

CASE REPORT

Live birth from a frozen–thawed pronuclear stage embryo almost 20 years after its cryopreservation

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Objective: To report a live birth after transfer of cryopreserved pronuclear embryos in cryostorage for almost 20 years.

Design: Case report.

Setting: Academic IVF center.

Patient(s): A 42-year-old female patient with low ovarian reserve receiving donated embryos. These embryos were the result of an anonymous donation from an infertile couple who had conceived during her IVF treatment.

Intervention(s): Cryopreservation (slow-freeze method) and thawing of pronuclear stage embryos, and ultrasound-guided uterine ET.

Main Outcome Measure(s): Live birth.

Result(s): Five pronuclear embryos were thawed; two embryos survived, cleaved, and were transferred on day 2. A singleton term pregnancy was achieved with the delivery of a healthy boy.

Conclusion(s): A healthy live birth was documented after uterine transfer of pronuclear stage cryopreserved (slow freeze)–thawed embryos that were in storage for 19 years and 7 months. To our knowledge this case represents the “oldest” cryopreserved human embryos resulting in a live birth to date. (Fertil Steril® 2010; ■: ■–■. ©2010 by American Society for Reproductive Medicine.)

Key Words: Embryo cryopreservation, cryostorage, pronuclear stage, live birth

Cryopreservation increases the total reproductive potential in the assisted reproductive technology setting (1) and is responsible for expanding therapeutic options and improving treatment safety for assisted reproductive technology patients (2). Patients and their treating physicians can decrease treatment costs with the performance of multiple ETs from a single stimulation cycle, eliminate or decrease the effects of ovarian hyperstimulation syndrome, and minimize high-order gestations by limiting the number of transferred embryos (3, 4).

The number of cryopreserved embryos in storage has increased, as well as the amount of time in storage (4). The length of time that pronuclear or cleavage stage embryos are in cryopreservation storage does not seem to decrease the ability to produce a pregnancy. This is important because of the dramatic increase in cryobiology activity in the clinical setting as we witness improved methods to freeze pronuclear, cleavage, and blastocyst stage embryos using slow-freezing methods, and as optimized vitrification protocols are being developed.

However, there are few reports on the effects of the length of storage on cryopreserved embryos. An early report by Testart et al. (5) found an increase in the rate of embryonic cell death after only a few months of cryostorage. However, Cohen et al. (6) showed that the increased length of cryostorage does not have an effect on embryo development and potential. Our center recently reported on a large number of frozen–thawed embryos (more than 11,000) and concluded that the length of storage did not effect survival, implantation, miscarriage, or live birth rates with pronuclear and cleavage stage frozen embryos (4).

In our program, patients with embryos in cryostorage and who do not wish to use these embryos have the opportunity to anonymously donate their “surplus” embryos, at their discretion, to an adequately “matched” couple. A disposition consent form is required to donate these embryos anonymously to another couple. This is a case report of a patient who achieved a pregnancy from cryopreserved pronuclear embryos (slow freezing) that were in cryostorage for 19.6 years.

CASE REPORT

Patients

The donated embryos were the result of an IVF cycle performed by an infertile couple using their own gametes in January 1990. The female patient (34 years of age) had bilaterally obstructed fallopian tubes. The husband’s sperm sample concentration was $26 \times 10^6/\text{mL}$, with 61% progressive motility and 4.5% normal morphology (strict criteria). This couple achieved a pregnancy and delivered a healthy baby boy after the transfer of cryopreserved embryos. After the birth of their

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child, the couple requested that the remaining five cryopreserved embryos be donated to another couple that would remain unknown to them, and they signed a disposition consent form for an “anonymous donation” in September 1993. These embryos remained in cryostorage for 19.6 years and were eligible for donation for almost 16 years. In 2009 a “recipient” patient was successfully matched, and she and her husband agreed to the anonymous donation.

After matching, the 42-year-old patient (“donor embryo recipient”), in overall good health, with low ovarian reserve, and a hysteroscopically normal uterus, received the cryopreserved–donated embryos. Both members of the “recipient couple” signed informed consent forms.

