Oocyte cryopreservation: the birth of the first Hungarian babies from frozen oocytes

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Abstract

Purpose To present data obtained with clinical application of oocyte cryopreservation.

Methods Slow freezing/rapid thawing in PBS based medium containing 1.5 M propanediol+0.3 M sucrose.

Results A total of 127 embryos were transferred into 54 patients (1.9 embryo/cycle, 64 transfer cycles). Clinical pregnancy rate of 20% per cycle (13/64) and 24.0% per patient were achieved. Up-to-date, six patients delivered seven healthy babies; there are four ongoing pregnancies. Three abortions (23%) and one biochemical pregnancy (0.7%) was obtained. Implantation rates of 11% per transferred embryos (14/127) and 6.5% (14/215) per thawed eggs were found. In each case, normal karyotype was detected. No difference was found in the ratio of spindle positive oocytes at the polscope analyses done before and after freezing (75.8% vs. 82.5%).

Conclusion Egg freezing is not a routine procedure yet, but there will certainly be a place for it in the future of assisted reproductive medicine.

Keywords Oocyte cryopreservation · Oocyte survival · Propanediol · Slow freezing · Sucrose

Capsule Three years experience with clinical application of oocyte cryopreservation using slow freezing and rapid thawing in PBS based medium supplemented with propanediol and sucrose.

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Introduction

It is now accepted beyond all doubt that cryopreservation (CP) of human gametes (spermatozoa/oocytes) and embryos is both mandatory and beneficial for the advanced techniques of assisted reproduction (AR). However, due to the low success and concerns over the damaged genetic material of the egg, CP of oocyte is still considered to be in research phase and more data is needed before it can be introduced into the clinical practice [1–4].

Basically two methods have been developed, and both of them is currently applied to human and animal oocytes and embryos [5–7]. The first method is the slow-freezing, which is considered to be an equilibrium procedure. At slow-freezing (traditional freezing), the cells are frozen with low concentration of cryoprotectant and therefore may result in small amount of—in intra- and extracellular—ice crystal formation. The second technique is the vitrification which is a non-equilibrium method and the cells are cryopreserved in a very small volume of freezing solution containing a mixture of permeating and non-permeating cryoprotectants in high concentration. At the vitrification, a very rapid freezing speed is used, therefore the small volume of concentrated freezing solution in which the cells are suspended solidifies without extra- and intracellular ice crystal formation and forms a glass-like solid state. Although, vitrification prevents the formation of intracellular ice crystals, but, exposes the cells to the toxic and osmotic effects of the highly concentrated freezing solution which may increase the probability of all other forms of cell injury.

The results of oocyte CP remain inconclusive and the reported inconsistent survival and pregnancy rates which were obtained by slow-freezing and/or vitrification indicate that more work is needed to find methods providing both excellent survival rates as well as maximal developmental

competence for the thawed oocytes [8–11]. The different authors agree in that further prospective trials are necessary to allow the evaluation of the two CP methods in terms of pregnancy achievement.

The aim of this retrospective series report of oocyte CP used in a clinical environment is to present the results obtained with frozen oocyte–embryo transfer cycles. Our work focused on the use of slow-freezing and rapid thawing protocol with PBS based medium containing 1,2-propanediol (PrOH) and sucrose combined with non-invasive spindle visualization with polscope and followed by intracytoplasmic sperm injection (ICSI).

Materials and methods

All patients taking part in this study declined embryo freezing for religious or other reasons. The number of eggs equivalent to the maximum number of embryos the patient requested to be transferred were fertilized by ICSI and the rest of the oocytes were frozen. Each patient had to sign a consent form before oocyte CP, in which they were informed about the lack of data concerning pregnancy outcome and the potential for increased risk of birth defects or chromosomal anomalies. In all cases chorion biopsy or amniocentesis was offered to the patients in order to evaluate the genetic material. The in vitro fertilization cycles combined with oocyte freezing are supported by the Collegium of the Gynecologists.

Only morphologically normal MII oocytes were selected for CP. The media used for freezing and thawing of oocytes were OocyteFreeze™ and OocyteThaw™ (MediCult, Mollehaven, Denmark). Equilibration was performed at room temperature in freezing medium based on PBS+20% HAS and containing 1.5 M PrOH+0.3 M sucrose (OocyteFreeze™, MediCult). Oocytes were frozen in straws (max. three oocytes per straw) in Planer III Kryo 10 cell freezer (Planer Products Ltd., Sunbury-on-Thames, UK). After seeding at minus 6°C, oocytes were slowly cooled (−0.3°C/min) to −30°C, then they were cooled at a higher speed (−50°C/min) to −150°C before plunging into liquid nitrogen (LN₂). After thawing, oocytes were de-hydrated and PrOH was removed in four steps from

the eggs by passage through mediums based on PBS+20% HAS supplemented with 0.5 and 0.3 M sucrose (OocyteThaw™, MediCult). Before freezing and after thawing prior to ICSI in all oocyte the spindle was visualized using the non-invasive spindle view technique (Polscope) [12]. In vitro fertilization with ICSI was performed 3–4 h after thawing, and fertilization was assessed 12–16 h later. Assisted hatching (AH) with laser was performed on all embryos prior to transfer [13]. Embryo transfer was carried out 48–72 h after ICSI at the 2 to 8 cell stages. Clinical pregnancy was defined by the presence of an intrauterine gestational sac and fetal heart beat on an ultrasound performed at 7 weeks of gestation.

