FERTILITY AND STERILITY®
VOL. 69, NO. 3, MARCH 1998
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Published by Elsevier Science Inc.
Printed on acid-free paper in U.S.A.

Pregnancy after human donor oocyte cryopreservation and thawing in association with intracytoplasmic sperm injection in a patient with ovarian failure

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Objective: To describe a pregnancy after human donor oocyte cryopreservation in association with intracytoplasmic sperm injection in a patient with ovarian failure.

Setting: Department of Reproductive Medicine, CER Medical Institute, Buenos Aires, Argentina.

Patient: A 48-year-old patient with ovarian failure.

Results: Ten donated oocytes were cryopreserved. Survival after thawing was 30%. Three oocytes were microinjected, and two embryos were obtained. The fertilization rate was 66%, and embryo development was 100%. Both embryos were transferred to a patient who had received hormonal replacement therapy. The attempt was successful, and a pregnancy was achieved after the transfer.

Conclusion: In association with intracytoplasmic sperm injection, an adequate technique of freezing and thawing of human oocytes might achieve encouraging results in ART programs. (Fertil Steril® 1998;69:555–7. ©1998 by American Society for Reproductive Medicine.)

Key Words: Oocyte cryopreservation, ovarian failure, intracytoplasmic sperm injection, oocyte donor program, pregnancy, ART

Human embryo cryopreservation has been shown to be a useful method in the treatment of infertility, allowing the transfer of a controlled number of embryos in order to avoid multiple pregnancies or to decrease the occurrence of ovarian hyperstimulation syndrome. Embryo cryopreservation has been successful but has led to ethical and legal problems.

Oocyte cryopreservation may be an alternative, not only by providing storage for the supernumerary oocytes, thus eliminating the ethical and legal complications of embryo banking, but also by giving a chance to patients at risk of losing ovarian function through surgical procedures, chemotherapy, or radiotherapy. Current methods for freezing oocytes are typical adaptations of protocols that have been successful with embryos.

Unfortunately, compared with the successful rates with embryos, survival rates of frozen-thawed oocytes are still low, and few pregnancies from cryopreserved oocytes have been reported in humans (1, 2). However, if such oocytes remain viable, the fertilization rate after intracytoplasmic sperm injection (ICSI) (3) and the rate of embryonic development is good.

After a report on unfertilized oocyte cryopreservation and thawing in experimental animals and humans was published (4), we used donated cryopreserved oocytes in our oocyte donation program.

MATERIALS AND METHODS

Ovarian Stimulation

A 34-year-old infertile patient with unexplained infertility was admitted to our ART

Received June 23, 1997; revised and accepted October 8, 1997.

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0015-0282/98/$19.00
PII S0015-0282(97)00530-X

program. Controlled ovarian hyperstimulation consisted of a lengthy protocol of GnRH agonist (0.2 mL daily of subcutaneous leuprolide acetate [Lupron; Abbott Laboratories, Buenos Aires, Argentina]) starting in the midluteal phase of the previous cycle in association with gonadotropin therapy (Metrodin or Pergonal; Serono Laboratories, Buenos Aires, Argentina) up to the day before hCG administration (Profasi; Serono Laboratories, Buenos Aires, Argentina). Monitoring and IVF procedures were performed as usual (5). Ten supernumerary oocytes were altruistically donated and cryopreserved in view of the absence of an adequate synchronized recipient. The couple signed a consent form. Like all study protocols designed and carried out at CER Medical Institute, the decision making related to this particular case was submitted to and approved by the CER Medical Institute Review Board.

Oocyte Cryopreservation

The oocyte cryopreservation protocol is an adaptation of the embryo-freezing method that has been used with success (4–6). Oocyte-cumulus complexes were transferred to human tubal fluid (HTF) supplemented with 0.5% human serum albumin (Irvine Scientific, Santa Ana, CA) at 37°C in an atmosphere of 5% CO_2 in air. Ten oocytes were frozen 5–6 hours after recovery using the slow freeze-rapid thaw 1,2-propanediol method. Following preequilibration of the oocytes for ten minutes in 1.5M 1,2-propanediol alone and transfer to 1.5M 1,2-propanediol containing 0.1M sucrose, the oocytes were immediately loaded into plastic straws and placed in a Planner Kryo 10 biological freezer (Planner Biomed, Sudbury, Middlesex, U.K.) and then cooled from 20°C to −8°C. Ice nucleation was induced manually by seeding each straw with precooled forceps. Gradual temperature reduction was continued to −30°C followed by rapid reduction to −150°C. Oocytes were stored in liquid nitrogen at −196°C prior to thawing.

