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Objective To review the outcomes of frozen-thawed embryo transfer cycles.

Design Retrospective review.

Setting Tertiary assisted reproduction centre, Hong Kong.

Patients Subfertile patients undergoing frozen-thawed embryo transfer between July 2005 and December 2007.

Main outcome measures Clinical and ongoing pregnancy rates.

Results A total of 983 frozen-thawed embryo transfer cycles performed during the study period were reviewed. The clinical pregnancy and ongoing pregnancy rates were 35% and 30%, respectively. Factors associated with successful outcome included younger maternal age (≤ 35 years) and 4 or more blastomeres at replacement, but not the method of insemination, the cause of subfertility, or the type of frozen-thawed embryo transfer cycle. The overall multiple pregnancy rate was 18%. For cycles with a single embryo replaced, embryos having 4-cell or higher stages at replacement gave an ongoing pregnancy rate of 25%, whereas those with less than 4 cells had a significantly lower ongoing pregnancy rate of 5% only. Blastomere lysis after thawing significantly reduced the clinical pregnancy and ongoing pregnancy rates of cycles with one embryo replaced.

Conclusions Clinical pregnancy and ongoing pregnancy rates of frozen-thawed embryo transfer cycles were 35% and 30%, respectively. Higher pregnancy rates were associated with younger maternal age (≤ 35 years), blastomere numbers of 4 or more, and no blastomere lysis after thawing.

Introduction

In order to reduce the chance of multiple pregnancy during in-vitro fertilisation (IVF)-embryo transfer treatment, there is an increasing trend towards transferring a lower number of embryos. Surplus good-quality embryos are then transferred in frozen-thawed embryo transfer (FET) cycles. The first pregnancy after FET was reported in 1983.¹ It has been estimated that in IVF programmes in which embryo cryopreservation is implemented, up to 42% of all implantations could be derived by FET.² Elective cryopreservation of all fresh embryos is also required in special circumstances where fresh embryo transfer is undesirable. Examples of the latter include: patients in whom the risk of ovarian hyperstimulation syndrome (OHSS) is high, or those involving oocyte or embryo donations where a quarantine period is advisable.

In our centre, the FET service was first introduced in 1992. The aim of this retrospective analysis was to review current pregnancy outcomes of FET cycles performed in the centre and the factors affecting outcomes.

Methods

All FET cycles using non-donor oocytes carried out between July 2005 and December 2007 at the Centre of Assisted Reproduction and Embryology, The University of Hong Kong, Queen Mary Hospital were reviewed. This review excluded: embryos transported from external centres, embryos derived from donated oocytes or embryos, and blastocyst transfers (including those after pre-implantation genetic diagnosis). Data were extracted from our computerised database on which information on every assisted reproduction treatment cycle was stored. Details of the long protocol pertaining to the ovarian stimulation regimen, gamete handling, assessment of embryo quality, embryo transfer,

Key words

Cryopreservation; Embryo transfer; Fertilization in vitro

Hong Kong Med J 2009;15:420-6

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and FET at our centre have been published.³

Stimulated cycles

All women were pre-treated for pituitary down-regulation with busereelin (Suprecur; Hoechst, Frankfurt, Germany) nasal spray 150 µg four times a day from the mid-luteal phase of the cycle preceding the stimulation cycle, and received human menopausal gonadotrophin or recombinant follicle-stimulating hormone for ovarian stimulation. The gonadotrophin-releasing hormone antagonist protocol was used instead of the long protocol in selected patients who had poor ovarian reserve. Human chorionic gonadotrophin (hCG) was given intramuscularly when the mean diameter of the leading follicle reached 18 mm and there were at least three follicles having a mean diameter of 16 mm or more.

