Cryopreservation of immature human oocytes

Mariabeatrice Dal Canto  
Giovanni Coticchio  
Fausta Brambillasca  
Paola Novara  
Monia Lain  
Diana Turchi  
Maria Cristina Guglielmo  
Manuela Sottocornola  
Mario Mignini Renzini  
Rubens Fadini

BIOGENESI Reproductive Medicine Centre,  
Istituti Clinici Zucchi, Monza, Italy

Address for correspondence:  
Mariabeatrice Dal Canto, MD  
BIOGENESI Reproductive Medicine Centre,  
Istituti Clinici Zucchi  
Via Zucchi 24  
Monza, Italy  
Phone/Fax: +39 39 8383314  
E-mail: dalcanto.biogenesi@grupposandonato.it

Summary

The success of reproductive technologies is facilitated by gamete and embryo cryopreservation. Recent advances in vitrification techniques have improved survival rate of oocytes after warming and the resulting pregnancy rates. Cryopreservation of oocytes is nowadays an established procedure for fertility preservation and oocyte donation programs.

A new challenge is represented by “immature oocyte vitrification”, which might be a supplemental or alternative fertility preservation approach for patients with a contraindication to ovarian stimulation. This is the case, for example, of women affected by hormone-sensitive tumours, or patient in whom the “urgency” of receiving cancer therapy conflicts with the time required by a standard IVF treatment.

In this review we analyse the state-of-the-art of the cryopreservation of immature oocytes.

KEY WORDS: oocyte cryopreservation, in vitro maturation, germinal vesicle.

Oocyte cryopreservation

The wide use of controlled ovarian hyperstimulation protocols in ART has allowed to obtain a huge number of oocytes. As a consequence, a high number of embryos have been produced. While all these efforts have significantly improved the success of IVF, surely they have also prompted researchers to find out suitable methods to cryopreserve surpernumerary embryos.

Embryo cryopreservation is at present a well-established procedure and certainly reproductive technologies have benefited from the cryopreservation of surplus embryos, with an improvement of the success of ART and reduction of multifoetal pregnancies.

Nowadays, oocyte cryopreservation is a procedure proposed for preserving fertility in patients at risk of ovarian failure, and is an alternative to embryo cryopreservation for overcoming ethical and religious problems and lastly is applied in oocyte donation programs for the establishment of “egg banks” (1, 2).

The main problems concerning oocytes cryopreservation are due to the extremely large size and consequent high water content. During freezing, the water retained in the cytoplasm may produce ice crystals which could irreparably damage the ultra-structure of the oocyte. Hence, to prevent the cell damage against chilling injuries, the oocytes must be exposed to permeable and non-permeable cryoprotectants (CPAs) that induce oocytes dehydration by removing most of water from the cytoplasm (3). The ultra-rapid cooling or vitrification is a technique that allows glasslike solidification of oocyte cytoplasm without risk ice-crystal formation.

It is applied in human oocytes cryopreservation programs with great success rates (4). From a clinical perspective, the development of an efficient oocyte cryopreservation program contributes to advances in infertility treatment and reproductive biology in autologous as well as donation treatments enables to create “egg banks” from
young donors. Furthermore, oocyte cryopreservation offers the opportunity to preserve fertility in women with cancer pathology, at risk of loss their ovarian function, or without partner.

Advances in the diagnosis and treatment of malignant diseases have increased the survival rates of cancer patients over the past decades and, as a consequence, the number of women at risk of premature ovarian failure has raised. For these women, ensuring their reproductive capacity after the oncological treatments has become a main concern, as it is directly related to their quality of life. In particular ovarian tissue cryopreservation is a strategy involving explantation and cryostorage of tissue before chemo-radiotherapy and plan a subsequent re-implantation of small slide of ovarian cortex in orthotopic or heterotopic position, in order to restore the endocrine and reproductive function of the tissue. Ovarian tissue cryopreservation with subsequent re-implantation has been shown to preserve fertility in adult women, leading to 24 babies born around the world. However, this practice remains experimental (5).

The last challenge to implement the success of fertility preservation program might combine cryopreservation of ovarian tissue with “immature” oocytes aspirated directly from small antral follicles present in the explanted slide cortex (6).

In current standard IVF protocols, for many days women are treated with large doses of hormones that can have significant side effects. In the IVM treatment, immature oocytes are retrieved from ovaries of “untreated” women, or following a short and mild gonadotropin priming (7).

The in vitro maturation strategy can also be applied to patients with a contraindication to ovarian stimulation eliminating, for example, the risk of stimulation of hormone-sensitive tumours, such as breast cancer, especially in countries where the use of aromatase inhibitors is not allowed for IVF treatment. Moreover women affected by hormone-sensitive tumours, or patient in whom the “urgency” of receiving cancer therapy conflicts with the time required by a standard IVF treatment.

