Live birth following transfer of a cryopreserved embryo generated from a cryopreserved oocyte and a cryopreserved sperm: Case report

Debra A. Gook · Lyndon Hale · David H. Edgar

Received: 27 October 2006 / Accepted: 6 November 2006 / Published online: 28 December 2006
© Springer Science+Business Media, LLC 2006

Abstract As is the case with non-frozen oocytes, the efficient and successful use of cryopreserved oocytes in human assisted reproduction is, in part, dependent on the ability to apply selection criteria when choosing the ‘best’ embryos for transfer from a cohort. In many cases this, in turn, will necessitate the cryopreservation of non-transferred embryos to minimise the risk of multiple pregnancy. It is therefore important to establish that an embryo, generated by fertilization of a frozen-thawed oocyte, can be capable of surviving subsequent cryopreservation while retaining the potential for normal development. In this case report, we document the delivery of a normal male infant following transfer of a frozen-thawed embryo, generated by the fertilization of a frozen-thawed oocyte by a frozen-thawed sperm.

Keywords Cryopreserved embryo · Cryopreserved oocyte · Human · Live birth

Introduction

The resurgence of interest in human oocyte cryopreservation as a consequence of a number of reports suggesting that it

may be a safe option in appropriate circumstances [1–4] has led to its clinical application and reports of a number of live births [5–10]. Although this approach has been adopted as an adjunct to routine IVF practice in Italy, this is predominantly a consequence of legal developments which resulted in the prohibition of the alternative, and more widespread, option of embryo cryopreservation.

Oocyte cryopreservation, however, may also be appropriate in circumstances where embryo freezing is not possible e.g in cases of malignant disease in young women where the imminent cytotoxic therapy is likely to inhibit subsequent oocyte development and where there is no male partner or in cases of routine IVF when no sperm are retrieved. The efficiency and success of subsequent use of frozen oocytes in such cases will, inevitably, be dependent on factors including the ability to select embryos for transfer. One consequence of generating sufficient embryos to allow selective criteria to be applied will be the possibility of supernumerary embryos being available after fertilisation of the frozen/thawed oocytes. For this approach to be successful without increasing the risk of multiple pregnancy by transferring multiple embryos, it may be necessary to cryopreserve embryos generated from previously cryopreserved oocytes.

We now report a live birth following transfer of a cryopreserved embryo which was, in turn, generated from a cryopreserved oocyte. Since the oocytes were frozen as a result of the absence of partner’s sperm at testicular biopsy, the subsequent fertilization also involved intracytoplasmic injection of cryopreserved sperm from a donor.

Case report

A 36 year old woman presented for treatment for infertility on the basis of a previous testicular needle biopsy

D. A. Gook · L. Hale · D. H. Edgar (✉)
Reproductive Services, Royal Women’s Hospital,
132 Grattan Street, Carlton, Victoria 3053, Australia
e-mail: david@mivf.com.au

L. Hale · D. H. Edgar
Melbourne IVF,
320 Victoria Parade, East Melbourne, Victoria 3002, Australia

D. A. Gook · D. H. Edgar
Department of Obstetrics & Gynaecology,
University of Melbourne,
Victoria 3010, Australia

diagnosis of Sertoli-Cell-Only Syndrome in her 37 year old partner. Ovarian stimulation was achieved by the daily administration of recombinant FSH (200 IU/day) following pituitary desensitisation with GnRH agonist. Meiotic maturation of preovulatory oocytes was induced by administration of human chorionic gonadotrophin (hCG; 10,000 IU) and cumulus-oocyte complexes were retrieved transvaginally 36 h later under ultrasound guidance. Twenty-one oocyte-cumulus complexes were identified in the aspirated follicular fluid. Multiple site open testicular biopsy was performed on her partner on the day of oocyte collection but no sperm was retrieved. The couple opted to have the oocytes cryopreserved pending a decision on future treatment. The oocytes were stored in 2002 and remained in storage for 3 years prior to thawing.

Materials and methods

Oocyte cryopreservation

Following digestion of cumulus-oocyte complexes using hyaluronidase (10 IU/ml), 19 of the 21 oocytes were confirmed as being at the metaphase II stage of meiosis and were prepared for cryopreservation. Following rinsing in phosphate buffered saline (PBS) containing 20% heat-inactivated maternal serum, oocytes were dehydrated in solutions of the same basal medium containing 1.5 M 1,2 –propanediol (PROH) for 10 min followed by 1.5 M PROH + 0.2 M sucrose for a further 5 min before being loaded into plastic straws. Dehydration was performed at room temperature and cooling was as previously described [1]. Oocytes were frozen 7.5 h after follicle aspiration.

