

Human pregnancy after transfer of intact frozen-thawed embryos

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Summary

Since the birth of the first baby as a result of *in vitro* fertilisation (IVF) in 1978, many clinics around the world have achieved pregnancies and births for their patients by using IVF and gamete intrafallopian transfer procedures. With the storage of excess embryos, multiple laparoscopies can be avoided; this has favoured the development of better cryopreservation techniques. In our clinic 8-cell human embryos are frozen in a 1,5M dimethyl sulphoxide solution as cryoprotectant using the slow freeze-thaw method. Sixteen thawed embryos were replaced in 8 patients, resulting in 1 pregnancy. Of the thawed embryos 51,6% survived the freezing process in that they had 50% or more of the original number of blastomeres and also the zona pellucida intact.

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It has been shown that embryo cryopreservation can increase the chance of pregnancy by making it possible to transfer embryos obtained from a single laparoscopy to the patient over several cycles.¹

Although a higher pregnancy rate is achieved with the placement of 3 - 4 embryos compared with 1 - 2,^{2,3} a higher pregnancy rate is not necessarily obtained with the transfer of more than 4 embryos.⁴ Therefore the need for cryopreservation facilities arise when numerous embryos are obtained simultaneously from a single cycle. Cryopreservation of excess embryos allows optimal patient and gamete management.

Recent articles^{1,5-7} have reported different success rates with the use of different freeze-thaw methods; these vary from an 8% to a 44% pregnancy rate per transfer.

Methods for embryo cryopreservation have been studied at Tygerberg Hospital since 1985; the technique was made available to patients in 1987. Experience with the first 8 patients treated by this method are discussed.

Patients and methods

Freezing method

The slow freeze-thaw method used in our laboratory has been described previously.^{5,8} Embryos are frozen in 1,5M dimethyl sulphoxide (DMSO) in sterile glass ampoules.

Cryoprotectant equilibration involved 10-minute incubations at room temperature in 0,25M, 0,5M, 1,0M and 1,5M DMSO solutions. Freezing is carried out in a biological freezer — the

ampoules are seeded at -7°C and the embryos are maintained at this temperature for 20 minutes and then cooled at a rate of $0,1 - 0,3^{\circ}\text{C}/\text{min}$ to -80°C , and at $-10^{\circ}\text{C}/\text{min}$ to -110°C . The ampoules are then transferred directly to liquid nitrogen.

Thawing is carried out at a rate of $+8^{\circ}\text{C}/\text{min}$ and the cryoprotectant removed by serial dilution at room temperature in 1,5M, 1,25M, 1,0M, 0,75M, 0,5M, 0,25M and 0M DMSO solutions. The embryos are examined for freeze-thaw damage and cultured for 2 hours in Ham's F10 medium supplemented with 20% serum before transfer to the patient's uterus.

The patient

The 31-year-old patient was entered into our programme after $1\frac{1}{2}$ years' primary infertility. Initially, she was accepted for an *in vitro* fertilisation (IVF) cycle because the right fallopian tube was reported to have a blind ending and the left had adhesions and abnormal fimbriae. A hysterosalpingogram done in 1987 showed obstruction and a hydrosalpinx of the left fallopian tube, but there was free spill of contrast medium on the right side.

In October 1987 the patient was stimulated for a possible gamete intrafallopian transfer (GIFT) cycle by being given clomiphene citrate 100 mg on days 5 - 9 of her cycle. She also received 150 IU of human menopausal gonadotrophin (HMG) from days 6 to 11. Blood samples were taken from day 9 and assayed for oestradiol and also luteinising hormone (LH) levels. A spontaneous LH surge was detected on day 12, but no β -subunit of human chorionic gonadotrophin (β -HCG) was administered. Laparoscopy and oocyte recovery were carried out on day 13 of the cycle, almost 35 hours after the onset of the LH surge.

