Clinical pregnancy from a vitrified/warmed human blastocyst

Abstract:
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Abstract
The first pregnancy after vitrification of a human blastocyst (day 5 of embryo culture) was reported by Yokota et al. in 2000. Since then more attention has been given to the technique of vitrification and its safe application in ART. To the best of our knowledge, this is the first report of a clinical pregnancy resulting in a live birth from the transfer of a vitrified/warmed human blastocyst in the Republic of Ireland.

Introduction
Modern cryopreservation techniques in assisted reproduction technology (ART) therapy include slow freezing or vitrification. The slow freezing process can lead to the formation of ice crystals with osmotic and chilling injury resulting in low embryo survival of approximately 60-70%, however it has proven itself over time to give rise to many healthy offspring. Vitrification however, eliminates ice crystals and survival rates are excellent, between 90-95%. In this case study we present a successful clinical pregnancy and live birth following vitrification, warming and elective transfer of a single human blastocyst.

Case Report
A couple were referred for ART to the HARI unit, Rotunda Hospital with a history of secondary subfertility for 3 years. Both partners were aged 32 years of age and the male partner had testicular cancer and required a left orchidectomy in the past. They previously had 2 ICSI treatments cycles abroad resulting in no ongoing viable pregnancy. Female investigations showed an AMH level of 5.15pmol/L, BMI=20.1. An antagonist protocol was used as described elsewhere. A total of 21 oocytes were injected. Fifteen oocytes fertilised and 10 blastocysts were suitable for cryopreservation. No blastocysts were transferred on this fresh cycle as a total of 28 oocytes were collected, and it was decided that there may be a risk of ovarian hyper-stimulation (OHSS).

Following informed consent we used the simplified blastocyst vitrification protocol (Irvine Scientific Santa Ana, CA, USA) and loaded the blastocysts individually into high security closed carrier devices, (HSV Cryo Bio system, France). Vitrification was undertaken by gently expelling solution 1, (20% HTF ) in the vicinity of the blastocyst before lifting the straw onto an equilibration solution. After 10 minutes, the vitrification solution (VS) was aspirated into the pipette and then gently expelled over the blastocyst before transferring them into the drop of VS. After 30 seconds, using a micropipette, each blastocyst was carefully deposited into the straw gutter. Immediately, the capillary rod was placed into the handler and sealed. The entire straw was then plunged into liquid nitrogen.

The couple returned for frozen blastocyst replacement 2 months after their ICSI cycle. A frozen embryo transfer hRT cycle protocol was used. Folltropin (Oestradiol valerate, Bayer Schering, UK) tablets, 8mg daily were commenced on day 1 of the period and continued until day 12 when progesterone supplementation (Crinone 8%, Serono, UK) was commenced. Final cycle monitoring showed an oestradiol level of 1717 pmol/L and endometrial thickness was 6.7mm. The patient was deemed ready for replacement and consented to an elective single frozen blastocyst transfer. One blastocyst was removed from storage and warmed using Irvine vitrification warming protocol. A 500 microlitre droplet of thaw solution was placed into the straw gutter and warmed to 37°C prior to the procedure. The straw containing the blastocyst was cut and the inner holding device plunged into the thaw solution for one minute. The blastocyst was then transferred into the dilution solution for 4 minutes at room temperature. It was then pipetted into two 50 microlitres of wash solution for 4 minutes each and transferred to a dish containing culture medium (20% SSM, Irvine Scientific Santa Ana, CA, USA). A single warmed day 5 blastocyst (grade BAa) was transferred under ultrasound guidance using a Sureview Wallace catheter (Smiths Medical, UK). A positive HCG was reported two weeks post transfer and a scan at 7 weeks gestation showed an intrauterine sac with a singleton viable fetus. A healthy male, weighing 3550 grams was delivered at 40 weeks gestation.

Discussion
To our knowledge this is the first reported clinical pregnancy resulting in a live birth from a vitrified/thawed blastocyst after ART therapy in Ireland. Vitrification of human reproductive material is an established technique which minimises cell injury and improves survival rates. The investigation into and subsequent introduction of the technique of vitrification into the HARI unit, has shown it to be a simple procedure but above all a reliable, successful and safe one.

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References