Oocyte slow freezing using a 0.2–0.3 M sucrose concentration protocol: is it really the time to trash the cryopreservation machine?

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Objective: To update results on outcomes with frozen/thawed oocytes using a differential sucrose concentration during dehydration (0.2 M) and rehydration (0.3 M), combined with a one-step propanediol exposure.
Design: Retrospective cohort study.
Setting: Private IVF centers.
Patient(s): Infertile couples undergoing IVF treatment.
Intervention(s): Oocyte thawing cycles between May 2004 and December 2010.
Main Outcome Measure(s): Survival, fertilization, and cleavage rates were reported to evaluate biological outcomes. Clinical pregnancy and implantation rates were analyzed as markers of efficiency.
Result(s): Three hundred forty-two patients and 443 cycles were monitored; the survival was 71.8%, fertilization 77.9%, and of the embryos obtained 83.8% were classified as grade 1 and 2. Three hundred ninety-four transfers were performed, resulting in 90 pregnancies. The pregnancy rate per transfer was 22.8% and per patient was 26.3%, with 122 gestational sacs. The implantation rate per embryo was 13.5%. Patients were divided into three groups according to their age: $\leq$34 years (group A), 35–38 years (group B), and $\geq$39 years (group C). Biological outcomes were comparable in all three groups, whereas the pregnancy rate per transfer was higher in the first group (27.7% vs. 21.4% and 17.6%). The implantation rates per injected egg were 11.8%, 8.0%, and 7.5% for the three groups, respectively.
Conclusion(s): The biological and clinical data obtained on 443 cycles are consistent with our previous results showing that slow freezing of oocytes can be a valid tool in IVF practice when performed with a suitable protocol. (Fertil Steril® 2012;97:1101–7. ©2012 by American Society for Reproductive Medicine.)
Key Words: Oocyte cryopreservation, slow freezing, ICSI, implantation/oocyte, clinical pregnancy

In the last 10 years there have been significant improvements in assisted reproductive technologies (ART), primarily concerning the field of oocyte cryopreservation. Since the first pregnancy in 1986 (1), several groups have published more consistent results, giving rise to a new wave of interest in this field (2–4). To date, oocyte freezing has been recommended primarily to preserve fertility in women diagnosed with cancer or other pelvic diseases that may compromise their chances to conceive. Presently early detection programs, combined with improved treatment regimens, have allowed women with cancer to live longer. Therefore, quality of life issues, such as maintaining fertility and future parenthood, have become extremely important. The optimization of oocyte freezing represents a major advancement for these women because embryo cryopreservation was previously the only option available. In cases in which female cancer patients did not have a partner, the only option to preserve fertility after remission was by embryo freezing, requiring them to choose a sperm donor, often resulting in moral and psychological distress. Currently the remarkable improvements in oocyte freezing techniques make it possible to guarantee higher chances of success for these patients to plan a family. Additionally, some countries have legal restrictions that require IVF clinics to adopt egg freezing as a standard practice to avoid repeated ovarian stimulations. Another benefit of oocyte cryopreservation is the possibility to circumvent moral and legal issues associated with embryo freezing, such as legal status and ownership of cryopreserved embryos in the event of divorce. Finally, for egg donation programs oocyte freezing avoids [1] synchronization issues between donor and recipient, and [2] the risk for transmission of infectious pathogens due to quarantine of the donor eggs.

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Nevertheless, the technique of oocyte cryopreservation remains under experimental status in terms of outcomes and biological variables involved in the process. Little is known about the intrinsic quality of oocytes, and much of the limited information available usually requires invasive procedures that compromise the oocyte; several reports in the literature to date have shown that the successful recovery and ongoing development of oocytes after thawing (5) is highly dependent upon the selection of good-quality oocytes before freezing.

Several studies on ultrastructural damages (6, 7), meiotic spindle repolymerization (8), and permeability evaluations (9) after thawing have been published to validate new protocols. The unique feature of the human egg as one of the largest cells in the body (approximately 130-μm diameter) is the main aspect to overcome during oocyte freezing. The low surface area to volume ratio reduces the efficacy of the exchange between cryoprotectant agents and water. In slow-cooling protocols the concentration of permeating cryoprotectants is approximately 1.5 M (usually propanediol [PROH]), whereas the nonpermeating agents (usually sucrose) are 0.1–0.3 M in concentration. The evaluation of cell volume dynamics is important to target improvements in freezing protocols; optimal exposure time combined with the minimizing of osmotic stress should allow sufficient dehydration to achieve protection from freezing injury.

