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Factors affecting the outcome of frozen–thawed embryo transfer

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STUDY QUESTION: Which clinical and laboratory factors affect live birth rate (LBR) after frozen–thawed embryo transfer (FET)?

SUMMARY ANSWER: Top quality embryo characteristics, endometrial preparation protocol, number of embryos transferred and BMI affected independently the LBR in FET.

WHAT IS KNOWN ALREADY: FET is an important part of present-day IVF/ICSI treatment. There is limited understanding of the factors affecting success rates after FET.

STUDY DESIGN, SIZE, DURATION: This is a two-centre retrospective cohort study. Analysis was carried out on 1972 consecutive FET cycles in 1998–2007, with embryos frozen on Day 2. The primary outcome was LBR per cycle.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We assessed the independent effect on LBR of the following variables: female age, female age at embryo freezing, BMI, diagnosis, primary versus secondary infertility, fertilization by IVF versus ICSI, pregnancy in the fresh cycle, type (spontaneous, spontaneous with luteal progesterone and estrogen/progesterone substitution) and rank of the FET cycle, as well as number and presence (yes versus no) of top quality embryo(s) at freezing, thawing and transfer, damaged thawed embryos and overnight culture.

MAIN RESULTS AND THE ROLE OF CHANCE: In 78% of the cycles with top quality embryos frozen ($n = 1319$), at least one embryo still had high-quality morphology after thawing. Top quality embryo morphology observed at any stage of culture improved the outcome even if high-quality characteristics disappeared before transfer. LBRs after the transfer of a top quality embryo were similar in the FET (24.9%) and fresh cycles of the same period (21.9%). The chance of live birth increased significantly if ≥ 1 top quality embryo was present at freezing (odds ratio (OR) 1.85, 95% confidence interval (CI) 1.10–3.14), at thawing (OR 1.93, CI 1.20–3.11) or at transfer (OR 3.41, CI 2.12–5.48). Compared with spontaneous cycles with luteal support, purely spontaneous cycles (OR 0.58, CI 0.40–0.84) and hormonally substituted FET (OR 0.47, CI 0.32–0.69) diminished the odds of pregnancy. BMI (OR 0.96, CI 0.92–0.99) and transfer of two embryos versus one (OR 1.45, CI 1.08–1.94) were other factors that improved LBR after FET.

LIMITATIONS, REASONS FOR CAUTION: The sample sizes available in some subanalyses were small, limiting the power of the study.

WIDER IMPLICATIONS OF THE FINDINGS: The presence of ≥ 1 top quality embryo at any step of the freezing and thawing process increases the chance of pregnancy. The data do not support the freezing of all embryos for transfer in order to improve the outcome. A top quality embryo transferred in FET may even have the same potential as in a fresh cycle. On the contrary, LBR in the group with no top quality embryos frozen was quite low (10.4%), raising the question of whether a re-evaluation of freezing criteria is necessary to avoid costly treatments with a low success rate.

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Key words: Top quality embryo / frozen–thawed embryo transfer / pregnancy / single embryo transfer

Introduction

Frozen–thawed embryo transfer (FET) has become an essential part of IVF/ICSI treatment, increasing the cumulative pregnancy rate (PR) after ovum retrieval (Tiitinen *et al*., 2001). Used with elective single embryo transfer (eSET), it keeps the multiple birth rate low and minimizes overall costs of treatment (Martikainen *et al*., 2001; Veleva *et al*., 2009).

Various factors are known to affect the outcome of FET. Among the clinical factors, increased female age at embryo freezing worsens the outcome after FET (Karlström and Bergh, 2007) and hormonal substitution is associated with an elevated miscarriage rate (Veleva *et al*., 2008). A pregnancy in the fresh IVF/ICSI cycle has been shown to be associated with an improved PR in subsequent FET (Toner *et al*., 1991; Karlström *et al*., 1997; El-Toukhy *et al*., 2003).