Transvaginal ultrasound-guided oocyte retrieval was carried out 36 hours after the hCG triggering. Fertilisation was carried out in-vitro either by conventional insemination or intracytoplasmic sperm injection (ICSI) depending on the semen parameters and previous fertilisation history. Patients were allowed to have a maximum of two embryos replaced into the uterine cavity 2 days after oocyte retrieval. Embryo transfer was performed under transabdominal ultrasound guidance. Prior to embryo transfer, embryos were examined for the number/regularity of blastomeres and the degree of fragmentation, and graded according to the criteria of Veeck.⁴ To reduce the risks of OHSS, all fresh embryos with good quality were cryopreserved whenever patients developed symptoms suggestive of OHSS or the serum oestradiol concentration on the day of hCG injection exceeded 20 000 pmol/L.

Cryopreservation and thawing protocols

Excess good-quality (grades 1 to 4) embryos were frozen on the day of embryo transfer, that is, the second day post-insemination, whereas embryos of poor quality (grades 5 and 6) were discarded. Grade 1 embryos had equal-sized blastomeres and no cytoplasmic fragments. Grade 2 embryos had equal-sized blastomeres and less than 25% fragmentation. Grade 3 embryos had blastomeres of distinctly unequal size and no cytoplasmic fragments. Grade 4 embryos had blastomeres of distinctly unequal size and less than 25% fragmentation. About 60% of our stimulated IVF cycles had embryo(s) available for cryopreservation.

A slow-freezing protocol using a programmable freezer (Planer Products Ltd, Sunbury-On-Thames, UK) was adopted, which employed 1,2-propanediol (1.5 M) and sucrose (0.1 M) as cryoprotectants. The embryos were frozen in straws. Each straw contained

凍融胚胎移植週期技術

目的	回顧凍融胚胎移植週期技術的結果。
設計	回顧研究。
安排	香港一所生育轉介中心。
患者	2005年7月至2007年12月期間，接受凍融胚胎移植的不育患者。
主要結果測量	臨床妊娠率和繼續妊娠率。
結果	研究期間進行了983次凍融胚胎移植，臨床妊娠率為35%，繼續妊娠率為30%。與成功受孕有關的因素包括孕婦較年青（35歲或以下），以及在移植階段時卵裂球含4個或以上的細胞；但授精方法、不育原因、和凍融胚胎移植週期技術的種類並無關係。總多胎率為18%。接受單一胚胎移植的人，胚胎中含4個或以上細胞卵裂球的繼續妊娠率為25%，而胚胎中含4個以下細胞卵裂球的繼續妊娠率明顯較低，只有5%。接受單一胚胎移植的人中，解凍後破裂的卵裂球明顯地減少了臨床及繼續妊娠率。
結論	凍融胚胎移植週期的臨床妊娠率為35%，繼續妊娠率為30%。與成功受孕有關的因素包括孕婦較年青（35歲或以下）、卵裂球含4個或以上的細胞，以及解凍後卵裂球沒有分裂。

one or two embryos, except for those cryopreserved before mid-2005, where some might have been frozen in threes. The freezing programme consisted of four steps: (1) cooling from 20°C to -7°C at a rate of -2°C per minute; (2) soaking for 5 minutes followed by seeding and soaking for a further 10 minutes; (3) cooling from -7°C to -30°C at a rate of -0.3°C per minute; (4) cooling from -30°C to -120°C at a rate of -30°C per minute. The straws were then immersed directly into liquid nitrogen for storage.

The frozen embryos were thawed on the morning of FET, which entailed removal from the liquid nitrogen and exposure to air for 40 seconds, followed by immersion in a 30°C water bath for another 40 seconds. Propanediol was then removed by washing in 1.0 M propanediol and 0.2 M sucrose solution in two steps, 5 minutes each, followed by final rehydration in phosphate-buffered saline for at least 10 minutes. Embryo survival, defined by lysis or degeneration of less than 50% of the original blastomeres, was then checked microscopically. Embryos not fulfilling this criterion were discarded.

Frozen-thawed embryo transfer

Frozen-thawed embryos were transferred in natural cycles for women having regular ovulatory cycles. During such times, the patients were monitored daily for serum oestradiol and luteinizing hormone (LH) concentrations from 18 days before the expected date of the next period. Frozen-thawed embryo transfer was performed on the third day after the LH surge.

