Successful pregnancy following blastocyst vitrification

Y.Yokota 1,3, S.Sato1, M.Yokota1, Y.Ishikawa1, M.Makita1, T.Asada1, and Y.Araki2

1Yokota Ob/Gyn Clinic, 1-16-5, Shimokoide, Maebashi, Gunma 371-0031, and 2The Institute for ARMT, 2-39-3, Kamikoide, Maebashi, Gunma 371-0037, Japan
3To whom correspondence should be addressed: Yokota Ob/Gyn Clinic, 1-16-5, Shimokoide, Maebashi, Gunma 371-0031, Japan. E-mail: yaaraki@mb.infoweb.ne.jp

A 32 year old woman and her 32 year old spouse were referred to our IVF programme. Following recovery of 12 mature oocytes, nine were fertilized following conventional IVF. Three fresh embryos were transferred to the uterus, but all failed to result in pregnancy. Six supernumerary embryos were cultured in vitro until day 5 in order to create blastocysts. Two grew to the blastocyst stage and were vitrified using a modification of a previous method. Two blastocysts survived the freeze–thaw process and were transferred to the patient's uterus during a natural cycle, 3 months after the previous retrieval cycle. Implantation resulted in a healthy pregnancy; delivery is expected in June 2000. This report documents the first successful pregnancy in Japan, achieved via blastocyst vitrification. Key words: blastocyst/cryopreservation/IVF/vitrification

Introduction

Assisted reproductive technology has undergone significant advances and embryo freezing has become a widely used routine procedure that may contribute to increasing cumulative pregnancy rates from every cycle of a successful oocyte recovery and transfer. Cryopreservation has become a necessary part of IVF programmes that helps to avoid the risk of multiple pregnancies following the transfer of large numbers of embryos, as well as preventing wastage of supernumerary embryos arising from the large number of oocytes primed by ovarian stimulation. In Japan, an average of three embryos was replaced in patients of all ages in 1996 (Minaguchi, 1996). On the other hand, recent studies have indicated the possibility of producing viable human blastocysts in a sequential culture medium (Gardner and Lane, 1997; Gardner et al., 1998). In IVF, the transfer of blastocysts facilitates high pregnancy rates, thereby reducing the number of embryos needing to be transferred. Thus, this procedure minimizes the risk of multiple gestation (Gardner et al., 1998). A simple procedure for the cryopreservation of supernumerary blastocysts for infertility treatment is needed.

While the slow cooling method of cryopreservation is now widely used, there has been some recent experimentation with ultra rapid freezing (vitrification) of human embryos. The technique of vitrification may be improved with regard to both the laborious nature of the technique itself and the survival of embryos upon recovery. Previously reported attempts to vitrify human embryos have been unsuccessful in achieving pregnancy (Quinn and Kerin, 1986; Trounson et al., 1988). In the 1990s, there were reports of vitrification of human cleavage stage embryos and some successful deliveries following thaw and transfer using several cryosolutions (Barg et al., 1990; Gordts et al., 1990; Feichtinger et al., 1991; Ohta et al., 1996; Mukaida et al., 1998; Kosuge et al., 1999). However, to the best of our knowledge, there have been no reports of pregnancies following human blastocyst vitrification.

In the present case, human blastocysts were successfully vitrified using modified VSED cryosolution (Ishimori et al., 1993), containing modified-human tubal fluid (m-HTF) with 20% serum, ethylene glycol and dimethyl sulphoxide (DMSO) at a 2:1:1 ratio. After thawing, viable blastocysts were transferred in utero, resulting in one healthy pregnancy, now in its 25th week.

Case report

A 32 year old woman and her 32 year old spouse were referred to our IVF programme in July 1998. The husband's semen characteristics were found to be within normal parameters according to World Health Organization criteria (WHO, 1999), i.e. volume 2.0 ml, sperm concentration 120×106/ml, motility 80%, abnormal morphology 10%.