Embryos of good quality before freezing have a better cryopreservation survival rate (Mandelbaum *et al*., 1987; Testart *et al*., 1987; Karlström *et al*., 1997) and are associated with a higher PR (Hartshorne *et al*., 1990; Salumets *et al*., 2006). The outcome of treatments is also improved if thawed embryos resume cleaving (Ziebe *et al*., 1998; Guerif *et al*., 2002), but cryopreservation-associated damage of embryos worsens the chance of pregnancy (Salumets *et al*., 2006). In fresh cycles, the transfer of top quality embryos is associated with increased PR and live birth rate (LBR) (Van Royen *et al*., 1999), but the criteria used to identify top quality embryos have not been applied to cryopreserved embryos. In addition, the relative impact of embryo morphology and clinical characteristics on clinical outcomes has not been evaluated.

The aim of the present study was to evaluate the importance of grading top quality embryos in FET and at the same time to identify which other characteristics in fresh and FET cycles, and cycle protocols in FET, affect the LBR after FET.

Materials and Methods

Analysis was initiated in 2525 consecutive FET cycles with embryos frozen on Day 2 after ovum retrieval. The cycles were performed in 1998–2007 at the Infertility Unit of Oulu University Hospital and the Family Federation of Finland in Oulu. We excluded from analysis 553 cycles with more than one top quality embryo transferred in FET, more than two embryos transferred and/or missing data as well as cases with top quality embryos transferred in previous FET cycles but with none left for the study cycle. In all, we analysed data of 1253 couples who underwent 1.3 $\pm$ 0.5 (mean $\pm$ SD) fresh IVF/ICSI cycles (range 1–4 cycles) and 2.0 $\pm$ 1.3 FET cycles (range 1–10 cycles) per woman.

The final analysis was performed on data from 1972 cycles. Of these, 1588 cases were used for the main analysis: these were either cycles with top quality embryos thawed or cases with no top quality embryos generated after ovum pickup. A subanalysis of the effect of embryo cohort quality on outcome was performed on the remaining 384 cycles, in which all top quality embryos were transferred in fresh cycles and only non-top quality embryos were frozen from the same cohort.

Protocols

Details of ovarian stimulation and FET protocols have previously been described (Veleva *et al*., 2008, 2009). In 1276 cycles (80.4%), transfer was performed after spontaneous ovulation was detected by means of a urinary luteinizing hormone (LH) test. Embryo transfer was carried out 3–5 days after a positive LH result. Luteal support with vaginal micronized progesterone (200 mg/day Lugesteron (Leiras, Helsinki, Finland)) was initiated at embryo transfer and continued for 2 weeks in 974 (76.3%) of these cycles. No luteal support was administered in the other 302 (23.7%) cycles because, at the time they were performed, it was not clear whether exogenous progesterone was beneficial in cases with spontaneous ovulations.

Hormonal substitution with estradiol and progesterone was carried out in 312 cycles (19.6%) with the main indication of oligo/amenorrhea. Estradiol valerate (Merimono, Novartis, Basel, Switzerland) was administered at a daily dose of 6 mg until cycle day 8. The dose was increased to 8 mg until embryo transfer, and changed to 6 mg thereafter. Vaginal micronized progesterone (800 mg Lugesteron/day) was started when the endometrial thickness was $\geq$8 mm on cycle day 11–13 and, in cases of pregnancy, both estradiol and progesterone were continued until the 10th gestational week. Clinical pregnancies were assessed at 6–7 gestational weeks, at which time the number of heartbeats was recorded.

Embryos were frozen on the day of fresh transfer (Day 2) if they had $<$50% fragmentation and no multinucleated blastomeres. Freezing and thawing were performed with 1,2-propanediol and sucrose as cryoprotectants, according to recommendations of the freezing and thawing kits (Vitrolife Sweden AB). Nunc ampoules (Thermo Fisher Scientific, Inc.) were used for freezing and storage instead of straws. Embryo quality was typically evaluated at two stages: just before freezing and about 4 h before transfer. An additional evaluation was also performed immediately after thawing, mostly in cases in which embryos were further cultured overnight (1177 cycles, 74.1%). During the study period, use of overnight culture depended on the week day as embryos transferred on Mondays were typically thawed on the same day. Such a strategy was adopted in order to minimize work during weekends. Embryos were transferred if $\geq$50% intact blastomeres and no multinucleated blastomeres were observed after thawing.