For patients with irregular menstrual cycles or without ovulation demonstrated during natural cycle monitoring, clomiphene citrate (Clomid; Merrell, Staines, UK) 50 to 150 mg was given daily for 5 days from days 3 to 7. The cycle was monitored by blood tests from day 10 of the cycle and FET was arranged as detailed above. Hormonal replacement cycles were offered to patients who showed no ovulatory responses after taking 150 mg of clomiphene citrate daily for 5 days. After downregulation by buserelin nasal spray (150 µg four times a day), treatment with 6 mg of oestradiol (Estrofem; Novo Nordisk, UK) daily was started from the third day of the next menstrual cycle. Buserelin was discontinued and 400 mg of progesterone (Cyclogest; Cox Pharmaceuticals, Barnstaple, UK) vaginal pessaries were given twice daily if endometrial thickness measured 8 mm or more by ultrasound scanning on day 16 of the cycle. All FET procedures were carried out on the fourth day of progesterone therapy.

In our centre, a policy of allowing the transfer of no more than two frozen embryos in any one cycle was enforced from mid-2005, except for patients with three embryos cryopreserved in each straw (prior to the implementation of two-embryo policy). All FETs were carried out using a soft transcervical catheter (Sydney IVF Embryo Transfer Catheter, Cook, US) under transabdominal ultrasound guidance. A urine pregnancy test was performed 16 days after FET. If it was positive, ultrasound examination was performed 10 to 14 days later to confirm an intrauterine pregnancy and determine the number of gestational sacs.

Statistical analysis

The primary outcome measures were clinical pregnancy rate (CPR) and ongoing pregnancy rate (OPR). Clinical pregnancies were defined by the presence of one or more gestation sacs or the histological confirmation of a gestational product in case of early pregnancy failures. Ongoing pregnancies were those prevailing beyond 8 to 10 weeks of gestation, at which stage the patients were referred to the antenatal care.

Statistical comparison was carried out by the χ^2 test using the Statistical Package for the Social Sciences (Windows version 15.0; SPSS Inc, Chicago [IL], US). A two-tailed P value of less than 0.05 was considered statistically significant.

Results

During the study period, 1995 frozen embryos were transferred to 608 patients in 983 FET cycles. In 614 (62%) cycles, embryos were derived from conventional insemination, whereas in 369 (38%) cycles embryos were fertilised by ICSI. In 64 of the latter (ie 6.5% of the 983 cycles), surgically

retrieved sperms were used. Male-dependent and tubo-peritoneal factors were the primary causes of subfertility, accounting for 50% and 24% of the cases, respectively. Endometriosis accounted for 9% of instances and in 8% subfertility was unexplained. Approximately 10% were due to mixed or other factors. The mean age of the cohort was 36 (standard deviation, 4; range, 22-45) years. The mean number of embryos transferred per FET cycle was 2 (range, 1-3), and the number of resultant gestational sacs was 443. A total of 348 clinical pregnancies and 297 ongoing pregnancies were attained, giving an overall CPR of 35% and OPR of 30% per transfer. There were 63 multiple pregnancies, including 55 twins and 8 triplets; the overall multiple pregnancy rate was 18% per clinical pregnancy attained. In all, 10 FET cycles were abandoned because of lysis of all embryos.

There were no significant differences in CPR and OPR among patients related to the various causes of subfertility, and the insemination method (conventional vs ICSI) used (Fig 1). No significant differences in CPR and OPR were observed among natural, clomiphene-induced, or hormone replacement cycles (Fig 2).

Age of women and the pregnancy outcome

In younger women (aged ≤ 35 years), FET was associated with significantly higher CPR and OPR rates ($P < 0.05$). Those who were older had a significantly higher miscarriage rate ($P < 0.05$) [Table 1]. When only cycles with double FETs were analysed, the same significant association with lower rates of CPR ($P = 0.0098$, χ^2 test for trend) and OPR ($P = 0.005$, χ^2 test for trend) were evident with older maternal age at FET.

