

*Case report*

# Birth following replacement of frozen – thawed embryos in an *in vitro* fertilization programme

Sheena E M Lewis, A I Traub, Sheena Y Robinson, W Thompson

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Ovulation induction is used routinely in all *in vitro* fertilization (IVF) programmes<sup>1</sup> and as a result large numbers of oocytes, often up to 15, may be retrieved in one cycle. To avoid multiple pregnancies, no more than three embryos are transferred to the uterus at one time, so that a number of embryos may be unused in each treatment. These supernumerary embryos have been successfully cryopreserved for later use.<sup>2-4</sup>

Cryopreservation reduces the cost and risk to the patient as no further superovulation or oocyte recovery is necessary in the cycle during which the freeze – thawed embryos are replaced. In addition, it allows the possibility of embryo replacement during a natural cycle subsequent to that in which the oocytes were recovered. This avoids hormone induced changes in the endometrium which may render it antagonistic to implantation.<sup>5</sup> Thus, the introduction of cryopreservation to an IVF programme should complement it and improve its efficiency. We report the first successful pregnancy achieved in Northern Ireland following the replacement of frozen – thawed embryos.

**CASE REPORT.** A 30-year-old married woman presented to the sub-fertility clinic, Royal Maternity Hospital, Belfast, with a history of infertility over the previous nine years. She was admitted to the *in vitro* fertilization programme in May 1990 and given superovulation therapy. Six oocytes were recovered by follicular aspiration using a needle which was passed via the vagina into the ovary under ultrasonic guidance. The oocytes were inseminated with spermatozoa from her husband, whose semen had a normal profile according to WHO criteria.<sup>6</sup> The next morning five of the oocytes had fertilized, each showing two pronuclei. Eighteen hours after insemination three of these embryos were placed in the patient's uterine cavity using a fine catheter which was passed through the cervix.

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Royal Maternity Hospital, Belfast and Department of Obstetrics and Gynaecology, The Queen's University of Belfast, Belfast BT12 6BA.

Sheena E M Lewis, BSc, PhD, Research Fellow.

A I Traub, MD, FRCOG, Consultant Obstetrician.

Sheena Y Robinson, BSc, Senior Embryologist.

W Thompson, MD, FRCOG, Professor of Obstetrics and Gynaecology.

Correspondence to Dr Lewis.

Two of these embryos were at the six cell stage and of 'A' quality which meant that all the blastomeres were of regular shape and equal size. The other embryo had four cells, graded 'B' quality because it had slightly irregular blastomeres. The remaining two embryos were available for freezing. One had cleaved to form four grade 'B' blastomeres and the other also had four blastomeres but graded 'B' to 'C' quality because it had irregular blastomeres and some fragmentation.

### **Embryo freezing method**

To freeze the embryos they were placed in phosphate-buffered saline (PBS; Gibco, Paisley, Scotland) supplemented with 17% human albumin (Albuminar 20; Armour Pharmaceutical Co Ltd, Eastbourne, England). They were then passed to a further PBS solution containing 1.5 M 1,2-propanediol (PROH; Sigma Chemicals Ltd, St Louis, Missouri, USA) for up to 10 minutes and then transferred to the actual freezing solution of serum supplemented plus 1.5 M PROH and PBS 0.1 M sucrose (Sigma) for up to 10 minutes.<sup>4</sup> During exposure to this final solution, embryos were individually loaded into crystal straws (Rocket, London, England). The straws were cooled and frozen in a controlled rate freezer (Kryo 10.16, Planar Products, Sunbury-on-Thames, England) with the cooling rates within each temperature range chosen to give the embryo maximum protection against trauma occurring at that temperature.

The fresh embryo transfer did not result in pregnancy, and in October 1991 the patient requested that her two frozen stored embryos be thawed and replaced.