At all times, embryos were graded using the criteria for top/non-top-quality fresh embryos. A top quality embryo was defined as having four to five cells and $<$20% fragmentation if cultured for 2 days, or $\geq$8 cells and $<$20% fragmentation if cultured for 3 days (Van Royen *et al*., 1999). We applied Day 2 criteria to grade all embryos at freezing and at thawing. In addition, the quality at transfer on the day of thawing was assessed with the same criteria. Day 3 criteria were used for evaluation of embryos at transfer after overnight culture. An embryo was considered to be damaged if $>$50%, but not all, of its blastomeres had survived thawing. If $<$50% of the blastomeres survived, the embryo was considered dead and discarded.

Four groups were formed, depending on the quality of the embryos. The group with no top quality embryos frozen ($n = 269$) included cycles in which there were no top quality embryos after ovum retrieval. Other groups that were examined included cycles with top quality embryo(s) at freezing, in which high-quality morphology was not observed at thawing and transfer ($n = 289$); cycles in which high-quality morphology persisted until thawing but was not observed at the time of transfer ($n = 545$); and cycles with top quality embryo(s) at transfer, in which one top quality embryo was transferred in FET ($n = 485$).

A separate subanalysis aimed at studying the effect on LBR of the whole embryo cohort created in the fresh cycle. For this purpose, we compared cycles in which no top quality embryos were created after ovum retrieval ($n = 269$) with those in which all top quality embryos were transferred in fresh cycles and only non-top quality embryos were frozen from the same time period ($n = 384$).

Statistics

Analysis was performed using the IBM Statistical Package for the Social Sciences software package, version 19. Statistical significance was defined as a $P < 0.05$. Parameters in the different groups were initially assessed by chi-square analysis and analysis of variance with Bonferroni's *post hoc* test.

The primary outcome variable of the study was the LBR per cycle. Analysis was initiated using the following variables: female age at freezing, female age at FET treatment, BMI (linear and quadratic models), diagnosis, primary versus secondary infertility, fertilization by IVF versus ICSI, pregnancy in the fresh cycle, type of FET cycle, consecutive rank of the cycle as well as presence (yes versus no) and number of top quality embryo(s) at freezing, thawing and transfer, presence and number of damaged thawed embryos, overnight culture and number of transferred embryos in FET (1 versus 2). Preliminary evaluation found collinearity between age at freezing and number of top quality embryos, and between these and type of infertility and pregnancy in the fresh cycle. Consequently, age at freezing, type of infertility and pregnancy in the fresh cycle were excluded from further analyses. We assessed the independent effects of the remaining variables on LBR by univariate analysis first; those with a significant effect on LBR were then examined in multivariate logistic regression. The performance of the model was examined by means of Hosmer–Lemeshow goodness-of-fit statistic, with $P > 0.05$ indicating a good performance of the model, free of high-degree correlations.

Results

Table I shows the background characteristics of the studied cycles. On average, FET cycles were performed less than a year after cryopreservation. In 335 cycles (21.1%), there had been a pregnancy in the fresh cycle. The overall PR in all FET cycles was 24.6% (391/1588), the LBR was 19.2% (305/1588) and the multiple birth rate was 9.0% (35/391). Compared with all FET cycles, analysis of only the first FET cycle revealed a similar PR (217/858, 25.3%, $P = 0.7$), LBR (176/858, 20.5%, $P = 0.5$) and multiple birth rate (15/217, 6.9%, $P = 0.4$). In the fresh cycles with $\leq$1 top quality embryo transferred that were performed in our clinic during the same period the PR was 26.5% (918/3458, $P = 0.1$), the LBR was 19.0% (656/3458, $P = 0.8$) and the multiple birth rate was 12.6% (116/918, $P = 0.06$).

In 16.9% of the cycles studied, no top quality embryos were frozen (Table II). Of the remaining 1319 cycles, in 78.1% of cases at least one top quality embryo survived the freezing and thawing process intact. The number of damaged embryos thawed was highest in the group in which high-quality characteristics were not observed after thawing (1.5 ± 1.2) and lowest in the group with a top quality embryo transferred (0.5 ± 0.7).