Number of embryos transferred and the pregnancy outcome

A higher number of FETs was associated with a significant increase in rates of clinical pregnancy, ongoing pregnancy, and multiple pregnancy. Significant differences in CPR and OPR were found between replacing one and two embryos only, but not between transferring two and three embryos. The miscarriage rate was similar among the groups (Table 2). Older age-groups were associated with a significantly higher number of embryos transferred ($P < 0.001$, χ^2 test).

Blastomere number at embryo replacement and the pregnancy rate

As more than a single embryo was usually replaced, the one having the highest blastomere number was counted. A blastomere number of 4 or more was associated with a significantly higher CPR ($P < 0.001$) and OPR ($P < 0.001$), as well as a lower miscarriage rate

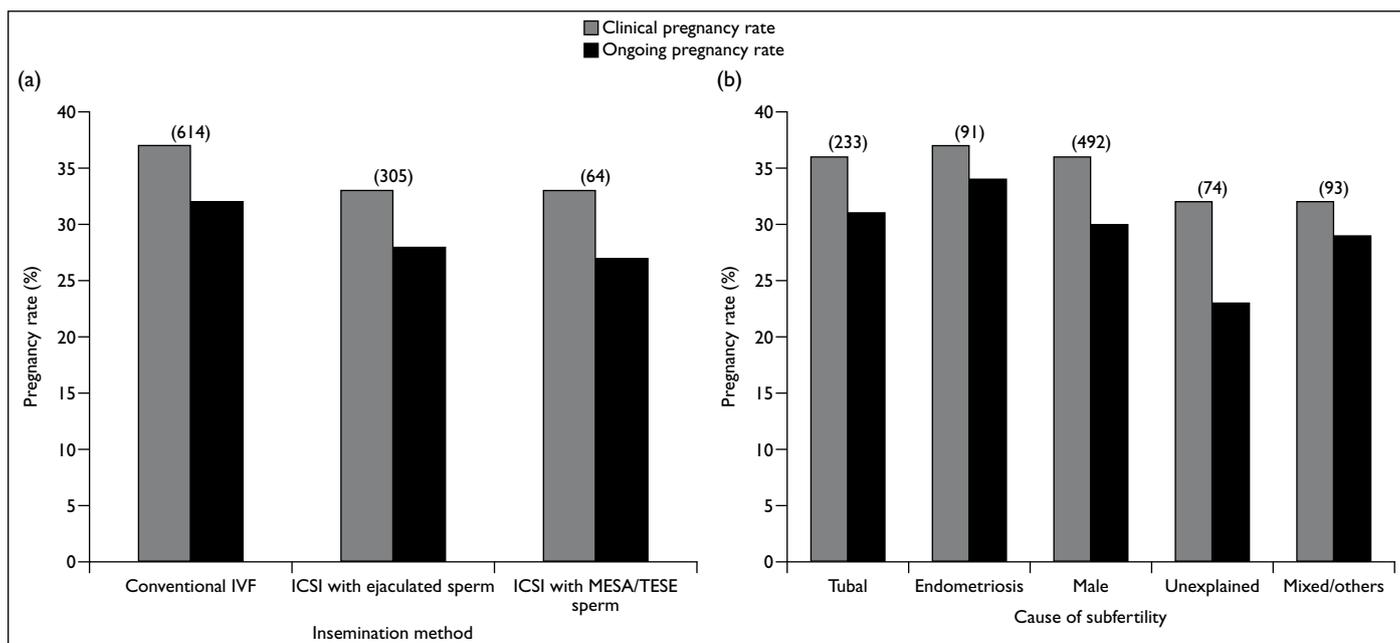


FIG 1. Pregnancy outcome with (a) different methods of insemination and (b) different causes of subfertility

Figures in brackets are the total numbers of transfers in the respective groups

IVF denotes in-vitro fertilisation; ICSI intracytoplasmic sperm injection; MESA/TESE sperm sperm retrieved by microsurgical sperm aspiration and/or testicular sperm extraction

(P=0.001) [Table 3]. There was no significant trend with respect to maternal age or the most advanced cell stage at FET (P=0.5334, χ^2 test for trend).