The approaches to address these issues have been varied. Yang et al. (10) increased cryoprotectant exposure temperature to achieve faster dehydration rates. Quintans et al. (11) adopted a stepwise addition of the permeating cryoprotectant (PROH) to reduce volume excursions, whereas Boldt et al. (12) tried to use a sodium-depleted freezing medium combined with a lower seeding temperature to improve postthaw recovery. The success of these methods seemed to be confined to sporadic cases and overall was not reproducible.

The approach conducted by our group, in collaboration with Paynter et al. (9), was based on measurements of oocyte osmotic response to cryoprotectants, and consequently a new protocol for slow freezing was designed in 2004. The objective of this article is to present data from an infertile cohort of patients who decided to cryopreserve their oocytes using a slow-freezing approach with differential sucrose concentration. The hypothesis was to confirm our preliminary data reported in 2007 (13).

MATERIALS AND METHODS

Study Design

This is a retrospective cohort study of patients who decided to freeze their supernumerary oocytes. Institutional approval for the present study was obtained by the Institutional Review Board of Tecnobios Procreazione, Centre for Reproductive Health, Bologna, Italy.

With the introduction in 2004 of a very restrictive law (40/2004) in Italy, a maximum of three oocytes could be inseminated because all the embryos produced must be transferred. As a consequence, egg freezing became a very important tool in IVF practice to avoid repeated ovarian stimulations.

However, on the May 8, 2009 the Italian Constitutional Court declared that the law (40/2004) was unconstitutional, affirming the need to empower the attending physician with the means to carry out a full evaluation regarding the number of eggs to inseminate, and eventually to freeze resulting embryos (14).

Setting

This study was carried out on 342 patients undergoing at least one thawing cycle from May 2004 to December 2010. Included among these subjects were 78 patients whose data had previously been published in 2007 (13) and 264 newly enrolled patients.

The biological parameters recorded were survival, fertilization, and cleavage rates. Oocytes with an intact membrane, clear cytoplasm, and no sign of degeneration were considered to have survived after thawing. All eggs were checked before culture and before injection.

The clinical variables considered were pregnancy rates (per patient, per thawing cycle, and per transfer) and implantation rates (per transferred embryo and per thawed and injected oocyte).

Clinical pregnancy was defined as the presence of a gestational sac at ultrasound examination. Patients who achieved a pregnancy were monitored during the entire period, and follow-up of the babies born was recorded. Neonatal outcomes have also been reported.

Participants and Variables

The study was carried out on 342 infertile patients (male, tubal, ovarian, or idiopathic infertility) with at least three supernumerary eggs to freeze; all oocytes were cryopreserved using a slow-freezing protocol previously described (13). All the subjects included in the cryopreservation program were informed about the procedure and provided written, informed consent.

Induction of multiple follicular growth was obtained by administering exogenous gonadotropins after pituitary desensitization with GnRH analogue, as previously described (15). Ovulation was induced with 10,000 IU hCG when at least two follicles of 22 mm in diameter were observed, with corresponding E_2 values detected in the bloodstream.

Egg retrievals were performed transvaginally via ultrasound guidance approximately 35 to 36 hours after hCG injection; oocytes were cultured in fertilization media (SAGE Cooper Surgical) supplemented with 5% human serum albumin (HSA; SAGE) at 37°C in a 5% CO_2 humidified incubator. Endometrial preparation for the thawing cycles was performed in all women as previously described (16). In case of pregnancy, hormone replacement therapy was continued for 60 days after transfer.

Oocyte Culture and Preparation Before Cryopreservation

The oocyte–cumulus complexes were isolated from their follicular fluid, washed in human tubal fluid medium supplemented with 10% HSA (Irvine Scientific), and cultured in 5% CO_2 at 37°C. Complete removal of the cumulus cells was performed enzymatically using hyaluronidase (80 IU/mL;