Results

The average ages of the female patients were: 36+5.9 (range 24 to 48). The outcomes of the frozen oocyte–embryo transfer cycles are presented in Table 1. Until now, 215 eggs have been thawed with a post thaw survival rate of 80.0% (172/215). From the 172 survived oocytes 145 fertilized (145/172; 84.0%) and out of the 145 fertilized oocytes 139 cleaved (139/145; 96%). In the frame of 64 embryo transfer cycles, 127 embryos (127/145; 87.5%) were transferred into 54 patients (1.9 embryos per cycle) resulting in 20.3% clinical pregnancy per cycle (13/64) and 24% clinical pregnancy per patient (13/54). Up-to-date, six patients delivered seven healthy babies and there are still four ongoing pregnancies being in the 8th, 9th, 16th and 32nd week of pregnancy. Three patients experienced a spontaneous abortion at 8–10 weeks of pregnancy (3/13; 23%) and one patient had biochemical pregnancy (1/14, 0.7%). Implantation rate of 11% (14/127) per transferred embryos, and 6.5% (14/215) per thawed egg was obtained. Chorion biopsy or amniocentesis on all cases performed did not yield any chromosomal abnormalities, and in each case, normal karyotype was detected. No difference was found in the ratio of spindle positive oocytes at the polscope analyses done before and after freezing. Before CP, the spindles were detected in 75.8% of the oocytes (163/215). After thawing just prior to ICSI the

Table 1 The outcomes of the clinical frozen oocyte–embryo transfer cycles

Oocyte					Embryo		Patient		
Thawed	Survived (%)	Fertilized (%)	Cleaved (%)	Implantation per thawed oocytes (%)	Transferred	Implanted (%)	Number	Av. number of embryos transferred	Pregnant (%)
215	172 (80)	145 (84)	139 (96)	14 (6.5)	127	14 (11)	54	1.9	13 (24)

spindles were visualized in 82.5% of the survived oocytes (142/172).

Discussion

One of the main concerns in cryopreserving oocytes is that the procedure may cause depolymerization of the meiotic spindle microtubules and other cytoskeletal elements leading to chromosomal abnormality. Apart from that no chromosomal abnormalities to date have been detected even though depolymerisation of spindle resulting in cytogenic alterations was an earlier concern [5, 6]. There have been reports of a higher incidence of disrupted spindles in cryopreserved oocytes compared to fresh oocytes [14, 15]. However, recently obtained results indicate that although the meiotic spindle transiently disappears immediately after thawing, it reorganizes after 3 to 5 h of culture in the majority of mature oocytes [5, 6, 16, 17]. Our result obtained with the non-invasive spindle view technique supports this observation and indicates that in the cryopreserved oocytes the spindles are capable to reorganize during a short term in vitro culture after thawing since the spindles were detected in the same ratio of oocytes prior to freezing and after thawing and a short in vitro culture just prior to ICSI (75.8% vs. 82.5%). In addition, if comparing the chromosome status of embryos originating from fresh non-frozen oocytes and cryopreserved oocytes no difference was found between the two groups [17, 18]. Furthermore no difference was found in the ratio of abnormal fertilization between them [18]. Our data is in agreement with the previous observations of others' that although the mammalian spindle disassembles during cooling/freezing, but it reforms when the oocyte is returned to 37°C, in most of the cases. Our observations provides further evidence of that oocyte CP is a safe procedure since the chorion biopsy and amniocentesis carried out in our study did not yield any chromosomal abnormalities similarly to the previously published data of others'. Comparing the abortion rates obtained in our study with the data previously published by others we found no difference between them [3, 6, 8].

Our data show that slow cooling of oocytes in freezing solution containing 1.5 M PrOH and 0.3 M sucrose combined with ICSI at 4 h post-thaw, results in favourable outcomes for survival, fertilization, cleavage and pregnancy. The results obtained by others and in our clinical study with frozen oocytes provide further evidence of that although oocytes have special structures making them very sensitive to low temperature and freezing procedure, but they are indeed freezable. This is indicated by the fact that more than 500 to 600 healthy children have been born from frozen oocytes worldwide. Our pregnancy result achieved with oocyte CP correlates with results worldwide and the

outcome of our frozen embryo transfer cycles (Day 3 embryos: 24%, Day 5 embryos: 36%), but somewhat lower [1–4, 6, 8, 11]. One of the explanations of the slightly lower pregnancy rate is that in the present study the average number of transferred embryos originating from frozen oocytes was lower (1.9 embryo per patient) than those published in the literature. Generally, three to four embryos are transferred per patient in the frozen oocyte+ET cycles. However, according to our policy max two to three embryos are transferred both in the frozen ET and in the frozen oocyte+ET cycles.

In our study, laser assisted hatching (LAH) was performed on all embryos originating from frozen oocytes prior to transfer. We decided to perform LAH because (1) CP of oocytes and embryos may lead to zona hardening that may compromise in vivo hatching and implantation, thus AH has been advocated as a means of assisting the natural hatching process and enhancing implantation; (2) Data indicate that LAH improves the outcome of frozen-thawed embryo transfer (implantation and clinical pregnancy rates) when performed before transfer on embryos that were allowed to cleave [19, 20]; (3) AH was indicated by the advancing female age, previous repeated implantation failures, and poor prognosis too.

In conclusion, in the past 5–8 years due to the modifications made in the procedure of oocyte CP the efficacy has significantly improved. Today 30–40 oocytes are needed to achieve one pregnancy, whereas in the past 100–150 were needed. The progress made indicates that there will certainly be a place for oocyte CP in reproductive medicine in the future. However, more work is needed since the survival and implantation rates should be further improved.

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