Materials and Methods

The “recipient” patient was prepared for the ET cycle as follows (7): transdermal 17β -E₂ patches were administered (Vivelle Dot; Novogyne Pharmaceuticals, Miami, FL; each patch delivering 0.1 mg/d of E₂) and replaced every other day. On cycle day 1, two patches were applied, and then E₂ administration was gradually increased on cycle days 7 (to three patches every other day) and on cycle day 11 (to four patches every other day). In addition, from cycle day 12 and every other day, 1 mg 17β -E₂ (Estrace; Warner Chilcot, Rockaway, NJ) was started vaginally and continued with the same dose every other alternate day to the estrogen patch. From cycle day 15, the transdermal E₂ dose was decreased (to two patches every other day), alternating with the vaginal Estrace pills (at the same dose). From cycle day 15 on, vaginal micronized P (Prometrium; Solvay Pharmaceutical, Baudette, MN) was added to the regimen (600 mg/d). Both steroids were continued until 9 weeks’ gestation.

All cryopreserved embryos were developed from oocytes that were at the metaphase II stage at the time of follicular aspiration and inseminated using standard IVF insemination techniques. Pronuclear embryos were cryopreserved 17 hours after fertilization and thawed with a slow-freeze method with a programmed biological freezer (Planer Kryo 10-7; T.S. Scientific, Perkasi, PA). The cryoprotective supplement added to the freezing medium (Dulbecco’s phosphate-buffered saline; GIBCO Laboratories, Grand Island, NY) was 1.5 M propanediol (1,2 propanediol; Fisher Scientific, Pittsburgh, PA) (4, 8). Embryos were thawed in the afternoon of day 16 and transferred on the morning of day 17. Survival of the pronuclear stage frozen embryo was defined as the ability of the zygote to enter syngamy and proceed to at least the first cleavage division. Embryo transfer was performed under transabdominal ultrasound guidance using a soft pass catheter (Cook Medical, Bloomington, IN) (9).

Results

The 34-year-old “donor” patient had nine pronuclear embryos originally cryopreserved in January 1990. Four of these pronuclear embryos were thawed in 1990 for the patient herself, two pronuclear embryos survived and were transferred, resulting in the birth of a healthy baby boy. The remaining five pronuclear embryos were donated to the 42-year-old “recipient” in August 2009. Two pronuclear embryos survived the thaw. These embryos were allowed to cleave overnight, and both cleaved to the two-cell stage. Both embryos were given a grade 3 according to Veck’s criteria (10) and were transferred in the morning of day 2. The presence of an intrauterine gestational sac with fetal heart was confirmed at 7 weeks by transvaginal

TABLE 1

Embryo donation cycles with average length of cryostorage > 10 years.

Variable	Rate
Patients age (y)	42.9 ± 5.2
Average length of storage (y)	14.5 ± 2.6 ^a
Embryos survived/thawed (%)	38/60 (63)
Pronuclear stage (PN)	27/48 (56)
Cleavage stage (CL)	11/12 (92)
Embryos transferred (n = 35)	2.5 ± 0.5
PN (n = 23)	2.5 ± 0.5
CL (n = 7)	2.3 ± 0.5
Mixed (PN + CL) (n = 5)	2.5 ± 0.7
Clinical pregnancy rate per cycle	3/14 (21)
Clinical pregnancy rate per patient	3/11 (27)
Implantation rate	4/35 (12)
Implantation rate PN	4/23 (17)
Delivery rate per cycle	2/14 ^b (14)
Delivery rate per patient	2/11 (18)

Note: N = 14 cycles, 11 patients. Values are mean ± SD or number (percentage).

^a Range, 11.7–19.6 years.

^b One clinical miscarriage with 17.7-year-old thawed embryos; one ongoing twin pregnancy with 13.2-year-old thawed embryos; one delivery with 19.6-year-old thawed embryos. All of these patients had a transfer with pronuclear stage frozen–thawed embryos.

Dowling-Lacey. Live birth from embryos stored two decades. *Fertil Steril* 2010.

ultrasound. The successful vaginal delivery of a healthy baby boy (6 lb, 15 oz) occurred on May 7, 2010 after 41.5 weeks’ gestation.

DISCUSSION

We report an IVF live birth resulting from the transfer of pronuclear embryos frozen for 19.6 years, which is the first pregnancy with embryos in cryopreservation storage (at any stage of development) for this length of time. In our program history, there were a total of 14 donor embryo cycles with embryos in cryostorage for more than 10 years (clinical pregnancy rate 21%; implantation rate 12%) (Table 1).

There are previous reports of pregnancies from thawed embryos that were in extended cryostorage. López-Teijón et al. (11) had previously reported a successful pregnancy and delivery from donated pronuclear embryos thawed after 13 years of cryostorage. Go et al. (12) had also reported a pregnancy from pronuclear embryos in cryostorage for 8 years. Quintas et al. (13) and Revel et al. (14) also reported pregnancies from frozen embryos at the cleavage stage thawed after 8.9 years and 12 years, respectively.