Oocyte Thawing

Thawing was carried out at room temperature. The straws were removed from liquid nitrogen, held at room temperature for 30 seconds, and transferred into a 30°C water bath for 40 seconds by which time complete thawing had occurred. The cryoprotectant was removed by transferring the oocytes through decreasing concentrations of propanediol solution (1.5–0.5 M in each) containing 0.2M sucrose, followed by a final step dilution of 0.2M sucrose alone. Oocytes were then transferred into fresh culture medium, and after an incubation period of 1 hour the cumulus corona cells were removed by briefly exposing the oocytes (for 30 seconds) to a culture medium containing 80 IU/mL of hyaluronidase type VIII; SIGMA (H-3757) and then gently aspirating them into and out of fresh medium using hard-drawn pipettes.

Recipient Hormonal Replacement

A 48-year-old patient was admitted to our oocyte donation program. She had a 15-year history of primary infertility due to ovarian failure. She had previously undergone five fresh oocyte donation cycles without success. At this last attempt she was prepared as a recipient using increasing doses of micronized oral estradiol. When the endometrium reached an ultrasound trilaminar pattern of 10 mm thickness, the two embryos obtained by ICSI of frozen-thawed oocytes were transferred. A consent form was signed by the couple.

RESULTS

Three of the ten oocytes (30%) that were cryopreserved survived the freeze-thawing procedure. These three oocytes were at metaphase II stage and were microinjected by ICSI. After sixteen hours, two of them exhibited pronuclei and extruded the second polar body. Fertilized oocytes were tranferred to fresh medium HTF plus 10% synthetic serum substitute (Irvine Scientific).

On day 2, the fertilized oocytes had cleaved and were at the 2-cell stage (both at grade 1 according to the Lucinda Veek classification). By day 3, one embryo was at the 4-cell stage and the other was at 6–8-cell stage (grades 3 and 2, respectively). Both of them were transferred. Fourteen and sixteen days after the embryo transfer, serum βHCG tests were performed. Serum concentrations reached 244 mIU/mL and 1013.5 mIU/mL, respectively.

The first transvaginal ultrasound performed 21 days after embryo transfer showed two normoinsert gestational sacs with the presence of embryo and cardiac activity in one. Ten days later the ultrasound showed one gestational sac containing a 5-mm embryo with cardiac activity. A healthy male baby was born at the 38th week of gestation by means of a cesarean section. The Apgar score was 9–10 and the body weight was 3,000 g. We also performed a chromosomal analysis that showed a normal 46,XY karyotype.

DISCUSSION

Nearly a decade has passed since the first reports of pregnancies following cryopreservation of human oocytes were published (1, 2). Scientific interest has remained consistently high with a view to improving not only cryosurvival of oocytes, but also their fertilization, embryo development, and the possibility of attaining pregnancy.

Our oocyte cryopreservation technique was similar to the one usually used for embryos (4–6). In our case, the oocytes were cryopreserved without removing the cumulus corona cells. In our experiments with hamster oocytes, which were cryopreserved randomly with and without the

cumulus and corona cells, 30% of the oocyte-cumulus complexes survived after thawing while none of the denuded oocytes did so. The relative benefits of the removal of cumulus and corona cells before cryostorage have yet to be fully established.

The oocyte survival rate after cryopreservation has been reported to be variable (27%–64%). Although our survival rate was 30%, fertilization rate and embryo development were satisfactory (66% and 100%, respectively). In agreement with other researchers, we believe that fertilization and cleavage rates can be significantly enhanced by using ICSI in frozen-thawed oocytes independently of the characteristics of the semen (3).

In the light of our encouraging results, we believe that oocyte cryopreservation should be reintroduced as a clinical procedure. In conclusion, we are reporting the birth of a healthy male baby resulting from frozen-thawed–donated oocytes in which the cumulus corona complex had not been removed, a strict thawing timing was applied, ICSI was performed, and the recipient was adequately prepared.

Acknowledgments: The authors thank Teodoro Fried, M.D., from the CER Obstetrical Team and Andriana Wajakowski, M.D., from the CER Ultrasound Department for their contributions.

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