Eight oocytes were obtained and 4 of these, together with 200 000 spermatozoa, were transferred into the ampulla of the left fallopian tube. The fimbriae of this tube had been reported to be normal during laparoscopy. The other 4 oocytes were fertilised and placed in culture. After 18 hours 2 pronuclei were visible in all the oocytes and after 59 hours 2 8-cell embryos and 1 6-cell embryo were obtained (1 oocyte remained a 1-cell embryo). The quality of the embryos was graded on a scale of 1 - 5, and these were considered good (grade 5) and frozen immediately.

No pregnancy resulted from the first procedure and the patient requested thawing of the frozen embryos 6 months later.

The time of transfer was chosen after the onset of the LH surge on day 16. No intercourse was allowed during follow-up. Thawing of the 3 frozen embryos took place 130 hours after the onset of the LH surge. No damage was apparent and all blastomeres and the zona pellucida were intact. We allowed 69 hours after the LH surge for the expected time of ovulation and 59 hours for the age of the embryos at the time of freezing.

Results

Pregnancy was confirmed by rising levels of the β -subunit of HCG on day 12 and 16 after transfer and also by ultra-

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sonography at 8,5 weeks, when 2 sacs were observed with 1 positive fetal heart. It was reported to be a normal intra-uterine pregnancy at this stage. Follow-up ultrasonography at 12 weeks confirmed normal development and growth of the fetus.

To date 78 embryos (from 6- to 10-cell stage) from 33 patients have been frozen. Of these patients, 11 have requested thawing of their embryos. In total, 31 embryos were therefore thawed, of which 16 (51,6%) had more than 50% of the original blastomeres and also the zona pellucida intact. These 16 embryos were transferred to 8 patients. Only 10 embryos remained totally intact. Two patients had 3 embryos replaced, 4 had 2 embryos replaced and 2 patients had only 1 embryo replaced.

Discussion

Major implications of embryo cryopreservation are not only an increased pregnancy rate per cycle but also an increase in the efficiency of IVF and reduction of cost and risks involved with repeated IVF treatments and laparoscopies. It is also possible for patients due to undergo radio- or chemotherapy and surgery to have their embryos cryopreserved.⁹

The 130 hours between the onset of the LH surge and the thawing of the embryos allowed for the ovulation interval and also the post-insemination age of the embryos. Thawing of embryos took place 4 - 5 hours before transfer to allow 2 - 3 hours' post-thaw incubation. Cohen *et al.*⁶ reported that embryos replaced a day earlier than their age have a better chance of implantation, but their study was based on very small numbers. Most clinics, however, tend to replace thawed embryos at a synchronised time calculated from the ovulation interval and post-insemination age of embryos.

Although all 3 thawed embryos were intact at transfer, blastomeres of embryos are often damaged after thawing. There are, however, no indications that abnormalities increase after embryo cryopreservation.⁹ The first pregnancies reported by Trounson and Mohr⁹ and Zeilmaker *et al.*¹⁰ occurred when intact embryos were transferred. A report from Freemann *et al.*⁵ stated that 3 of 5 embryos transferred that resulted in abortions had only 50% of the original number of blastomeres left, but the numbers are too small to conclude that damage may lead to pregnancy loss. Willadsen¹¹ studied sheep embryos and concluded that embryos with only half the original number

of blastomeres can give rise to normal conceptus. This was confirmed by Trounson and Mohr⁹ who said that the total number of blastomeres of pre-implantation embryos is not necessary for development to term.

The results of this study indicate a 51,6% survival rate, which compares favourably with the 58% survival rate obtained at Monash University⁵ and the 36% obtained by Cohen *et al.*⁶ — both studies used the slow method and 1,5M DMSO as cryoprotectant. Although the numbers are still small, the 12,5% pregnancy rate is promising and also correlates with that of Freemann *et al.*⁵ (11%) and Cohen *et al.*⁶ (21%).

This work opens new doors for infertile patients in the GIFT and IVF programmes in our hospital.

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