Frozen-thawed transfer cycles: are they comparable with fresh?

AUTHOR(S)

Edgar Mocanu, Evelyn Cottell, K Waite, Bernadette Hennelly, Claire Collins, Robert F. Harrison

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EV Mocanu[1], E Cottell[1], K Waite[1], B Hennelly[1], C Collins[3], RF Harrison[1,2]

[1]Human Assisted Reproduction Ireland, Rotunda Hospital, Dublin 1
[2]Royal College of Surgeons in Ireland, Dublin 2
[3]Rotunda Hospital, Parnell St, Dublin 1

Abstract
Cryopreservation of zygotes and subsequent thaw and transfer is an established ART treatment. We assessed if success rates with frozen-thawed (day 2) zygotes are comparable with the outcome in fresh cycles of treatment. We performed a prospective follow-up and analysis of all frozen (FZT) and fresh cycles of treatment during a 12 months period. One hundred and nineteen patients in the frozen-thawed and 652 in the fresh group had a transfer. The overall thaw-survival rate was 71.7%. Clinical pregnancy rates per thaw and transfer were respectively 15.1% and 21% in the frozen and 29.1% (per transfer) in the fresh group. Implantation rates in fresh and frozen cycles were 16% and 12.3% respectively. The pregnancy loss rate was higher in the FZT group (29% vs. 18.3%). Cryopreservation of good quality zygotes, after fresh transfer offers optimal success rates in subsequent frozen treatment. It also encourages consideration of elective single zygote transfers.

Introduction

The first pregnancy from frozen-thawed embryos was reported in 1983[1]. The following year, Zeilmaker et al.[2] reported the first baby born from a frozen-thawed embryo. Many studies[3-5], as well as the long-standing experience with the technique have shown that the procedure is safe and the incidence of fetal abnormalities is the same as that in fresh cycles of Assisted Reproductive Technology (ART).

One complication of ART treatment is the high incidence of multiple pregnancies, directly related to the number of zygotes transferred. There is a recent trend[6,7,8] towards single zygote transfer (both fresh and frozen) to reduce the incidence of multiple pregnancies. This pursuit would not be possible without the existence of safe cryopreservation processes and reliable scientific methods to identify suitable zygotes for storage.

The aim of our paper was to analyse the outcome and compare success rates in frozen-thawed (day 2, 2-6 cells zygotes) cycles with fresh cycles of treatment during a 12 month period. The data obtained allowed for

adequate counselling of subsequent patients at the time of their initial fresh cycle of treatment, when the decision to transfer as well as freeze is taken and also prior to their frozen cycle.

Methods

This study reviews data available on all Frozen Zygote Transfer (FZT) cycles of treatment carried out over a 12 months period (1.1.2001 and 31.12.2001) in the Human Assisted Reproduction Ireland Unit. All completed fresh cycles of ART during the same time period have been analysed for comparison. We considered a difference in excess of 10.5% as clinically significant in terms of success rates between fresh and FZT treatment. Hence the sample size required, based on the power to detect an absolute difference of 10.5% and given an expected pregnancy rate of 25% in the fresh treatment were 760 patients in the fresh treatment group and 152 patients in the frozen. All treatment data is entered routinely, prospectively in a register and also in a Computerised Database (Filemaker Pro for Macintosh).