Oocyte thawing, fertilization and culture

Straws containing cryopreserved oocytes were thawed rapidly as previously described [1] and rehydrated by sequential incubation (at 37°C) in 1.0 M PROH/0.2 M sucrose (5 min), 0.5 M PROH/0.2 M sucrose (5 min), 0.2 M sucrose (2.5 min) and 0.1 M sucrose (2.5 min) before being washed twice in the basal medium and incubated in Quinn's Advantage Fertilisation medium containing 4 mg human serum albumin (HSA) per ml. The basal medium used for all rehydration solutions was Quinn's Advantage HEPES-buffered medium (QHEPES; SAGE BioPharma, USA) supplemented with 20 mg HSA per ml.

Cryopreserved donor sperm was prepared by density gradient centrifugation and intracytoplasmic sperm injection was performed as previously described [11]. Post injection oocytes were cultured in Quinn's Advantage Fertilisation medium for 17 h and assessed for the presence of pronu-

clei before being transferred to Quinn's Advantage Cleavage medium. Timing of syngamy/first cleavage was assessed at 23 h post insemination (hpi) and embryo development was assessed at 41 hpi prior to selection for transfer or cryopreservation.

Embryo transfer, cryopreservation and thawing

Single embryo transfers were performed and embryos were transferred in natural menstrual cycles. Embryo dehydration prior to cryopreservation was carried out in a single step by immersion in QHEPES (with 10 mg HSA per ml) and containing 1.5 M PROH + 0.1 M sucrose at room temperature for 10 min before loading into a straw. Cooling and thawing were as previously described [12]. Post thaw rehydration was achieved by sequential incubation in QHEPES (with 4 mg HSA per ml) and sucrose at 0.5, 0.2, and 0 M respectively, followed by two rinses in Quinn's Advantage Cleavage medium (with 4 mg HSA per ml) and overnight culture prior to transfer.

Results

In 2005, three years after the oocytes were placed in storage (during which time the woman had a number of unsuccessful attempts to conceive via donor insemination) the couple decided to have oocytes thawed for treatment. Four of 6 thawed oocytes survived intact and a single sperm was injected into each oocyte 3 h later. All four had 2 visible pronuclei at 17 hpi. At 23 hpi, one fertilised oocyte had entered syngamy while the remaining 3 still had visible pronuclei. The fertilised oocyte which had entered syngamy at 23 hpi had cleaved to the 2-cell stage at 41 hpi and was transferred. Two of the other 3 (2 cells and 5 cells respectively) were discarded on the basis of multinucleated blastomeres. The final fertilised oocyte had 3 blastomeres at 41 hpi but had progressed to the 5 cell stage at 45 hpi and was cryopreserved. The serum β hCG level peaked at 498 IU/l on the 26th day after transfer but no ultrasound evidence of pregnancy was obtained and the β hCG level subsequently decreased to undetectable levels.

In a subsequent natural menstrual cycle, the cryopreserved embryo was thawed and survived with all 5 blastomeres intact. Following overnight culture, the embryo had developed to the 8-cell stage and was transferred. Fourteen days after embryo transfer the serum β hCG level was 612 IU/l. A single intrauterine gestation sac with a fetal heartbeat was confirmed by ultrasonography 32 days after embryo transfer. A healthy male infant weighing 3242 g was delivered by Caesarean section at 37 weeks gestation.

Discussion

Cryopreservation of human oocytes has long been postulated to be a potentially useful addition to the range of techniques available in assisted reproduction laboratories. It would permit the storage of reproductive potential for women at risk of loss of fertility when undergoing cytotoxic therapy, allow storage of oocytes when sperm retrieval is not possible in a cycle of IVF treatment, and, more controversially, may play a role in circumventing ethical and legal problems associated with the more established practice of embryo cryopreservation.

The number of babies born following in vitro fertilization of cryopreserved oocytes has steadily increased over the last few years [5–10]. Much of this growth can be attributed to changes in the law in Italy, which resulted in the prohibition of embryo cryopreservation. Under this new legal framework, only a small number of fresh oocytes can be inseminated since all resultant embryos must be transferred. Similarly, when thawing oocytes for insemination, the maximum number of embryos to be transferred will determine the maximum number of oocytes which can be thawed. Such restrictions inevitably result in the loss of the ability to apply selective criteria when choosing embryos for transfer. Criteria such as morphology and cleavage rate [13], and timing of the first cleavage division [14–16] have been applied successfully to select early cleavage stage embryos with enhanced implantation potential for transfer. This approach is only realistically feasible, however, when it is possible to cryopreserve the remaining embryos in a cohort which are not chosen for transfer.