The PR increased the longer the high-quality characteristics were observed (Table III). In cycles in which no top quality embryos were frozen, the PR was 13.4%, while in cases in which high-quality morphology was seen at transfer the PR was 31.3% ($P < 0.0001$). A similar increase was observed for implantation rate (from 8.0 to 21.5%, respectively, $P < 0.0001$) and in LBR (from 10.4 to 26.0%, respectively, $P < 0.0001$). There were no statistically significant differences in the rates of spontaneous abortion or multiple birth. There were 4 cases of miscarriage after the 12th gestational week, 15 cases of ectopic pregnancy and 5 cases of induced abortion due to genetic causes and/or malformations. Two cases were lost to follow-up.

Transfer of a single embryo was performed in 548 cycles (34.5%), with an LBR of 17.2% (94/548). In the remaining cases two embryos were transferred, resulting in an LBR that tended to be higher than for single embryo transfer (20.3%, 211/1040, $P = 0.1$). Among cycles with a single top quality embryo transferred, PRs in fresh and FET cycles (397/1286, 30.9% versus 69/225, 30.7%, respectively, $P = 1.0$) and LBRs (282/1286, 21.9% versus 56/225, 24.9%, respectively, $P = 0.3$) were similar.

The LBR in cycles with hormonal substitution was 11.9% (37/312). This was lower than the LBRs in spontaneous cycles (15.9%, 48/302) and in spontaneous cycles with luteal support (22.6%, 220/974) ($P < 0.0001$). A higher percentage of anovulation was observed among patients with hormonally substituted cycles (75/312, 24.0%), compared with the two types of spontaneous FET (spontaneous: 8/302, 2.6%, spontaneous with luteal support: 34/974, 3.5%, $P < 0.0001$).

Univariate logistic regression showed that BMI (linear model), the type of FET cycle, the presence as well as the absolute number of top quality embryos at freezing, thawing and transfer all affected the LBR after FET. Collinearity was found between top quality embryo numbers at freezing, thawing and transfer. In addition, the clinical importance of these variables was assumed to be limited since >1 top quality embryo was frozen in only 22% (352/1588), and thawed in only 11% (177/1588), of cases. For these reasons, the presence of top quality embryo(s) at different times before transfer was used in the model. Embryo quality at different times until transfer was examined as one variable (presence of top quality embryos at freezing, at thawing and at transfer) in order to establish the relative importance of high-quality morphology and also in order to avoid collinearity. The final model thus included the presence of $\geq$1 top quality embryo at freezing, thawing and transfer, the type of FET cycle and BMI. Because of the clinical importance of the number of embryos transferred, this variable was also included in the multivariate analysis, despite the lack of statistical significance in univariate regression. For the same reason, overnight culture was included as well.

The final adjusted model is shown in Table IV. The Hosmer–Lemeshow goodness-of-fit test indicated the model explained well the data analysed and no important correlations were noted, with $P = 0.4$. Compared with cycles with no top quality embryos, even cycles in which top quality embryos were frozen, but the morphology was lost upon thawing were associated with a higher chance of live birth. The cycles with the best odds for live birth were the ones in which top quality embryos were transferred. Compared with spontaneous cycles with luteal phase support, purely spontaneous cycles were associated with diminished odds of live birth. Hormonal substitution worsened the chance of live birth even more. Lower BMI and the transfer of two versus one embryo in FET were also associated with an increased chance of live birth. Overnight culture showed no independent effect on LBR.

In the subanalysis of the effect of embryo cohort quality on LBR, logistic regression with the same factors as in the main analysis showed that the transfer of a top quality embryo in the fresh cycle was not associated with an improved PR in subsequent FET (adjusted OR 1.09, CI 0.54–2.23). In fact, no significant differences were noted between the two groups. Compared with cycles in which no top quality embryos were obtained after ovum retrieval ($n = 269$, see Tables I, II and III), those cycles in which all top quality embryos were transferred in fresh cycles and only non-top quality embryos were frozen ($n = 384$) were characterized by a similar age at freezing (32.1 ± 4.5 years, $P = 0.2$), and similar numbers of embryos frozen (2.9 ± 1.3, $P = 0.9$), thawed (2.3 ± 1.1, $P = 0.3$) and transferred (1.7 ± 0.5, $P = 0.2$). Overnight culture was performed in 71.4% (274/384) of cases ($P = 0.9$) and LBR was 9.9% (38/384, $P = 0.9$).

