay can only determine the amount of DNA fragmentation that ensues, with the hypothesis that higher levels of DNA fragmentation are pathologic.[10] Nowadays, the only reliable test to determine sperm DNA fragmentation is SCSA. This test has validated clinical reference range and criteria to interpret the yielded results precisely. Using the SCSA test one can test 5,000 individual sperm with a high-precision flow cytometer. To interpret the results of SCSA test DNA fragmentation index (DFI) is used, which represents the population of cells with DNA damage.[11,12]

Finally a major limitation of present study is absence of pictures both from TUNEL results and vitrified sperms.

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