In contrast to the circumstances which are prevalent in Italy, i.e. in situations where embryo cryopreservation is not prohibited, it should be possible to increase the efficiency of treatment by fertilizing an increased number of cryopreserved oocytes and applying the above selective criteria. Prior to adopting this approach, however, it is necessary to demonstrate that embryos generated by in vitro fertilization of human cryopreserved oocytes can survive further cryopreservation and undergo subsequent development to term when transferred in utero. Partial proof of principle in relation to this question was provided by a study in a mouse model which demonstrated that mouse 4 cell stage embryos generated from frozen-thawed oocytes can successfully survive a second cryopreservation and develop to the blastocyst stage in vitro [17].

In the present case report, we have now demonstrated that a human embryo, generated by fertilization of a frozen-thawed oocyte (by a frozen-thawed sperm), can survive subsequent cryopreservation, resume development in vitro and develop to term when transferred in utero.

References

1. Gook DA, Osborn SM, Johnston WI. Cryopreservation of mouse and human oocytes using 1,2-propanediol and the configuration of the meiotic spindle. *Hum Reprod* 1993;8:1101–9
2. Gook DA, Osborn SM, Bourne H, Johnston WI. Fertilization of human oocytes following cryopreservation; normal karyotypes and absence of stray chromosomes. *Hum Reprod* 1994;9:684–91
3. Gook DA, Osborn SM, Johnston WI. Parthenogenetic activation of human oocytes following cryopreservation using 1,2-propanediol. *Hum Reprod* 1995;10:654–8
4. Gook DA, Schiewe MC, Osborn SM, Asch RH, Jansen RP, Johnston WI. Intracytoplasmic sperm injection and embryo development of human oocytes cryopreserved using 1,2-propanediol. *Hum Reprod* 1995;10:2637–41
5. Porcu E, Fabbri R, Damiano G, Giunchi S, Fratto R, Ciotti PM, Venturoli S, Flamigni C. Clinical experience and applications of oocyte cryopreservation. *Mol Cell Endocrinol* 2000;169:33–7
6. Borini A, Bonu MA, Coticchio G, Bianchi V, Cattoli M, Flamigni C. Pregnancies and births after oocyte cryopreservation. *Fertil Steril* 2004;82:601–5
7. Chen SU, Lien YR, Chen HF, Chang LJ, Tsai YY, Yang YS. Observational clinical follow-up of oocyte cryopreservation using a slow-freezing method with 1,2-propanediol plus sucrose followed by ICSI. *Hum Reprod* 2005;20:1975–80
8. Boldt J, Tidswell N, Sayers A, Kilani R, Cline D. Human oocyte cryopreservation: 5-year experience with a sodium-depleted slow freezing method. *Reprod Biomed Online* 2006;13:96–100
9. Borini A, Sciajno R, Bianchi V, Sereni E, Flamigni C, Coticchio G. Clinical outcome of oocyte cryopreservation after slow cooling with a protocol utilizing a high sucrose concentration. *Hum Reprod* 2006;21:512–7
10. Levi Setti PE, Albani E, Novara PV, Cesana A, Morreale G. Cryopreservation of supernumerary oocytes in IVF/ICSI cycles. *Hum Reprod* 2006;21:370–5
11. Bourne H, Richings N, Harari O, Watkins W, Speirs AL, Johnston WI, Baker HW. The use of intracytoplasmic sperm injection for the treatment of severe and extreme male infertility. *Reprod Fertil Dev* 1995;7:237–45
12. Edgar DH, Bourne H, Speirs AL, McBain JC. A quantitative analysis of the impact of cryopreservation on the implantation potential of human early cleavage stage embryos. *Hum Reprod* 2000;15:175–9
13. Ziebe S, Petersen K, Lindenberg S, Andersen AG, Gabrielsen A, Andersen AN. Embryo morphology or cleavage stage: how to select the best embryos for transfer after in-vitro fertilization. *Hum Reprod* 1997;12:1545–9
14. Shoukir Y, Campana A, Farley T, Sakkas D. Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability. *Hum Reprod* 1997;12:1531–6
15. Sakkas D, Percival G, D'Arcy Y, Sharif K, Afnan M. Assessment of early cleaving in vitro fertilized human embryos at the 2-cell stage before transfer improves embryo selection. *Fertil Steril* 2001;76:1150–6
16. Lundin K, Bergh C, Hardarson T. Early embryo cleavage is a strong indicator of embryo quality in human IVF. *Hum Reprod* 2001;16:2652–7
17. Revel A, Moshe N, Helman A, Safran A, Simon A, Koler M. Mouse embryos generated from frozen-thawed oocytes can successfully survive a second cryopreservation. *Hum Reprod* 2004;19:666–9