Discussion

To our knowledge, this is the first study evaluating the feasibility of applying high quality grading to cryopreserved embryos. Results showed that

Table I Characteristics of the FET cycles for each study group.

Characteristics	Study groups				*P*-value
	No top quality embryos frozen	Top quality embryo(s) frozen	Top quality embryo(s) frozen and thawed	Top quality embryo(s) frozen, thawed and transferred	
Cycles (*n*)	269	289	545	485	–
Age at freezing, years	32.5 ± 5.0[a,b]	31.2 ± 4.5[a]	32.0 ± 4.5	31.3 ± 4.6[b]	0.001
Age at transfer, years	32.8 ± 4.8	32.1 ± 4.5	32.9 ± 4.4	32.2 ± 4.5	0.02
BMI, kg/m^2	23.9 ± 4.1	23.9 ± 4.0	23.8 ± 4.0	23.4 ± 3.9	0.1
ICSI[f]	118 (43.9%)	121 (41.9%)	211 (38.7%)	164 (33.8%)	0.04
Main diagnosis[f] *n* (%)					0.2
Endometriosis	35 (13.0%)	37 (12.8%)	75 (13.8%)	77 (15.9%)	
Anovulation	16 (5.9%)	16 (5.5%)	42 (7.7%)	43 (8.9%)	
Male factor	93 (34.6%)	98 (33.9%)	191 (35.0%)	147 (30.3%)	
Tubal factor	49 (18.2%)	52 (18.0%)	87 (16.0%)	82 (16.9%)	
Unexplained	48 (17.8%)	69 (23.9%)	121 (22.2%)	109 (22.5%)	
Other	28 (10.4%)	17 (5.9%)	28 (5.1%)	27 (5.6%)	
Primary infertility[f]	165 (61.3%)	168 (58.1%)	289 (53.0%)	252 (52.0%)	0.04
Pregnancy in fresh cycle[f]	44 (16.4%)	66 (22.8%)	127 (23.3%)	98 (20.2%)	0.1
Rank of FET cycle	1.3 ± 0.7[c,d,e]	1.5 ± 0.9[c]	1.5 ± 0.8[d]	1.6 ± 0.9[e]	0.001
Type of FET cycle[f]					0.04
Spontaneous, luteal support	160 (59.5%)	163 (56.4%)	333 (61.1%)	318 (65.6%)	
Spontaneous	57 (21.2%)	72 (24.9%)	101 (18.5%)	72 (14.8%)	
Hormonal substitution	52 (19.3%)	54 (18.7%)	111 (20.4%)	95 (19.6%)	

Study Groups: The group with no top quality embryos frozen ($n = 269$) included cycles in which there were no top quality embryos after ovum retrieval. In the group with top quality embryo(s) at freezing ($n = 289$) top quality morphology was not observed at thawing and transfer. The group in which top quality morphology persisted until thawing but was not observed at the time of transfer ($n = 545$) included cycles with top quality embryos frozen and thawed. Finally, in the group with a top quality embryo at transfer ($n = 485$), one top quality embryo was transferred in FET.

Data are presented as mean ± SD unless otherwise stated. Groups with the same superscript show significant differences in Bonferroni's *post hoc* test:

[a]$P = 0.005$.

[b]$P = 0.004$.

[c]$P = 0.01$.

[d]$P = 0.009$.

[e]$P < 0.0001$ (a–e by one-way analysis of variance (ANOVA)).

[f]Chi-square test.

transfer of a top quality embryo is the most important factor as regards improving the chance of live birth after FET. The type of protocol also affected results as progesterone supplementation after a spontaneous ovulation doubled the odds of a live birth, compared with purely spontaneous or hormonally substituted cycles.

High quality criteria include several factors that have previously been reported to affect the outcome: good morphology at freezing (Hartshorne *et al.*, 1990; Salumets *et al.*, 2006; Sole *et al.*, 2010), lack of damage at thawing (Salumets *et al.*, 2006; Sole *et al.*, 2010) and resumed cleavage after overnight culture (Ziebe *et al.*, 1998; Guerif *et al.*, 2002; Gabrielsen *et al.*, 2006). The present study thus demonstrates that the same criteria for high quality as in fresh cycles can also be applied to frozen and thawed embryos, which significantly simplifies clinical practice. This is especially important given the wide application of high quality grading in fresh transfers.