The two types of medical treatment offered in the HARI Unit for couples who have zygotes stored and wish transfer are natural and hormonal replacement therapy (HRT) controlled. Natural cycle frozen zygote transfer is suggested to patients who have regular periods and where two or less zygotes are frozen. The aim of treatment is to monitor follicular growth and confirm ovulation after which time the zygotes are thawed and the ones that cleaved are transferred following discussion with the couple. Luteal support in the form of progesterone vaginal pessaries 400µg b.d. (Cyclogest, Shire, UK) was given to women with low day 21 progesterone levels. The advantage of this treatment consists in avoidance of intense medical intervention when the chances of zygote survival are poor (50%). HRT controlled frozen zygote transfer cycles of treatment involved down regulation with a GnRH analogue, Buserelin acetate (Suprecur, Aventis, Ireland) 1.2mg nasal spray 6 hourly starting on day one of a period followed by stimulation of the endometrium carried out with oestradiol valerate oral tablets (Progynova, Schering, UK) in a dose of 6mg daily. The dose of GnRH analogue was reduced in the second part of the cycle.
The advantage of HRT controlled treatment lies in better control of both anatomical and biochemical milieu prior to thawing of good quality zygotes. The zygotes were thawed the day before transfer and allowed to cleave, three being the maximum number transferred. Daily hormonal treatment was continued after transfer in the form of oestradiol valerate oral tablets (Progynova, Schering, UK) 6mg daily and progesterone vaginal pessaries 400µg b.d. (Cyclogest, Shire, UK) or vaginal gel 1.125g b.d. (Crinone 8%, Serono Pharmaceuticals, UK). Positive pregnancy test patients were scanned 3 weeks after to confirm an intrauterine pregnancy.

A clinical pregnancy was defined as the presence of an intrauterine sac with a viable fetus at 5 weeks post transfer. A biochemical pregnancy was diagnosed when vaginal bleeding followed an initial positive pregnancy test and the uterus was empty at the time of scheduled scan (5 weeks post transfer). The implantation rate was defined as the number of gestational sacs per 100 zygotes transferred. Absence of a fetal pole or absence of fetal cardiac activity at 5 weeks post transfer scan diagnosed a miscarriage, while a pregnancy implanted outside the uterine cavity was termed ectopic. Pregnancy loss represented the sum of biochemical pregnancies, ectopic pregnancies and miscarriages.

Statistical analysis

The parameters analysed were: number of cycles of thawing, percentage of zygotes surviving thaw, clinical pregnancy rates per thaw and transfer, success rates according to number of zygotes transferred, implantation rates, incidence of multiple pregnancy in fresh and frozen cycles and final pregnancy outcome from frozenthawed cycles. The data of the frozen transfers has been analysed separately as natural and HRT controlled and also as a whole. Statistical analysis was performed using chi-square test with $p<0.05$ considered significant.

Results

Number of thaws

One hundred and sixty-six cycles of thawed zygotes (2-6 cells at freeze) were performed in the HARI Unit, Rotunda Hospital during the studied period, with 119 patients (71.7%) proceeding with a zygote transfer. Because the vast majority of the frozen cycles (87%, n=144) were controlled by HRT and only 13% (n=22) were natural, comparison between natural and HRT was precluded and as the indication for one treatment over the other could bias the data the two groups were taken as one. During the same period, 790 fresh cycles of ART were initiated, of which 652 had a zygote transfer (82%).

Table 1 Clinical pregnancy rates in FZT and fresh cycles

Cycle	Thaws	Transfers	Positive hCG	Clinical/thaw or cycle started	Clinical/ transfer
Natural	22	10	3 (30%) (8.3%–62.0%)	3 (13.6%) (3.6%-32.8%)	3 (30%) (8.3%–62.0%)
HRT	144	109	32 (29.4%) (21.4%-38.4%)	22 (15.3%) (10.1%-21.9%)	22 (20.2%) (13.4%-28.5%)
Total FZT	166	119	35 (29.4%) (21.8%-38.1%)	25 (15.1%) (10.2%-21.1%)	25 (21%) (14.4%-29.0%)
FRESH	–	652	225 (34.5%)[a] (30.8%-38.1%)	190 (24%) (20.5%-27.6%)	190 (29.1%)[b] (25.8%-32.7%)

Note: NS = not significant.
a,b Total FZT vs. FRESH
a,b p = NS

Table 2 Clinical pregnancy rates per number of zygotes transferred

All frozen cycles	1 zygote	2 zygotes	3 zygotes
Transfers	36 (30.3%)	30 (25.2%)	53 (44.5%)
Positive hCG /transfer	5 (13.9%) (5.3%-28.1%)	9 (30%) (15.7%-48.0%)	21 (39.6%) (27.2%-53.2%)
FZT Clinical rate	3 (8.3%) (2.2%-21.0%)	6 (20%) (8.5%-37.0%)	16 (30.2%) (19.0%-43.5%)
Fresh Clinical rate	4/60 (6.7%)[a] (2.2%-15.3%)	89/266 (33.5%)[b] (28.0%-39.3%)	97/326 (29.8%)[c] (25%-34.9%)

Note: NS = not significant.
a,b,c FZT clinical rate vs. Fresh clinical rate.
a,b,c p = NS.

