A high-tech closer look to evaluate the impact of oocyte vitrification on embryo quality

From the early times of assisted reproductive technologies (ART), a great effort has been put into introducing and optimizing laboratory procedures for best gamete and embryo handling. In recent years, a simple although highly effective cryopreservation procedure for human oocytes, called vitrification (ultra-rapid freezing), has been implemented. A report by Kuwayama et al. (1) published in 2005, summarized the study done in a cohort of 64 vitrified human oocytes that revealed over 91% normal cell morphology after oocyte warming, an 89.7% fertilization rate after intracytoplasmic sperm injection, a 61.5% embryonic development to the blastocyst stage in vitro, and a total of 12 initial pregnancies, 7 healthy babies and 3 ongoing pregnancies after 29 embryo transfers (2.2 vitrified-embryos per transfer). Human oocyte vitrification using the Cryotop method involves a very small volume of cryoprotectant that minimizes its cytotoxic effect, and ultra-rapid cooling and thawing rates that reduces the chance of ice nucleation. Since then, the Cryotop method has been implemented in numerous ART centers worldwide.

In 2012, experts from the Practice Committee of the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology performed a systematic literature search using the MEDLINE site to evaluate the efficacy and safety of mature oocyte cryopreservation. The analysis done on four randomized controlled trials comparing fresh versus vitrified human oocytes, revealed comparable fertilization and pregnancy rates in in vitro fertilization/ intracytoplasmic sperm injection procedures done in young women with fresh and vitrified/warmed oocytes. From these results, experts concluded human oocyte vitrification and warming should no longer be considered experimental (2).

Based on the results achieved worldwide, human oocyte vitrification is currently offered for fertility preservation in several clinical conditions, among them in patients with genetic conditions or diagnosed with cancer and treated with gonadotoxic chemotherapies. In addition, oocyte vitrification is available to couples undergoing ART procedures, when no spermatozoa are recovered the day of oocyte retrieval. In addition, oocyte vitrification has been used to adapt to specific regulations in countries that prohibit the production and storage of surplus embryos. As a growing practice, oocyte vitrification is being performed in women that decide to delay pregnancy for medical or social reasons, as pregnancy rates depend more on the age of the patient at the time of oocyte retrieval than when the patient decides to have a child. However, elective or social oocyte cryopreservation is still considered controversial, and additional studies from large cohorts of heterogeneous samples are needed to help assess its use and safety, as well as to assist specialists in providing adequate counseling.

Human oocyte vitrification has also become very useful in procedures involving donor oocytes. By creating oocyte cryobanks, the complexity of coordinating the oocyte donor and the recipient(s) is being eliminated, and waiting lists may be better managed. A relevant issue regarding oocyte donation, which may be addressed with oocyte-banking, is the screening for infectious diseases among donors. From the recipient's standpoint, women may have more choices in selecting a donor and flexibility in timing their pregnancy, and may be better adjusted when the ART procedure is done. Currently, vitrified oocytes are part of ovum donation programs in several centers worldwide.

Regarding human vitrified-banked oocytes, two reports from the Instituto Valenciano de Infertilidad, have shown results comparing the performance of vitrified-banked and fresh oocytes. These two studies were included in the 2012 American Society for Reproductive Medicine's mature oocyte cryopreservation guideline (2). The first study by Cobo et al. (2008) is a cohort prospective randomized study done with 231 vitrified and 219 fresh metaphase II oocytes. The second study by Cobo et al. (2010) is a randomized, prospective, triple-blind, single-centre, parallel-group controlled-clinical trial done with 3,286 viitrified and 3,185 fresh oocytes. Both studies analyzed, in addition to fertilization and pregnancy rates, early embryonic development using standard procedures that relied on morphological criteria and involved conventional static observations limited to specific time point assessments and subjective evaluations. A third report by Cobo and colleagues (3) included around 3,500 oocyte donation cycles with more than 40,000 vitrified oocytes, and summarizes their 6 year-period experience with the technology of oocyte cryobanking, although no details of early embryo development results are presented in the report.

In the year 2009, time-lapse imagining (TLI) was incorporated in the evaluation of early human embryonic development in ART. This powerful technology allows embryo assessment in a non-invasive fashion, and provides a wide range of morphological and dynamic parameters from individual embryos, which can be used to design algorithms and to identify predictive markers for best embryo selection. Compared to conventional image acquisition, TLI has already demonstrated several advantages, since qualitative and quantitative data of the biological samples cultured on the imaging device is obtained from images captured at defined time intervals. In any case, some limitations still do exist to maintain embryos on the dish within the field and in an optimal culture environment. In addition, a major concern exists on the potential DNA damage caused by continuous embryo light exposure during long periods of time. With TLI, specific cell events (i.e., cytokinesis) and their specific timing and alterations in oocyte and embryo evolution can be monitored. Moreover, there is software coupled to TLI for analysis that enables image capture and storage as well as assessment of specific embryo features (i.e., blastomere area, perimeter and diameter, as well as fragmentation). These assessments are still tedious and time consuming, and multicenter validation of new technologies designed to automate the tracking quantitative measurements of TLI will be required to gain clinical utility.

Then, can TLI analysis be used to assess the effect of oocyte vitrification on embryo quality? The work by Cobo

et al. (4) published in this issue of *Fertility and Sterility*, investigated the effect of oocyte vitrification upon embryo quality using TLI and a morphokinetic evaluation with an algorithm of analysis previously developed by the same group (5). The observational two consecutive year cohort study was conducted in 1,359 ovum donation cycles (n=9,936 embryos) done with fresh oocytes, and 631 ovum donation cycles (n=3,794 embryos) carried out with vitrified oocytes. Embryo development was analyzed in a TLI incubator and the variables studied included timing to two cells (t2), three cells (t3), four cells (t4), five cells (t5), morula (tM) and cavitated, early and hatching blastocyst (tB, tEB, tHB), and second cell cycle duration (cc2=t3 − t2), based on criteria previously reported by the group in 2011. All the embryos were classified according to the hierarchical tree model and were subjected to statistical analysis. Moreover, implantation, clinical and ongoing pregnancies were included in the analysis. Similar proportions of embryos derived from fresh and vitrified oocytes were allocated to categories A-E in the hierarchical tree. Interestingly, authors reported that embryos that originated from vitrified oocytes showed a significant delay of around 1 h from the first division to two cells (t2) to the time of blastulation (tB), results that suggested morphokinetic differences between embryos from fresh and vitrified oocytes.

The report presented by Cobo et al. (4) in this issue of *Fertility and Sterility* describes the results from the largest sample size reported to date with embryo transfers performed on day 3 or in the blastocyst stage. The study has systematically addressed the evaluation of embryo quality in a large population of donated fresh and vitrified oocytes using an objective evaluation of embryonic development. As mentioned by the authors in the Discussion section of the paper, differences observed may be related to changes in gene expression. In the future, studies combining TLI with molecular expression analysis may shed some light on how vitrification specifically affects the oocyte and the early embryo.

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