Frozen-thawed embryo transfer cycles with single embryo replaced

During the study period, there were 125 FET cycles with single embryos replaced, which resulted in 26 (21%) clinical pregnancies of which 21 (17%) were ongoing. Both the CPR and OPR were significantly higher when the blastomere number was 4 or higher at replacement, and if no blastomere was lysed upon thawing. Both CPR and OPR were not correlated with embryo grading at freezing (Table 4). There appeared to be a trend towards higher pregnancy rates in which the cell stage was 4 or more at freezing and in women aged 35 years or less, and yet statistical significance was not reached probably because the small numbers rendered the analysis underpowered.

Discussion

We reviewed our FET cycles from July 2005 onwards, because we had enforced a policy of replacing a maximum of two frozen-thawed embryos in newly initiated cycles since then. Our CPR and OPR figures of 35% and 30%, respectively, compared favourably with other reports. For instance, the most recently reported CPRs and live birth rates from FET in the United States were 35.1% and 27.7% respectively,⁵ whereas in Europe they varied widely with the best

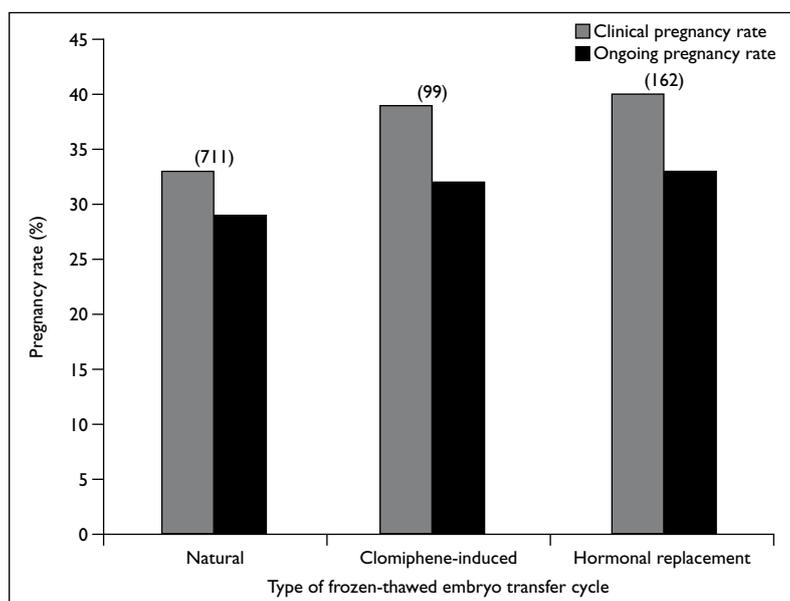


FIG 2. Types of frozen-thawed embryo transfer cycle regimens and pregnancy outcomes

Figures in brackets are the total numbers of transfers in the respective groups

reported pregnancy and live birth rates both being around 30%.⁶

Our analysis revealed a higher pregnancy rate for FETs associated with (1) younger maternal age, (2) higher numbers of embryos transferred, and (3) blastomeres with 4 cells or more at replacement, but was independent of the method of insemination

TABLE 1. Maternal age at frozen-thawed embryo transfer (FET) and pregnancy outcomes

Outcome	Age of women at FET (years)				P value (χ^2 test for trend)	P value (≤ 35 vs > 35 years)
	<31	31-35	36-40	>40		
Clinical pregnancy rate	40/87 (46%)	141/370 (38%)	155/470 (33%)	12/56 (21%)	0.001	0.01
Ongoing pregnancy rate	35/87 (40%)	126/370 (34%)	125/470 (27%)	11/56 (20%)	<0.001	0.001
Miscarriage rate	5/40 (13%)	15/141 (11%)	30/155 (19%)	1/12 (8%)	0.171	0.048
Multiple pregnancy rate	10/40 (25%) [all twins]	34/141 (24%) [29 twins + 5 triplets*]	16/155 (10%) [14 twins + 2 triplets*]	3/12 (25%) [2 twins + 1 triplet*]	-	-