Applying this strategy, the collected oocytes are mainly germinal vesicle (GV) stage oocytes surrounded by compacted cumulus cells (70%) and few metaphase II (MII) stage oocytes (8). MII oocytes are “ready to use” while GV oocytes have to be matured in vitro in specific culture system or cryopreserved as “immature” (9).

Although the clinical application of oocyte vitrification has resulted in reasonable success rates in assisted reproduction programmes using MII oocytes, the experience with “immature oocytes” is in its infancy.

### Immature oocyte cryopreservation

In dependence of the maturation stage, oocytes have different specific physiological and biophysical properties that make them more or less sensitive to cryoinjuries and CPAs toxicity during cryopreservation procedures. This different properties entail that biological implications of cryopreservation can be different depending on the maturation stage of the oocyte (10).

In the mature oocyte, the most characterized cytoskeletal damage caused by cryopreservation concerns disruption of the sub-cortical actin meshwork, microtubule depolymerization, abnormal spindle configuration and chromosome scattering. Several studies demonstrate that some cytoskeletal alterations may be reversible. Under specific conditions, spindle fibres may depolymerize and re-polymerize after thawing, re-establishing the physiological interactions with chromosomes, which can be repositioned at the equatorial plate (11).

In immature oocytes microtubules are not organized in the MII spindle. Therefore, in principle the cryopreservation of immature GV-stage oocytes bypasses the risk of chromosome aberrations because at this stage the chromatin is decondensed and protected by a nuclear envelope (12). However, in some cases loss of DNA integrity may be an implication of the cryopreservation of in vitro matured oocytes (13).

Oocytes are surrounded by, and metabolically coupled with, adjacent cumulus cells by transzonal processes (TZPs). A critical difference between MII- and GV-stage oocytes regards the importance of these intercellular contacts between germinal and somatic cells. While MII-stage oocytes do not rely on support from surrounding cumulus cells and therefore it is irrelevant whether cryopreservation can assure viability of the somatic compartment, cryopreservation of GV-stage oocytes requires maintenance of their communication with viable cumulus cells to preserve a mutual interaction through TZPs that is essential for the process of maturation (14).

In particular, cryostorage mechanical stresses and toxic effect of CPAs on the microfilaments and microtubules cytoskeleton forming the TZPs can re-
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result in oocyte-cumulus cells membrane injury and TZPs disruption (15, 16). Furthermore it is essential to guarantee the cumulus cell survival after cryopreservation. It is important to optimize cryopreservation protocols considering that the large volume difference between oocyte and cumulus cells leads to diverse responses to CPAs (17) and that the presence of a somatic vestment can modify the penetration of CPAs into the oocyte. However, studies on this subject are not consistent.

Some reports have highlighted a protective effect of cumulus cells inducing an increment in survival and fertilization rates (18). On the other hand, other studies have suggested that cumulus cells do not have protective effects and that they interfere with the CPAs passage through ooplasm. However, even if cumulus cells can influence negatively the cryopreservation of GV oocytes, their presence is absolutely required for subsequent IVM. All these considerations underline the importance to maintain the integrity of TZPs during the cryopreservation of immature cumulus oocyte-complex. Cryopreservation at the immature stage is therefore an extremely problematic practice.

During the maturation process, oocytes undergo a change in lipid composition of their plasma membrane modifying the sensitivity of the plasma membrane to chilling injury (19). In particular, the elevated concentration of saturated fatty acids (79.22%) and a content of polyunsaturated fatty acids of 6.5% of mature oocytes makes the mature oocytes membrane more fluid and so more resistant to low temperature in comparison with immature stages, decreasing their sensitivity to chilling injury (20).

Clinical application of immature oocyte cryopreservation

Some studies investigated the efficiency of cryopreservation of immature or in vitro matured oocytes obtained from stimulated cycle. Oocytes cryopreserved at the GV stage showed a lower maturation rate with an increased rate of spontaneous activation then fresh control (21, 22). Cryopreservation of immature oocytes derived from IVM cycles represents an alternative fertility preservation approach for patients in whom gonadotropin stimulation is not recommended. In recent years, some experience has been gained in this field showing that also oocytes from IVM cycles cryopreserved at the GV-stage show a lower maturation rate than control. Vitrification of in vitro matured oocytes derived from IVM cycles seems to be less effective than vitrification of mature oocytes from ovarian stimulation treatment in terms of oocyte survival and fertilization rate (23). In conclusion, current evidence does not suggest an advantage in cryopreserving oocytes at the GV-stage.

The early hypothesis was that the presence of the nucleus and the absence of a spindle could be an advantage for cryopreserving oocytes at GV stage because the risk of chromosome aberrations could be bypassed. Recent data have shown that other important cellular structures may be damaged. In particular, as discussed above, the damage to the cumulus cell compartment and TZPs may explain the poor performance of oocyte cryopreserved at the GV stage in terms of maturation, fertilization and embryo developmental capacity as reported in the 90’s. It is our opinion that cryopreservation of in vivo or in vitro matured MII-stage oocytes is a better option than GV-stage cryopreservation.

References


