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Human Blastocysts Developed From Thawed Zygotes: Refreezing Does Not Affect Their Survival Or Implantation

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Table 1

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Abstract

The thawing of frozen human zygotes and culture to the blastocyst stage often generates supernumerary blastocysts which can be refrozen. A total of 9 recipients of donated oocytes and 35 women originally at risk of ovarian hyperstimulation syndrome had such blastocysts thawed. There were respective survival rates of 72.0% and 66.7%, and on-going pregnancy rates of 23.5% and 18.8% after transfer, which did not differ from the general IVF population of women receiving thawed blastocysts generated from fresh zygotes. The refreezing of blastocysts from thawed zygotes can therefore be undertaken with confidence.

Introduction

Human embryos within in vitro fertilisation (IVF) programmes have often been frozen in the past as zygotes (ie pronuclear oocytes) because of their apparent superior survival rates [4, 9]. This stage is particularly suited to the “freeze-all” strategy in which all embryos are frozen and the transfer is postponed, as in cases at risk of ovarian hyperstimulation syndrome (OHSS) [3] or where fertilised donated oocytes are to be quarantined [8]. However, the relatively recent availability of commercially prepared sequential media and the extended culture of embryos to the blastocyst stage has resulted in increased pregnancy rates per embryo transfer [10], and the improved selection of high quality embryos to maintain an effective single embryo transfer policy to reduce the risks of multiple pregnancy [7]. An increase in the proportion of laboratories undertaking blastocyst culture has occurred over the last 5 years [21].

Laboratories that have frozen zygotes in storage but now use extended culture are presented with a dilemma at thawing: how many zygotes should be thawed and cultured on? Unfortunately, less than half of the embryos will become good quality blastocysts [14, 20] and some patients may even have none form for transfer [19]. It would therefore seem prudent to thaw a sufficient number of zygotes to ensure a reasonable chance of getting at least one blastocyst for transfer, although there is then risk of having too many blastocysts formed if a maximum of only one or two are transferred [15]. In such cases, the refreezing of supernumerary blastocysts would seem a viable option as indicated by an isolated case report [5]. The present report describes our clinical experience of thawing frozen blastocysts derived from thawed zygotes for women that were initially at risk of OHSS or recipients of donated oocytes.

Methods

Women treated between 2000 and 2007 were included if their frozen/thawed embryos were cultured to the blastocyst stage and supernumerary blastocysts refrozen. These were either:

a) Fertility patients who had all embryos frozen because the serum estradiol was >20,000 pmol/l or >20 oocytes were collected, putting them at risk of developing OHS.

b) Oocyte recipients whose donated oocytes had been fertilised, cryopreserved and stored for at least six months before use due to quarantine restrictions.

All embryos were stored with standard operating procedures and according to West Australian law. Embryos were initially frozen and thawed as zygotes using a previously published slow rate freezing protocol with sucrose and propoanediol as the main cryoprotectants [11]. Up to 7 zygotes were thawed at any one time. Following culture for a further 4-5 days and the transfer of a maximum of two embryos, supernumerary blastocysts were frozen using glycerol as the principle cryoprotectant [2]. In subsequent cycles, blastocysts were thawed on the day of transfer and embryo transfers were performed under ultrasound-guidance when the patient had a half-full bladder. Serum hCG was measured 12 days after the embryo transfer, with a positive pregnancy test result being recorded if the hCG concentration >25iu/l. The pregnancies were monitored weekly by serum hCG, estradiol and progesterone, and an ultrasound performed at 7 weeks to confirm the presence of a fetal heart.

Proportions were compared with the $\chi 2$ test, and Yates’ correction for continuity was applied if >20% of

expected frequencies were

Results

The fate of the refrozen supernumerary blastocysts upon thawing is shown in Illustration 1. Comparison of the performance of refrozen blastocyst from donated oocyte recipients and patients originally at risk of OHS showed that there was a similar survival rate of blastocysts ($\chi 2=0.3$, $p=0.62$) and incidence of pregnancy after transfer ($\chi 2=0.0$, $p=0.94$). There were also no significant differences in survival and pregnancy rates when compared to patients with once frozen blastocysts.

Discussion

Many IVF laboratories will have cryopreserved zygotes in storage, largely from cases at risk of developing OHSS or from recipients of donated oocytes. Furthermore, there are some countries whereby the local legislation prevents the cryopreservation of embryos and so freezing of zygotes is undertaken prior to syngamy, eg Germany [12] and Italy [13]. The technology for cryopreservation of zygotes is fairly robust and effective, but the difficulty really comes at thawing when deciding the number to thaw for any given woman. It is important for the embryos and the uterine environment to be synchronised and so more embryos cannot be thawed in that cycle should the thawed zygotes fail to develop to the blastocyst stage. One solution is to thaw several zygotes, culture, choose the best for transfer, and then refreeze the remainder. Such an approach would seem feasible based on an earlier case report in which supernumerary blastocysts following the thaw and culture of cryopreserved zygotes can be refrozen [5].

The present study has confirmed that freezing zygotes with propanediol as the principle cryoprotectant, followed by refreezing the blastocysts using glycerol, gives good results in a cohort of women attending the Hollywood Fertility Centre. The refreezing strategy should also be suitable for other combinations of developmental stages and cryoprotectants as has been suggested by a number of isolated case reports, although they should be confirmed by larger studies. These case reports include thawed zygotes refrozen [1], early cleavage embryos thawed and refrozen at blastocyst [6], thawed blastocysts refrozen [16], thawed zygotes refrozen at the morula or blastocyst stage [22], or thawed early cleavage embryos refrozen as blastocysts [18]. The increased efficiency of vitrification of blastocysts [17] will make this strategy more attractive.

In conclusion, the freezing of zygotes using propanediol and then the refreezing of blastocysts with glycerol can be undertaken with confidence.

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Illustrations

Illustration 1

Table 1

Table 1. The fate of frozen blastocysts after thawing. Once frozen blastocysts were from IVF patients having supernumerary blastocysts frozen. Refrozen blastocysts were developed from thawed zygotes for women who were either recipients of donated oocytes or fertility patients deemed at risk of ovarian hyperstimulation (OHSS) in the original IVF cycle.

	Once frozen	Refrozen	
	IVF patients	Recipients	OHSS
No. women	430	9	35
No. thaw cycles	789	19	57
No. blastocysts:			
- thawed	1137	25	81
- survived/transferred	757 (66.6%)	18 (72.0%)	54 (66.7%)
No. transfers	709	17	48
No. +ve pregnancy tests	191	4	10
No. on-going pregnancies	144 (20.3%)	4 (23.5%)	9 (18.8%)

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