Thaw survival
In 119 of the 166 thaws zygotes were available for transfer (71.7%). The figure was higher in HRT controlled cycles (109/144; 75.7%) compared with the natural ones (10/22; 45.5%).

Pregnancy rates
Pregnancy rates for the two groups of treatment are presented in Table 1. We present the results from FZT overall and separately, as the selection criteria for the two groups are very different. Clinical pregnancy rates per transfer in frozen cycles (21%) are comparable with the ones in fresh cycles of treatment (29%). As the confidence intervals show (Table 2), there is considerable overlap between the groups in terms of pregnancy rates, thus the differences do not reach significance. The pregnancy rates per number of zygotes transferred are detailed in Table 2. This shows that even when the clinical
pregnancy rates are analysed according to the number of zygotes transferred the success rates are similar in fresh and frozen cycles. Implantation rates in fresh and frozen zygote transfer groups were 16% (252/1567) and 12.3% (31/252) respectively.

Multiple pregnancy
We analysed the incidence of multiple pregnancies in FZT and fresh cycles and correlated the chance of a multiple pregnancy according to the number

of zygotes transferred, Table 3. An increased risk of multiple pregnancy in the 3 zygote transfer FZT group was noted.

Pregnancy loss rates

Pregnancy loss rates were calculated per positive hCG (Table 4). This shows a higher risk of pregnancy loss in FZT cycles compared to fresh ones mainly due to a high biochemical pregnancy rate, although this did not reach significance.

Pregnancy outcome

Of the 25 clinical pregnancies after frozen-thawed transfers 24 had a successful outcome with 30 babies delivered. One patient miscarried at 11 weeks. Both sets of twins and triplets delivered at 37 weeks. There were 13 boys and 17 girls delivered. No cases of congenital abnormalities were recorded. In fresh cycles, of the 190 clinical pregnancies 132 were singleton, 54 sets of twins and 4 sets of triplets. Of clinical pregnancies in fresh cycles of treatment 23 (12%) did not proceed to delivery. A total of 226 babies were born after 24 weeks. There were 106 girls and 118 boys. Of the four sets of triplets 2 delivered as twins and one set of triplets.

Discussion

Freezing has many advantages and indeed is now considered as essential safety element for all those practising IVF/ICSI[9]. It reduces the risk of multiple pregnancy allowing a low order zygote transfer with cryopreservation of remaining good quality zygotes. A freeze all protocol in high-risk patients avoids the risk of late (pregnancy related) ovarian hyperstimulation. Fresh transfers are also avoided where implantation may be jeopardised during treatment as in uterine bleeding, unfavourable endometrium, endometrial polyps and technically impossible zygote transfers. All couples are provided with a further chance of pregnancy by maximising the number of zygote transfers per oocyte retrieval and in cases of patients with an indication for single zygote transfer such as congenital uterine abnormalities, cryopreservation allows use of zygotes in subsequent cycles of treatment.

Table 3 Multiple pregnancy rates according to the number of zygotes transferred (FZT)

No. Zygotes transferred Pregnancy	1	2	3	Total
Singleton (frozen) *Singleton (fresh)*	3/3 (100%) 3/4 (75%)	6/6 (100%) 64/89 (72%)	12/16 (75%) 65/97 (67%)	21/25 (84%) 132/190 (69.5%)
Twins (frozen) *Twins (fresh)*	0/3 1/4 (25%)	0/6 24/89 (27%)	2/16(12.5%) 29/97 (30%)	2/25 (8%) 54/190 (28.5%)
Triplets (frozen) *Triplets (fresh)*	0/3 0/4	0/6 1/89 (1%)	2/16 (12.5%) 3/97 (3%)	2/25 (8%) 4/190 (2%)