* All triplets were derived from replacement of three embryos (Table 2)

TABLE 2. The number of frozen-thawed embryos transferred and pregnancy outcomes

Outcome	No. of embryos transferred			P value (χ^2 test for trend)	P value (1 vs 2 embryos, χ^2 test)	P value (2 vs 3 embryos, χ^2 test)
	1	2	3			
Clinical pregnancy rate	26/125 (21%)	262/704 (37%)	60/154 (39%)	0.003	<0.001	0.685
Ongoing pregnancy rate	21/125 (17%)	225/704 (32%)	51/154 (33%)	0.006	<0.001	0.781
Miscarriage rate	5/26 (19%)	37/262 (14%)	9/60 (15%)	0.760	0.482	0.861
Multiple pregnancy rate	1/26 (4%)*	41/262 (16%)*	21/60 (35%)†	<0.001	0.104	<0.001

* All were twins

† 13 (22%) were twins and 8 (13%) were triplets

TABLE 3. Blastomere numbers at replacement and pregnancy outcomes

Outcome	Cell stage				P value (χ^2 test for trend)	Odds ratio of cell stage ≥ 4 vs cell stage < 4 (95% confidence interval)
	<3	3	4	>4		
Clinical pregnancy rate	22/108 (20%)	26/130 (20%)	232/569 (41%)	68/176 (39%)	<0.001	2.669 (1.883-3.783)
Ongoing pregnancy rate	14/108 (13%)	20/130 (15%)	201/569 (36%)	62/176 (35%)	<0.001	3.274 (2.210-4.850)
Miscarriage rate	8/22 (36%)	6/26 (23%)	31/232 (13%)	6/68 (9%)	0.001	0.342 (0.168-0.696)

TABLE 4. Single frozen-thawed embryo transfers

Parameter	Pregnancy outcome		
		Clinical pregnancy rate	Ongoing pregnancy rate
Blastomere number at freezing	<4 cell	7/45 (16%)	4/45 (9%)
	≥ 4 cell	19/80 (24%)	17/80 (21%)
	P value*	0.361	0.086
Blastomere number at replacement	<4 cell	6/58 (10%)	3/58 (5%)
	≥ 4 cell	19/67 (28%)	17/67 (25%)
	P value*	0.014	0.003
Embryo grading at freezing†	1-2	19/98 (19%)	15/98 (15%)
	3-4	7/27 (26%)	6/27 (22%)
	P value*	0.436	0.394
Lysis of blastomere	No	25/98 (26%)	21/98 (21%)
	Yes	1/27 (4%)	0/27 (0%)
	P value*	0.0143	0.007
Maternal age at cryopreservation (years)	≤ 35	19/76 (25%)	16/76 (21%)
	≥ 36	7/49 (14%)	5/49 (10%)
	P value*	0.1797	0.144

* Fisher's exact test

† Embryos with grading 5 or 6 were discarded and not cryopreserved according to our protocol

(conventional or ICSI), causes of subfertility, and type of replacement cycle regimen (natural, clomiphene-induced, or hormone replacement cycle). These findings were in line with reports in the literature.^{7,8}

There was only one reported study comparing pregnancy outcomes of clomiphene-induced and hormone replacement FET cycles, which revealed no significant difference,⁹ despite the possible anti-oestrogenic effect of clomiphene on the endometrium.

While there was a positive correlation between number of embryos transferred and the pregnancy rate, this needs to be balanced against the risk of multiple pregnancy. From our data, replacing two embryos yielded significantly higher CPRs and OPRs than replacement of a single embryo, but did not differ significantly from replacing with three frozen embryos. On the other hand, replacing three embryos resulted in a significantly higher risk of multiple pregnancy, including triplets, all of which would put the women at much higher perinatal risk and greatly increase foetal risks. For newly initiated IVF cycles (since mid-2005), we ceased replacing more than two frozen embryos in one transfer. This did not compromise our pregnancy rate, which actually showed an increasing trend. Since then, our rate of multiple pregnancies derived from FET cycles has decreased, although there was still a multiple pregnancy rate of 18%, with eight triplets derived from cycles where three embryos were

replaced. Despite occasional debates as to whether twin pregnancies should be accepted as a treatment success, most authorities regard it as undesirable, as it is associated with higher obstetric and neonatal complications. This view is also reflected in major treatment guidelines, and the current trend in Europe is to encourage single embryo transfers.^{2,10-12} Overall, there is still room for further reduction in the twinning rate.

