Live birth of a normal healthy baby after a frozen embryo transfer with blastocysts that were frozen and thawed twice

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Objective: To report the birth of a normal healthy baby after a frozen ET (FET) with blastocysts that were frozen and thawed twice.

Design: Case report.

Setting: Private infertility practice.

Patient(s): A 26-year-old female who presented with male factor infertility and polycystic ovarian disease.

Intervention(s): One cycle of IVF-ET and FET no. 1 followed by the refreezing of blastocysts and FET no. 2.

Main Outcome Measure(s): Clinical pregnancy and live birth.

Result(s): Thirty-six months after a successful IVF-ET cycle, FET no. 1 was performed with eight frozen blastocysts that were thawed. One blastocyst was transferred to the uterus and four were refrozen. The first FET resulted in a singleton pregnancy that ended in a spontaneous abortion after 7 weeks’ gestation. Six months later, FET no. 2 was performed. All four refrozen blastocysts were thawed and transferred to the uterus. The second FET resulted in a singleton pregnancy and the birth of a normal healthy baby (male) weighing 3.005 g after 38 weeks’ gestation.

Conclusion(s): Human blastocysts can be refrozen/thawed and produce a normal healthy baby after an FET. Further studies will be required to determine survival, implantation, and live birth rates with refrozen/thawed human blastocysts. (Fertil Steril® 2005;83:198–200. ©2005 by American Society for Reproductive Medicine.)

Key Words: Cryopreservation, frozen embryo transfer, FET, refrozen, blastocysts

The culture of human embryos in vitro to the blastocyst stage using either coculture (1) or sequential culture media (2–6) is an important advance in assisted reproductive technologies, which has resulted in increased implantation rates and a reduction in the number of embryos transferred in IVF-ET cases. Supernumerary blastocysts are normally cryopreserved in IVF-ET cases, giving the patient the potential of a future pregnancy with an FET. Human blastocysts have been successfully cryopreserved using either slow-freezing methods (7, 8) or vitrification (9). Many live births occur each year using blastocysts that have been frozen and thawed once. However, little, if any, information exists on FETs with human embryos frozen and thawed more than once at the blastocyst stage. We report here the case of a successful pregnancy and the live birth of a normal healthy baby from human blastocysts frozen and thawed twice at the blastocyst stage.

MATERIALS AND METHODS

IVF-ET was performed using a normal ovarian stimulation protocol. The patient was placed on oral contraceptives (OCPs) and a GnRH agonist (20–10 IU/day), which was added after 14 days. Recombinant FSH was begun on cycle day 1, and ovulation was induced with a single dose of recombinant hCG (5,000 IU). Thirty-six hours after hCG, an ultrasound-guided transvaginal oocyte aspiration/retrieval was performed with a 17 g × 30 cm oocyte retrieval needle and 20-mL syringes.

Oocyte insemination was performed with 200,000 motile sperm/mL for 18 hours in 100 µL drops of G1.2 media (Vitrolife, Denver, CO) overlaid with mineral oil. All 2-pronuclei embryos were cultured in 50 µL drops of G1.2 media overlaid with mineral oil for 48 hours. On day 3, all viable embryos were transferred to 50 µL drops of G2.2 media (Vitrolife) overlaid with mineral oil and cultured to day 6. All embryos were cultured at a density of one embryo/drop in a 37°C incubator with 6% CO₂.

Blastocyst freezing was performed on day 6 on all expanded/hatching/hatched blastocysts with a visible inner cell mass using a two-step, slow-freeze protocol (Menezo mod-
All freezing/thawing media were from SAGE Biopharma, Trumbull, CT. Blastocysts were incubated for 10 minutes in a HEPES-buffered 5% glycerol solution followed by 10 minutes in a HEPES-buffered 9% glycerol solution with 0.2 M sucrose. During these incubations, the temperature was lowered from 37°C to 20°C by opening the IVF chamber door and turning the heater off. The blastocysts were frozen in cryopreservation vials with 200 μL of the HEPES-buffered 9% glycerol solution with 0.2 M sucrose using a controlled-rate LN2 vapor freezer. The freezing program lowered the temperature from 20.0°C to -7.0°C at -2.0°C/minute. The temperature was held at -7.0°C for 10 minutes, and the vials were manually seeded with 1.1,1,2-tetrafluoroethane spray and maintained at -7.0°C for another 10 minutes. The temperature was then lowered to -37.0°C at -0.3°C/minute. Finally, the vials were plunged and stored in LN2 at -196°C.