There are few reports on long-term storage of human embryos. However, there is an indication that embryos left in cryostorage for extensive periods of time can result in healthy, live births. Animal models have already shown that extended cryostorage does not effect embryo survival or healthy deliveries (15, 16). Further human studies are necessary to improve the safety of long-term storage of embryos. Currently even less is known about the long-term effects of vitrification, an alternative to the slow-freezing methods. More studies are needed to determine whether embryo vitrification can result in improved survival and pregnancy rates, as well as its impact of long-term storage (17, 18).

REFERENCES

1. Toner JP, Brzyski RG, Oehninger S, Veeck LL, Simonetti S, Muasher SJ. Combined impact of the number of pre-ovulatory oocytes and cryopreservation on IVF outcome. *Hum Reprod* 1991;6:284–9.
2. Society for Assisted Reproductive Technology, American Society for Reproductive Medicine. Assisted reproductive technology in the United States: 2001 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology registry. *Fertil Steril* 2007;87:1253–66.
3. Oehninger S, Mayer J, Muasher SJ. Impact of different clinical variables on pregnancy outcome following embryo cryopreservation. *Mol Cell Endocrinol* 2000;169:73–7.
4. Riggs R, Mayer JF, Dowling-Lacey D, Ting-Fung C, Jones EL, Oehninger S. Does storage time influence postthaw survival and pregnancy outcome? An analysis of 11,768 cryopreserved human embryos. *Fertil Steril* 2008;93:109–15.
5. Testart J, Lassalle B, Forman R, Gazengel A, Belaisch-Allart J, Hazout A, et al. Factors influencing the success rate of human embryo freezing in an in vitro fertilization and embryo transfer program. *Fertil Steril* 1987;48:107–12.
6. Cohen J, Inge KL, Wiker SR, Wright G, Fehilly CB, Turner TG Jr. Duration of storage of cryopreserved human embryos. *J In Vitro Fert Embryo Transf* 1988;5:301–3.
7. Arslan M, Bocca S, Jones E, Mayer J, Stadtmayer L, Oehninger S. Effect of coasting on the implantation potential of embryos transferred after cryopreservation and thawing. *Fertil Steril* 2005;84:867–74.
8. Veeck LL, Amundson CH, Brothman LJ, DeScisciolo C, Maloney MK, Muasher SJ, et al. Significantly enhanced pregnancy rates per cycle through cryopreservation and thaw of pronuclear stage oocytes. *Fertil Steril* 1993;59:1202–7.
9. Mirkin S, Jones EL, Mayer JF, Stadtmayer L, Gibbons WE, Oehninger S. Impact of transabdominal ultrasound guidance on performance and outcome of transcervical uterine embryo transfer. *J Assist Reprod Genet* 2003;20:318–22.
10. Veeck LL. Atlas of the human gametes and conceptuses. New York: Parthenon Publishing, 1991.
11. López-Teijón M, Serra O, Olivares R, Morages M, Castello C, Alvarez JG. Delivery of a healthy baby following the transfer of embryos cryopreserved for 13 years. *Reprod BioMed Online* 2006;13:821–2.
12. Go KJ, Corson SL, Batzer FR, Walters JL. Live birth from a zygote cryopreserved for 8 years. *Hum Reprod* 1998;13:2970–1.
13. Quintas CJ, Donaldson MJ, Bertolino MV, Godoy H, Pasqualini RS. Birth of a healthy baby after transfer of embryos that were cryopreserved for 8.9 years. *Fertil Steril* 2002;77:1074–6.
14. Revel A, Safran A, Laufer N, Lewin A, Reubinov BE, Simon A. Twin delivery following 12 years of human embryo cryopreservation: case report. *Hum Reprod* 2004;19:328–9.
15. Glenister PH, Thornton CE. Cryoconservation-archiving for the future. *Mamm Genome* 2000;11:565–71.
16. Fogarty NM, Maxwell WM, Eppleston J, Evans G. The viability of transferred sheep embryos after long-term cryopreservation. *Reprod Fertil Dev* 2000;12:31–7.
17. Loutradi KE, Kolibianakis EM, Venetis CA, Papanikolaou EG, Pados G, Bontis I, et al. Cryopreservation of human embryos by vitrification or slow freezing: a systematic review and meta-analysis. *Fertil Steril* 2008;90:186–93.
18. Al-Hasani S, Ozmen B, Koutlaki N, Schoepper B, Diedrich K, Schultze-Mosgau A. Three years of routine vitrification of human zygotes: is it still fair to advocate slow-rate freezing? *Reprod Biomed Online* 2007;14:288–93.