Bearing in mind the potential advantages of single FET, we further analysed the factors affecting treatment success in single FET cycles. In the latter, embryos with blastomere numbers of 4 or more at cryopreservation yielded a 2.5-fold increase in OPR, although this change did not reach statistical significance (possibly due to the limited sample size). On the other hand, embryos with blastomere numbers of 4 or more after thawing attained significantly better pregnancy rates. This could be explained by differences in blastomere lysis upon thawing; the blastomere number at cryopreservation not being an exact reflection of what was actually replaced. Indeed, subgroup analysis demonstrated that upon thawing, lysis of blastomeres led to a significantly poorer pregnancy rate, with only one resulting clinical pregnancy that eventually ended in a miscarriage. Previous reports have also shown that the implantation potential of cryopreserved, early stage embryos is directly related to blastomere cell survival rates. Embryos which are intact after thawing have the same implantation potential as fresh embryos and it is the loss of blastomeres which determines the decrease in implantation potential.¹³⁻¹⁵ Conforming to reported evidence,¹⁶ morphological embryo grading did not reflect the pregnancy rate of FET. This could be partly due to a selection bias, such that embryos with the poorest grading were usually discarded. This information has implications for patient counselling and further management. We suggest that it is reasonable to encourage elective single FET, where the blastomere number is 4 or more.

The age of the women has generally been considered to be an important determinant of treatment success following both fresh and frozen embryo transfers. Our results showed that women aged 35 years or below had higher CPRs and OPRs than older women, and the latter also had a

significantly higher miscarriage rate. We did explore for possible interactions between age and other factors in predicting the pregnancy rate. Although older subjects in our cohort tended to have higher numbers of frozen embryos replaced, their pregnancy rate was lower. Moreover, in the subgroup analysis of double FET, the same trend of lower pregnancy rates with higher maternal age was noted, indicating what appears to be a genuine independent correlation between maternal age and outcomes. In the subgroup analysis of single FET, there was also a two-fold higher pregnancy rate in women aged 35 years or below, although this did not attain statistical significance, possibly due to the underpowered sample size.

In our Centre, we routinely perform fresh embryo transfers and embryo freezing on day 2 post-fertilisation. There are suggestions that better selection of good-quality embryos can be achieved on day 3, thus leading to higher pregnancy rates after both fresh embryo transfers and FETs, and apparently this does not compromise post-thaw embryo survival rate.¹⁷ However, a day-3 transfer protocol would lower the number of embryos available for transfer per stimulation cycle, and would compromise the pregnancy rate especially for women with relatively poor ovarian reserve (and smaller number of embryos).^{18,19} A systematic review also reported that day-3 fresh embryo transfer did not confer a higher live birth rate compared to day-2 transfers.²⁰ Another recent report also revealed no significant difference in the cumulative (fresh plus frozen) live birth rate between single day-2 embryos versus blastocyst transfers.²¹ With our good outcome of FET cycles, the day-2 transfer protocol used at our Centre seems justified, as a means of maximising the number of embryos for cryopreservation and hence the overall cumulative pregnancy rates per stimulation cycle.

To summarise, CPR and OPR of FET cycles were 35% and 30%, respectively. Higher pregnancy rates were associated with younger maternal age (≤ 35 years), blastomere numbers of 4 or more, and no lysis of the blastomeres after thawing.

Acknowledgements

The authors would like to thank other staff members of the Centre for their contribution to the assisted reproduction service throughout all these years.