### **Embryo thawing and replacement**

The thawed embryos were replaced in a subsequent natural menstrual cycle. The age of the embryos was three days post-ovulation at the time of freezing and thawing was timed to allow their transfer to synchronise with the post-ovulatory age of the endometrium. It has been shown that the most favourable 'window' of endometrial receptivity is between days 17 and 19.<sup>7</sup> The patient was scanned on days 13, 14 and 15 of her cycle to monitor the growth of the follicle within the ovary. On day 15 the follicle was seen to have ruptured and so embryo transfer was arranged for two days later. Thawing was undertaken rapidly ( $200-300^{\circ}\text{C min}^{-1}$ ), taking the embryos from liquid nitrogen temperatures of  $-180^{\circ}\text{C}$  and warming them in a  $37^{\circ}\text{C}$  water bath. This prevents the growth of small intracellular ice crystals which might damage the blastomeres. Next, the embryos were rehydrated in a series of PBS solutions, each containing 0.2 M sucrose but with decreasing concentrations of the cryoprotectant, so that in the final solution there was no PROH present. The addition of sucrose controlled the degree of swelling during cryoprotectant removal by acting as a non-toxic counterforce.

Immediately after thawing one embryo was graded as a three 'B', because one of its four blastomeres had not survived the freezing and thawing processes, but 30 minutes later the embryo had cleaved in culture, forming five healthy cells. Only one blastomere of the other, poorer quality, embryo had survived. However, both embryos were transferred to the patient after 2½ hours in culture. Fifteen days later the patient had a positive pregnancy test and after a further week ultrasound scan showed a single intrauterine pregnancy sac. Thereafter serial scans showed a healthy single fetus. The patient's antenatal care was undertaken at the

Royal Maternity Hospital and progressed satisfactorily until the patient had a spontaneous vaginal delivery of a healthy male infant weighing 3150 grams at term.

## **DISCUSSION**

The first pregnancy to result from the transfer of a frozen–thawed embryo occurred in Australia in 1983.<sup>8</sup> Since then many different cryopreservation techniques have been employed and it is now common practice to freeze super-numerary embryos after GIFT and IVF if facilities and staff are available.

There are at least two prerequisites for a successful pregnancy in a freezing programme — embryo quality and endometrial suitability. The embryos most likely to survive the trauma of freezing and thawing are those of high quality, with all their blastomeres intact and with no signs of fragmenting.<sup>9</sup> The advantage of freezing embryos in early cleavage stages, when they have divided to form two, four or eight cells, is that these embryos have more blastomeres and can maintain their viability with 50% survival. In contrast, pronucleates which have not begun to divide need 100% cell survival to maintain their viability. Testart<sup>10</sup> reported high survival rates (88%) for pronuclear oocytes but with a progressive decline in survival with increasing cleavage divisions. Troup and colleagues claim that the number of embryos surviving when frozen–thawed at the pronucleate stage is not significantly higher than when frozen at earlier cleavage stages (60%) but the implantation rates of pronucleates are substantially greater (47%) than those of early cleavage embryos (14%).<sup>11</sup>

The frozen–thawed embryos may be replaced in a natural menstrual cycle without artificial regulation if, as in this case, the patient has a regular cycle ( $28 \pm 1$  days). If her periods are irregular, pituitary function is suppressed by the administration of a GnRH analogue and the ovaries are then stimulated by gonadotrophin injections. Studies have shown that pregnancy rates are similar whether in natural or simulated cycles.<sup>12</sup>

There are varying degrees of success in attaining pregnancies with frozen – thawed embryos in different IVF centres. Some larger and better staffed centres report pregnancy rates as high as 32%<sup>13</sup> and 47%,<sup>11</sup> whereas an overall success rate in the range of 3–6% has been suggested.<sup>14</sup> Another consideration is that a patient requesting replacement of frozen–thawed embryos is excluded from a further cycle on the IVF programme until the frozen embryo cycle is completed. As her chances of becoming pregnant with fresh embryos are greater ( $\approx 30\%$ /cycle) than with frozen–thawed embryos, it may be in the patient's interest to opt for another cycle of IVF rather than using frozen embryos. This decision must be made by the couple.

Following the lead of Troup in Manchester,<sup>11</sup> there is an increasing preference to freeze only pronuclear oocytes and to give priority to the timing of the freezing procedure. Initial reports of this modified technique claim greater success and it is hoped that this improvement will allow cryopreservation of human embryos to achieve the very high success rates obtained in mouse embryos and thus fulfil its potential. Embryo freezing combined with IVF can improve conception rates per cycle for infertile couples, as it did for this couple, for the first time in Northern Ireland.

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