Middle East Fertility Society Journal (2013) **18**, 223–232

Middle East Fertility Society

Middle East Fertility Society Journal

www.mefsjournal.org
www.sciencedirect.com

REVIEW

Cryopreservation of human oocytes, zygotes, embryos and blastocysts: A comparison study between slow freezing and ultra rapid (vitrification) methods

Tahani Al-Azawi [a], Safak Tavukcuoglu [a], Amir Afshin Khaki [b], Safaa Al Hasani [a,*]

[a] *Reproductive Medicine Unit, University of Schleswig-Holstein at Luebeck, Ratzburger Alle 160, 23538 Luebeck, Germany*
[b] *National Health Management Center (NPMC), Tabriz Iran and Tabriz University of Medical Science, Tabriz, Iran*

Received 24 June 2012; accepted 31 October 2012
Available online 11 October 2013

KEYWORDS

Vitrification;
Cryopreservation;
Oocyte;
Zygote;
Embryo;
Blastocysts

Abstract Preservation of female genetics is currently done primarily by means of oocyte and embryo cryopreservation. The field has seen much progress during its four-decade history, progress driven predominantly by research in humans. It can also be done by preservation of ovarian tissue or entire ovary for transplantation, followed by oocyte harvesting or natural fertilization. Two basic cryopreservation techniques rule the field, slow-rate freezing, the first to be developed and vitrification which in recent years, has gained a foothold. The slow-rate freezing method previously reported had low survival and pregnancy rates, along with the high cost of cryopreservation. Although there are some recent data indicating better survival rates, cryopreservation by the slow freezing method has started to discontinue. Vitrification of human embryos, especially at early stages, became a more popular alternative to the slow rate freezing method due to reported comparable clinical and laboratory outcomes. In addition, vitrification is relatively simple, requires no expensive programmable freezing equipment, and uses a small amount of liquid nitrogen for freezing. Moreover, oocyte cryopreservation using vitrification has been proposed as a solution to maintain women's fertility by serving and freezing their oocytes at the optimal time. The aim of this research is to compare slow freezing and vitrification in cryopreservation of oocytes, zygotes, embryos and blastocysts during the last twelve years. Therefore, due to a lot of controversies in this regard, we tried to achieve an exact idea about the subject and the best technique used.

* Corresponding author.
E-mail address: sf_alhasani@hotmail.com (S. Al Hasani).
Peer review under responsibility of Middle East Fertility Society.

Production and hosting by Elsevier

1110-5690 © 2012 Production and hosting by Elsevier B.V. on behalf of Middle East Fertility Society.
http://dx.doi.org/10.1016/j.mefs.2012.10.008

Contents

1. Introduction

Great scientific efforts are being made to discover more logical, ethical and economical procedures in order to maintain and advance the success of assisted reproduction techniques. Cryopreservation of human oocytes, zygotes, embryos and blastocysts is one of the necessary procedures. This tool has been widely used since the publication of the first reports about successful biochemical and clinical pregnancies with frozen–thawed human embryos in the early 1980s (1,2). The characteristics of cryopreservation methods, such as exposure time of cells to the different cryoprotectant solutions and to their different concentrations, as well as the rate of formation of extra- and intracellular ice crystals, have critical effects on survival and viability of human oocytes and embryos (3–5).

2. Indications for fertility preservation in women

Any condition which threatens to destroy all the follicles in both ovaries, or even destroy a large number of them, is an indication for fertility preservation. Both gynecological cancer and non-gynecological cancer may affect the ovarian reserve and ovarian function. The pathology itself may damage the ovarian tissues and its follicles. On the other hand, the therapies and their long-term complications cause gonadal toxicity. As the cancer treatment and therapeutics improve and more young women survive recently, many women consider fertility preservation as a part of their plan of treatment (6). Moreover, the reproductive behavior of women has been changed. There is a delay in the age of marriage and motherhood due to changes in the life style, women's education and career. Normal ovaries show a progressive decrease in the antral follicle count after the age of 35 years leading to a depletion of the ovarian reserve. So, in the future women wishing to postpone the motherhood will requisite fertility preservation. Thus, oocyte cryopreservation at a younger age using vitrification has been proposed as a solution to maintain women fertility (7,8).

3. Advantage of cryopreservation

Cryopreservation of embryos produced during human IVF is a logical way to provide an opportunity for patients to have repeated attempts at conception following a single drug stimulation cycle, preventing wastage of valuable genetic material and improving cumulative pregnancy rates. This approach may have several advantages to the patient (9,10). Firstly, it provides an opportunity to limit the number of embryos transferred while maximizing the usable embryo per oocyte retrieval cycle ratio at each stimulation attempt, a procedure that is costly and potentially difficult for patients. Secondly, the number of drug stimulation cycles in order to obtain oocytes can be decreased; consequently, the potential risk to the patient from exposure to anesthesia and the possible development of hyperstimulation syndrome can be reduced. In addition, storage of embryos from a cycle allows the patient to space the timing of sibling pregnancies, and improve their potential to achieve a pregnancy at an advanced maternal age, since the eggs were retrieved when the patient was younger (11). Moreover, Li et al. (12) noticed in their study that more human blastocysts survived with higher DNA-integrity index after vitrification/warming than after slow freezing/thawing.

4. Methods of cryopreservation

The slow-rate freezing method previously reported low survival and pregnancy rates, along with the high cost of cryopreservation. Although there are some recent data indicating better survival rates, cryopreservation by the slow freezing method has started to discontinue. Vitrification of human embryos, especially at early stages, became a more popular alternative to the slow rate freezing method due to reported comparable clinical and laboratory outcomes (13–15). In comparison, vitrification is more simple and cost effective than the slow freezing technique. Vitrification requires no expensive programmable freezing equipment and it needs a very small volume of vitrification medium which must be cooled at extreme rates not obtainable in regular enclosed cryostraws and cryovials (16,7). Vitrification can be observed and analyzed while slow freezing cannot. Unlike the slow freezing method, vitrification offers the ability to control the solute penetration, control the dehydration rate, and the maintenance of physiological temperature during the equilibrium procedure. The duration out of incubator is about 3 h in the slow freezing method, while it is about 10 min in vitrification. Fracture of zona pellucida and capture by growing ice crystals are possible during the slow freezing process, but they are not possible with vitrification (17,18). On comparing pregnancy and neonatal outcomes after the frozen or fresh embryo transfer, Aflatoonian et al (19) had concluded that vitrification has similar neonatal outcomes with no significant difference in pregnancy rates for frozen as compared with those after fresh embryo transfer.