Cryopreservation of human oocytes: hopes, no hypes?

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"Although the first pregnancy from slow-rate frozen oocytes was reported 23 years ago by Chen, the overall efficiency has remained exasperatingly low..."

The development of an efficient cryopreservation technique for human oocytes is probably the most important achievement in human assisted reproductive technology (ART) today. Although the first pregnancy from slow-rate frozen oocytes was reported 23 years ago by Chen [1], the overall efficiency has remained exasperatingly low and has resulted in the cessation of major clinical activity in this area for almost 20 years [2]. Paradoxically, the first wave of advancement was the result of a law that was created to undermine certain advancement in human ART. Skilled and ambitious embryologists in Italy tried to find alternative ways to compensate the negative effects of the strict regulations and, thus, the efficiency of traditional freezing expanded considerably and acheived the level of clinical applicability [3]. However, the breakthrough in the technology was provided by the alternative cryopreservation approach, vitrification.

Vitrification

Despite its current overwhelming success, the introduction of vitrification to human embryology cannot be regarded as a cloudless success story. To date, it is often stigmatized with epithets as 'new', 'experimental', 'developed empirically, without thorough basic research' and 'may have unknown long-term consequences'. Part of this characterization is completely false; others could be applied to many – if not all – procedures in laboratory embryology [4].

In fact, vitrification has been used in mammalian embryology since 1985 [5], and its improved versions then resulted in breakthroughs in domestic animal embryology in the 1990s [6]. Vitrification for human oocytes was first reported in 1995 [7]. The first live birth was published only in 1999 [8] and did not result in a breakthrough, as no further pregnancies were reported with exactly the same parameters.

The slow advancement can also be attributed to the lack of an appropriate animal model. Mouse oocytes are too easy to cryopreserve, while the extreme amount of lipids in most domestic animal oocytes necessitates a different approach. One can interpret as a wicked joke of nature the observation that the seemingly optimal test objects, the *in vitro*-matured and failed-fertilized metaphase II human oocytes become more resistant against cryoinjuries during *in vitro* culture, but the painstakingly developed parameters may become completely useless when applied to fresh oocytes [2,4,9].

Ultimate approach

The final solution has become the result of optimization and summary of all previous achievements: minimum volume, practical open carrier, optimal cryoprotectant concentration and combination, extended equilibration with lower concentration at room temperature, warming at core temperature of the human body, and a stepwise rehydration, again, at room temperature [10,11]. The procedure is inexpensive, easy to learn and produces consistent results. Survival rates evaluated by morphology are close to 100%, cleavage rates after intracytoplasmic sperm injection approach 75% and, in an oocyte donation program, no difference was detected between results achieved with fresh versus vitrified oocytes [12,13]. More

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Box 1. Indications that necessitate oocyte vitrification.

Medical reasons:
- Malignant diseases
- Surgical ovary removal
- Polycystic ovary
- Poor responders
- Hyperstimulation syndrome
- Premature menopause

Logistic reasons:
- Sperm-collection problems

Legal reasons:
- Restrictions in embryo cryopreservation

Social reasons:
- Wish to delay motherhood

Moral reasons:
- To compensate the handicapped situation of women in reproduction
- Wish to avoid embryo freezing (prefers oocyte freezing instead)

and more laboratories confirm these data, and growing evidence is provided that the procedure does not increase the rate of developmental abnormalities [14]. Additionally, vitrification can be applied several times to the same gamete and embryo without decreasing viability, as indicated by pregnancies obtained after two cryopreservation procedures one at oocyte and onc at blastocyst stage [15].

> "...vitrification can be applied several times to the same gamete and embryo without decreasing viability, as indicated by pregnancies obtained after two cryopreservation procedures, one at oocyte and one at blastocyst stage."

These impressive achievements might indicate that the problem has been resolved once and for all. Unfortunately, we are still far from that.

Tasks to accomplish

First, there are still concerns regarding the safety of oocyte vitrification. All current efficient systems require direct contact between the medium and the liquid nitrogen, which may consequently result in disease transmission [16]. Even if this risk is extremely low, almost negligible [17], it will not be indefinitely neglected by the authorities. The next evident step is to create a standard procedure that eliminates even this minimal danger while preserving the simplicity, efficiency and reproducibility of the present procedure.

Second, the realization of the possibility, the spreading of the method and the worldwide application of oocyte vitrification seems to be a far slower process than expected. Strangely, countries that are leading in other areas of human ART (including the whole of Scandinavia, Europe in general – except for Spain and Italy – Australia and most states of the USA) have been very slow to embrace these achievements, while Colombia, Mexico and other Latin American countries have obtained a leading role and high international reputation in this field. One may refer to legislation and financial issues, but the main problem might be more complicated, relating to challenges faced in both the scientific and business fields.

However, this situation cannot be maintained for long. The popular argument that "there is actually no need for oocyte vitrification here" is simply unacceptable and reflects a deliberate ignorance of the existing situation. There are dozens of medical, social or legal indications that necessitate oocyte vitrification, some of which occur in any ART practice. Box 1 lists only the most important ones.

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Clearly, one of the first and most significant applications for oocyte cryopreservation is for fertility preservation of patients battling with cancer (or with other malignant diseases), where the disease itself or the therapy would significantly reduce the reproductive ability of the person. In the USA alone, a total of 713,220 new cancer cases are expected in women in 2009, with approximately 8% of cases being in women younger than 40 years of age [101], where many would benefit from oocyte cryopreservation (i.e., women without children or established families). Startlingly, only a few dozen oocyte cryopreservations were performed for this indication, suggesting that not only the general public but also medical peers (particularly oncologists) need to be educated about the breakthrough improvement in this technology and to refer these patients to a procedure that may help them to fulfill their future dreams of having children. Paradoxically, fertility preservation through oocyte cryopreservation may gain a faster dissemination and greater use in healthy women, who want this procedure to be performed in order to 'create insurance' so that they are able to have children later in life ('social' indications, due to individual, family or professional conditions). Social indications for oocyte cryopreservation (i.e., to stop the biological clock) may be understandable, but this is also likely to create some moral/ethical dilemmas.

However, independently from indications, since the solution is now available at all ART clinics, it is not only a possibility but our destiny and moral duty to help those who need it.

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