Table 4 Pregnancy loss rate					
Cycle	**Positive test**	**Miscarriage**	**Biochemical**	**Ectopic**	**Pregnancy loss**
Natural	3	0	0	0	0
HRT	32	4 (12.5%) (4.1%-27.5%)	5 (15.6%) (6.0%-31.3%)	1 (3.1%) (0.2%-14.5%)	10 (31.3%) (17.1%-48.7%)
Total FZT	35	4 (11.4%) (3.7%-25.3%)	5 (14.3%) (5.4%-28.9%)	1 (2.9%) (0.1%-13.3%)	10 (29%) (15.5%-45.1%)
Fresh	224	23 (10.3%)[a] (6.8%-14.8%)	11(4.9%)[b] (2.6%-8.4%)	1 (0.5%)[c] –	35 (15.6%)[d] (11.3%-20.8%)

Note: NS = not significant
a,b,c,d Total FZT vs. Fresh
a,c,d p = NS
b p <0.05

In terms of the cost effectiveness of ART it has been shown that the cost of frozen embryo transfers are approximately one quarter of those associated with repeat IVF cycles[10]. Thus, as others6, we see a lot of scope in freezing all good quality supernumerary zygotes in order to maximise the chance of pregnancy from a single oocyte retrieval. This is based not only on financial grounds but also on emotional trauma, comfort and patient satisfaction points of view.

Because of the low numbers of patients having natural cycles and also the significant reasons why natural or HRT treatments were chosen it is not possible to compare one with the other and draw firm conclusions, although interesting trends are noted when the two are consolidated together as frozen transfers for comparison against the fresh.
Twenty-eight percent of the thawed frozen zygotes in our study did not reach the stage of a transfer. This is similar to previous reports in the literature[11,12,13]. The initial assessment of suitability for either Natural of HRT treatment is very important. The correct assessment of survival chance can facilitate allocation of less invasive treatment to patients with a predicted poor zygote survival. As previously shown[14], morphological zygote quality is the most important factor for successful implantation of cryopreserved zygotes, thus our policy of suggesting natural cycles for couples with poorer quality zygotes is justified although the numerical imbalance in the natural versus HRT precludes analysis. Indeed of the 10 natural transfers 30% had clinical pregnancies while in the 109 HRT transfers 20.2% had clinical pregnancies.
The outcome in frozen thawed cycles of treatment is comparable with the one in fresh cycles where transfers were carried out (21% vs. 29%) but because of the loss at thawing, is halved when the data is analysed per cycle started (27% vs.15%). Nevertheless, our results show that natural cycle FZT is a desirable and successful treatment option in a selected group of patients.

The overall pregnancy loss rate was 29% for FZT cycles and 18.3% for fresh cycles. It is interesting to note that miscarriage and ectopic pregnancy rates were similar in the two groups. The biochemical pregnancy rates were significantly different but low numbers preclude firm conclusions. This finding suggests the zygotes had the potential to grow and implant but the early development was defective. The reasons are only speculative but could involve poorer quality zygotes left after initial fresh transfer when "the best" are selected, traumatic freeze-thaw process, physical damage due to ice crystals or indeed genetic causes.

The old myth of significantly poor success rates with frozenthawed zygotes compared with fresh treatment has no support. More so, there are obvious clinical and financial benefits from offering frozen-thawed zygote treatment to our patients and they need to be informed about them. The implementation of a single zygote transfer programme depends on the capacity to cryopreserve available good quality zygotes. Since the year 2003, the HARI Unit now replaces only a maximum of 2 zygotes in both fresh and frozen cycles if the female partner is less than 40 years old, in keeping with current best practices. Despite this policy, success rates have stayed the same.
The fact that pregnancy rates are similar in fresh and frozen cycles makes single zygote transfer and cryopreservation of surplus zygotes a financially and clinically attractive alternative to multiple zygote transfers in good prognosis patients.

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Author's Correspondence
EV Mocanu
Human Assisted Reproduction Ireland, Rotunda Hospital, Parnell St, Dublin 1 Tel: 01 807 2732 E-mail: emocanu@rcsi.ie

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