Although the patient had regular ovulatory cycles, a bilateral hydrosalpinx was also demonstrated by hysterosalpingography. Ovarian stimulation was achieved via a combination of gonadotrophin-releasing hormone (GnRH), human menopausal gonadotrophin (HMG; Nikken, Tokyo, Japan) and 10 000 IU human chorionic gonadotrophin (HCG, Pregnyl; Organon, Oss, The Netherlands). Both were administered when the leading follicle had reached a mean diameter of 18–20 mm.

Following recovery of 12 mature oocytes, nine were fertilized following conventional IVF. Three days after insemination, three fresh embryos were transferred to the uterus, but all failed to result in pregnancy. The remaining six embryos continued to be cultured in vitro until day 5. Two grew to the blastocyst stage, and the resulting blastocysts were...
cryopreserved in VSED solution. Blastocysts were exposed to 10% ethylene glycol for 5 min, then placed into 50% VSED for 1 min, and finally (within 30 s), loaded into straws containing VSED at room temperature. The straws were placed in liquid nitrogen vapour for 2 min, and then plunged immediately into liquid nitrogen.

The thawing procedure involved warming the straws in a 25°C water bath, after a one-step dilution of the cryoprotectant was carried out using 0.5 mol/l sucrose solution; the blastocysts were cultured in vitro overnight. Informed consent was obtained from the couple before the use of VSED.

The two frozen–thawed blastocysts were transferred to the patient’s uterus during a natural cycle, 3 months after the previous retrieval cycle. Pregnancy was achieved, and a HCG concentration of 50 mIU/ml in the urine was detected 2 weeks after the blastocysts had been transferred. A singleton pregnancy with a visible heartbeat was seen at 6 weeks gestation and is now ongoing in its 35th week.

**Discussion**

Previously reported attempts to vitrify human embryos have not resulted in pregnancy (Quinn and Kerin, 1986; Trounson *et al.*, 1988). In the 1990s, there were reports of successful vitrification of human cleavage stage embryos and deliveries following thaw and transfer using various cryosolutions (Barg *et al.*, 1990; Gordts *et al.*, 1990; Feichtinger *et al.*, 1991; Ohta *et al.*, 1996; Mukaida *et al.*, 1998; Kosuge *et al.*, 1999). However, another study (Ishimori *et al.*, 1993), described 23 morula to blastocyst-stage bovine embryos that had been equilibrated in 50% VSED solution for 1 min and then transferred into VSED drops at room temperature (20–24°C); the resulting calves were normal (delivery rate 39%). Hence, a mixture of ethylene glycol and DMSO has been shown to be effective in vitrifying morula to blastocyst-stage embryos. Both ethylene glycol and DMSO are agents which can permeate a cell (Kasai, 1997). Therefore, the relatively short equilibration period (1 or 2 min) in 50% VSED probably permitted the development of intracellular conditions conducive to vitrification, while minimizing any potential toxicity of the cryoprotectant. The survival rates of vitrified embryos depend on several mechanisms of injury, e.g. the chemical toxicity of the cryoprotectant, intracellular ice formation, fracture damage, and osmotic swelling during the removal of cryoprotectant.

Recently, a case involving a unique combination of high survival and meiotic normality together with good preservation using egg yolk was described (Isachenko and Nayudu, 1999). Another author (Park *et al.*, 1999) demonstrated that higher survival of vitrified–thawed bovine blastocysts can be obtained using electron microscope grids rather than plastic straws as embryo containers during freezing. Furthermore, vitrification has another advantage in that it may improve survival if the procedure is further optimized for human blastocysts, since all of the physical and chemical injuries caused by extracellular ice are eliminated in vitrification.

The present case report demonstrated the effectiveness of a simple vitrification method using VSED solution for the cryopreservation of human blastocysts. This is the first report of a pregnancy resulting from vitrification of a human blastocyst in Japan.

**Note added in proof**

During the reviewing process of this manuscript, another two pregnancies have been achieved via vitrification vitrification out of seven patient transfers. One of these is now ongoing.

**References**


Received on January 4, 2000; accepted on May 2, 2000