Blastocyst thawing was performed using a two-step, rapid-thaw protocol (Mencez modified two-step). The vials were incubated at room temperature for 1 minute, followed by 2 minutes in a 30°C water bath. The embryos were then incubated for 3 minutes in a HEPES-buffered 0.5-M sucrose solution followed by 2 minutes in a HEPES-buffered 0.2-M sucrose solution. During these incubations, the temperature was increased from 30°C to 37°C by closing the IVF chamber door and turning the heater on. The embryos were then incubated in 1 mL of G2.2 media for ≥4 hours at 37°C with 6% CO2. Only blastocysts that demonstrated reexpansion after 4 hours were used for the FETs. The ETs were performed with an 18 cm Wallace ET catheter containing ≤15 μL of G2.2 media.

The patient was prepared for the FETs using an E2 patch protocol. She was placed on OCPs, and a GnRH agonist (20 IU/day) was added after 14 days. Transdermal E2 patches (0.1 mg/patch) were applied every 3 days starting at cycle day 1 (1–4 patches/application). After 14 days, daily IM injections of P in oil (50 mg) were started along with E2 patches (0.2 mg/3 days). The FETs were performed on the sixth day of P injections. Both P injections and E2 patches were continued until the tenth week of pregnancy.

**RESULTS**

A 26-year-old female (gravida 0) with a diagnosis of male factor infertility and polycystic ovarian disease underwent IVF-ET. The oocyte aspiration/retrieval resulted in the retrieval of 19 intact metaphase II oocytes. After insemination, 15 were normally fertilized (2 pronuclei). After 6 days of culture, 12 embryos reached the blastocyst stage, with eight exhibiting spontaneous hatching in vitro. Two hatched zona-free (HZF) blastocysts were transferred to the uterus on day 6. Eight other blastocysts were frozen on day 6. The fresh transfer resulted in a twin pregnancy that spontaneously reduced to a singleton pregnancy after 17 weeks’ gestation. The patient gave birth to a normal healthy baby (male) weighing 2.069 g after 32.5 weeks’ gestation.

Thirty-six months later, FET no. 1 was performed. Because our IVF laboratory normally obtains a survival rate of approximately 50% after the freezing/thawing of blastocysts and to maximize the patient’s probability of success, all eight frozen blastocysts were thawed. At 4 hours post-thaw, only one blastocyst had reexpanded. This HZF blastocyst was transferred to the uterus, and the remaining seven were cultured in 1 mL of G2.2 media overnight (18 hours). The next morning, four of the seven blastocysts had reexpanded. These four blastocysts (three HZF blastocysts and one expanded blastocyst) were refrozen. FET no. 1 resulted in a singleton pregnancy that ended in a spontaneous abortion after 7 weeks’ gestation.

Six months later, FET no. 2 was performed. All four refrozen blastocysts were thawed. At 4 hours post-thaw, all four had reexpanded and were transferred to the uterus. Ten days later, the patient’s serum hCG was 197 mIU/mL. Three weeks after the positive hCG, a transvaginal ultrasound revealed two sacs, one with cardiac activity (153 bpm). The singleton pregnancy progressed uneventfully, with the patient giving birth to a normal healthy baby (male) weighing 3,005 g after 38 weeks’ gestation.

**DISCUSSION**

We have demonstrated in this case report that it is possible for human blastocysts to be frozen/thawed twice and produce a normal healthy baby after an FET. There have been at least two reports of live births of healthy babies after the refreezing/thawing of human embryos (10, 11). The first group performed the primary freeze/thaw cycle with cleavage-stage embryos (6–8 cells) and the secondary freeze/thaw cycle at the blastocyst stage, using slow-freeze protocols for both (10). The second group performed the primary freeze/thaw cycle with pronuclear-stage embryos with a slow-freeze protocol and the secondary freeze/thaw cycle at the morula stage with a vitrification protocol; the embryos were then cultured and transferred at the blastocyst stage (11).

To our knowledge, there have been no reports demonstrating that human embryos can be successfully frozen/thawed twice at the blastocyst stage. To date, we have refrozen three other cohorts of blastocysts from FETs. All three remain in storage and have not yet been thawed.

Since this report is only a single case, it will require more data on FETs performed with refrozen/thawed blastocysts to determine the survival, implantation, and live birth rates from these embryos. In addition, the risk of biochemical pregnancies, spontaneous abortions, and birth defects must be determined. Until these data are collected and analyzed, refreezing human blastocysts should be approached with caution.

However, if refrozen/thawed blastocysts can produce implantation and live birth rates that are not significantly different from primary frozen/thawed blastocysts, it may alter
the way we approach some FETs. One change may be to aggressively thaw all cryopreserved blastocysts for an FET, having the confidence that any supernumerary blastocyst(s) that have survived and reexpanded can be refrozen with a realistic expectation of survival. Thawing all available blastocysts at the time of the initial FET would allow the selection of the embryos with the greatest potential for implantation (similar to an IVF-ET case), which should increase the implantation, pregnancy, and live birth rates for FETs.

REFERENCES