References

1. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature* 1983;305:707-9.
2. Borini A, Cattoli M, Bulletti C, Coticchio G. Clinical efficiency of oocyte and embryo cryopreservation. *Ann N Y Acad Sci* 2008;1127:49-58.
3. Ng EH, Yeung WS, Lau EY, So WW, Ho PC. High serum oestradiol concentrations in fresh IVF cycles do not impair implantation and pregnancy rates in subsequent frozen-thawed embryo transfer cycles. *Hum Reprod* 2000;15:250-5.
4. Veckel LL. Oocyte assessment and biological performance. *Ann N Y Acad Sci* 1988;541:259-74.

5. Wright VC, Chang J, Jeng G, Chen M, Macaluso M; Centers for Disease Control and Prevention. Assisted reproductive technology surveillance - United States, 2004. *MMWR Surveill Summ* 2007;56:1-22.
6. Andersen AN, Goossens V, Ferraretti AP, et al. Assisted reproductive technology in Europe, 2004: results generated from European registers by ESHRE. *Hum Reprod* 2008;23:756-71.
7. Salumets A, Suikkari AM, Mäkinen S, Karro H, Roos A, Tuuri T. Frozen embryo transfers: implications of clinical and embryological factors on the pregnancy outcome. *Hum Reprod* 2006;21:2368-74.
8. Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. *Cochrane Database Syst Rev* 2008;23:CD003414.
9. Loh SK, Ganesan G, Leong N. Clomid versus hormone endometrial preparation in FET cycles. Proceedings of the 17th World Congress on Fertility and Sterility (IFFS); 2001 25-30 November; Melbourne, Australia; 2001: 3.
10. Dickey RP, Sartor BM, Pyrzak R. What is the most relevant standard of success in assisted reproduction?: no single outcome measure is satisfactory when evaluating success in assisted reproduction; both twin births and singleton births should be counted as successes. *Hum Reprod* 2004;19:783-7.
11. National Collaborating Centre for Women's and Children's Health. Fertility: assessment and treatment for people with fertility problems. NICE Clinical Guideline. London: RCOG Press; 2004.
12. European Society of Human Reproduction and Embryology. Good clinical treatment in assisted reproduction: an ESHRE Position Paper. *The Parliament Magazine* 2008;274:85-9.
13. Edgar DH, Bourne H, Jericho H, McBain JC. The developmental potential of cryopreserved human embryos. *Mol Cell Endocrinol* 2000;169:69-72.
14. Edgar DH, Archer J, McBain J, Bourne H. Embryonic factors affecting outcome from single cryopreserved embryo transfer. *Reprod Biomed Online* 2007;14:718-23.
15. Olivius C, Lundin K, Bergh C. Predictive factors for live birth in cryopreservation single embryo transfer cycles. *Reprod Biomed Online* 2008;17:676-83.
16. Granne I, Child T, Hartshorne G; British Fertility Society. Embryo cryopreservation: evidence for practice. *Hum Fertil (Camb)* 2008;11:159-72.
17. Sifer C, Sellami A, Poncelet C, et al. Day 3 compared with day 2 cryopreservation does not affect embryo survival but improves the outcome of frozen-thawed embryo transfers. *Fertil Steril* 2006;86:1537-40.
18. Shen S, Rosen MP, Dobson AT, Fujimoto VY, McCulloch CE, Cedars MI. Day 2 transfer improves pregnancy outcome in in vitro fertilization cycles with few available embryos. *Fertil Steril* 2006;86:44-50.
19. Bahceci M, Ulug U, Ciray HN, Akman MA, Erden HF. Efficiency of changing the embryo transfer time from day 3 to day 2 among women with poor ovarian response: a prospective randomized trial. *Fertil Steril* 2006;86:81-5.
20. Oatway C, Gunby J, Daya S. Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection. *Cochrane Database Syst Rev* 2004;(2): CD004378.
21. Guerif F, Lemseffer M, Bidault R, et al. Single day 2 embryo versus blastocyst-stage transfer: a prospective study integrating fresh and frozen embryo transfers. *Hum Reprod* 2009;24:1051-8.