Cryobiology 78 (2017) 119–127

Contents lists available at ScienceDirect

Cryobiology

journal homepage: www.elsevier.com/locate/ycryo

# Safety and efficiency of oocyte vitrification

Neelke De Munck [a, *], Gábor Vajta [b]

[a] Universitair Ziekenhuis Brussel (UZ Brussel), Centrum voor Reproductieve Geneeskunde, Laarbeeklaan 101, 1090 Brussels, Belgium
[b] Central Queensland University, Bruce Highway, North Rockhampton QLD 4702, Australia

ARTICLE INFO

*Article history:*
Received 20 March 2017
Received in revised form
25 July 2017
Accepted 25 July 2017
Available online 1 August 2017

ABSTRACT

As the oocyte is the starting point for a new life, artificial reproductive technology (ART) techniques should not affect the (ultra) structural and functional integrity, or the developmental competence. Oocyte vitrification -one of the most significant achievements in human ART during the past decade- should therefore be a safe and efficient technique. This review discusses the principles and developments of the existing and future techniques, applications possibilities and safety concerns.

The broad range of vitrification media and devices that are currently available, show differences in their effects on the oocyte ultrastructure and preimplantation development. It is not yet fully decided whether this has an influence on the obstetric and neonatal outcome, since only limited information is available with different media and devices. For autologous oocytes, the obstetric and neonatal outcomes appear promising and comparable to pregnancies obtained with fresh oocytes. This however, is not the case for heterologous fresh or vitrified oocytes, where the immunological foreign foetus induces adverse obstetric and neonatal outcomes. Besides the oocyte vitrification process itself, the effect of multiple stimulations (for oocyte banking or for oocyte donors), seems to influence the possibility to develop gynaecological cancers further in life.

Automated vitrification/warming should offer a consistent, cross-contamination free process that offers the highest safety level for the users. They should also produce more consistent results in survival, development and clinical pregnancies between different IVF clinics.



**Contents**



* Corresponding author.
*E-mail address:* neelke.demunck@uzbrussel.be (N. De Munck).

http://dx.doi.org/10.1016/j.cryobiol.2017.07.009
0011-2240/

## 1. Introduction

Widespread use of vitrification for cryopreservation of oocytes and embryos is one of the most significant achievements in human ART during the past decade. Although often called as “new” technology, vitrification has been applied successfully for mammalian embryos 33 years ago [76]. The delay in routine application can be explained by the slow advancement in optimization of parameters, as well as the aversion of professionals to use an experimental procedure for valuable human reproductive cells. In fact, in spite of the watershed that happened approximately ten years ago, commonly used vitrification techniques are still extremely primitive ones, based entirely on the simple manual work of operators. Although the outcome justifies the application, there is an increasing demand for more sophisticated procedures that may eliminate potential dangers and inconsistencies related to human factors. The purpose of this review is to summarize the principles and the development of the existing techniques, to discuss various application possibilities and safety concerns. Finally, an attempt is made to outline the route for elimination of these concerns and to introduce new approaches that meet the level of the 21st century technology and the demand of modern assisted reproduction in humans.

### 1.1. Vitrification

In contrast to traditional slow-rate freezing, where a delicate balance is maintained through the induction of extra-cellular ice crystals, vitrification focuses on the total elimination of ice crystal formation in both the extra- and intracellular solutions. In embryology, this goal is usually achieved by transferring the oocytes to a solution with a relatively high concentration of cryoprotectants, as well as by using extremely high cooling and warming rates and by using small (<1 μl) solution volumes that are exposed directly to liquid nitrogen. The small volume also prevents heterogenous ice crystal formation, and the high cooling and warming rate at relatively high temperatures decreases chilling injuries.

In spite of the extensive basic research that explains in detail events during vitrification, all steps in the development of the procedure have happened empirically, including the selection of the most efficient cryoprotectant mixture, development of the proper carrier tool, finding the right final cryoprotectant concentration and outlining parameters of optimal equilibration. For permeable cryoprotectants, equal proportions of dimethylsuphoxide and ethylene glycol were found highly efficient and reliable [43]. Concerns related to the toxicity of the former component were mostly based on misunderstandings and were not justified by the outcome; moreover, the suggested alternative (propylene glycol) was proven to be more toxic by various investigations [5,97]. For non-permeable cryoprotectants, no convincing evidence is available to prove the superiority of either sucrose or trehalose, both sugars are used widely in solutions for cooling and warming. Development of the carrier tool was retrospectively a simple task, however, the discovery of the first model in bovine oocytes [56] and the first purpose-made designs required almost ten years [41,48,93]. Although new carrier tools are introduced almost every year, none of them was/is found essentially superior to these first devices. The minimal volume - direct contact to liquid nitrogen has helped to decrease the required concentration of permeable cryoprotectants by 25–30% [56,93]. However, the adjustment of the optimal multistep equilibration parameters, required further years, almost a decade [48,72].

Retrospectively the key of the success was (i) to reach a full equilibrium with a relatively low concentration (7.5% for both components) of permeable cryoprotectants with a relatively long (possibly multistep) exposure time at relatively low (25 °C) temperature, to avoid osmotic and toxic injuries, respectively; (ii) to make a short and aggressive compression-dehydration of the cells with exposure to a higher concentration (15–17% for both components) of permeable cryoprotectants mixed with a concentrated (0.7–1 M) non-permeable cryoprotectant and (iii) loading the sample to the carrier tool and immersion into liquid nitrogen - or analogue cooling agent.

Among various agents tested for additional protection, human serum derivates and recently a semisynthetic plant derivate, hydroxypropyl-methylcellulose [62] were found useful, most probably by protecting membranes during the cryopreservation process. On the other hand, cytoskeleton relaxants and antifreeze proteins were eventually found of little or no use [31,82]. Attempts to further increase cooling rates by using liquid nitrogen supercooled in vacuum, or helium were found unpractical and their application was not justified by the overall outcome [85].

Warming is usually performed by direct immersion of the sample in pre-heated solutions (37 °C) to ensure the highest warming rate. The solution contains a highly concentrated (0.5–1 M) non-permeable cryoprotectant to avoid osmotic damage. Subsequently, a slow and careful decrease of the cryoprotectant concentration is applied to ensure a mild rehydration without causing osmotic damage in membranes that are rather fragile as a consequence of the cryopreservation. By applying the complete procedure correct, close to 100% survival rates should be obtained with preservation of the developmental competence comparable to that of fresh human oocytes.

### 1.2. The oocyte and the developing embryo

The mature human oocyte has a specific chromatin and cytoplasmic arrangement that is important to achieve fertilization and adequate development [25,90].

During the process of vitrification, the oocyte is exposed to numerous physical and chemical processes that fluctuate over a wide non-physiological range which may impact structural and genomic integrity [47]. Since the oocyte -as a single cell structure-is the starting point for a new life, the repeated volumetric changes