The processes of freezing and thawing damage embryos, but the extent of this damage varies from one embryo to another. Previous studies have shown that only ~30–48% of embryos survive cryopreservation intact (Guerif *et al.*, 2002; Sole *et al.*, 2010). However, top quality embryos seem to do better, as in 79% (1088/1377) of the cycles in the present study with top quality embryos frozen, at least one embryo still had high-quality morphology after thawing, indicating that top quality embryos are more viable than embryos of a poorer quality. In total, top quality embryo morphology was observed in 66% (1088/1646) of cycles after thawing. The results indicate that the transfer of a frozen–thawed top quality embryo may lead to a similar LBR as in the fresh cycles. However, pregnancy odds remained higher even in cycles in which high-quality morphology did not persist until transfer, suggesting partial preservation of the high pregnancy potential of these embryos. This should be considered when selecting embryos for transfer.

We also wanted to study whether or not the presence of top quality embryo is associated with improved quality of the whole embryo cohort created in the fresh cycle. For this purpose, we compared cycles in which no top quality embryos were created after ovum retrieval with those in which all top quality embryos were transferred in fresh cycles and only

Table II Embryo characteristics for the study groups.

Characteristics	Study groups				*P*-value
	No top quality embryos frozen, *n* = 269	Top quality embryo(s) frozen, *n* = 289	Top quality embryo(s) frozen and thawed, *n* = 545	Top quality embryo(s) frozen, thawed and transferred, *n* = 485	
Embryos frozen	2.9 ± 1.2[a]	3.2 ± 1.5[a,b,c]	2.9 ± 1.3[b,d]	2.7 ± 1.1[c,d]	<0.0001
Frozen top quality embryos	–	1.4 ± 0.8[e,f]	1.5 ± 0.8[e]	1.1 ± 0.9[f]	<0.0001
Embryos thawed	2.4 ± 1.1	2.4 ± 1.1	2.6 ± 1.1[g]	2.3 ± 0.9[g]	<0.0001
Damaged thawed embryos	0.6 ± 0.9[h]	1.5 ± 1.2[h,i,j]	0.6 ± 0.9[i]	0.5 ± 0.7[j]	<0.0001
Top quality embryos thawed[n]	–	–	1.3 ± 0.5	1.1 ± 0.6	<0.0001
Overnight culture[o]	191 (71.0%)	226 (78.2%)	521 (95.6%)	239 (49.3%)	<0.0001
Embryos transferred	1.7 ± 0.5[k]	1.6 ± 0.5[k,l]	1.8 ± 0.4[l,m]	1.5 ± 0.5[k,m]	<0.0001
Top quality embryos transferred	–	–	–	1.0	–

Data are presented as mean ± SD unless otherwise stated. Analysis using one-way ANOVA.
Groups with the same superscript show significant differences in Bonferroni's *post hoc* test.
[a]$P = 0.03$.
[b,d,k]$P = 0.02$.
[c,e,f,g,h,i,j,l,m]$P < 0.0001$.
$P = 0.04$.
[k]$P = 0.001$.
[n]*T*-test.
[o]Chi-square test.

Table III Clinical outcome following FET.

Characteristics	Study groups				*P*-value
	No top quality embryos frozen, *n* = 269	Top quality embryo(s) frozen, *n* = 289	Top quality embryo(s) frozen and thawed, *n* = 545	Top quality embryo(s) frozen, thawed and transferred, *n* = 485	
Clinical pregnancies	36/269 (13.4%)	63/289 (21.8%)	140/545 (25.7%)	152/584 (31.3%)	<0.0001
Implantation rate	37/462 (8.0%)	60/463 (13.0%)	138/958 (14.4%)	160/745 (21.5%)	<0.0001
Spontaneous abortion <12 gestational weeks	4/36 (11.1%)	9/63 (14.3%)	27/545 (19.3%)	20/485 (13.2%)	0.4
Live births	28/269 (10.4%)	49/289 (17.0%)	102/545 (18.7%)	126/485 (26.0%)	<0.0001
Multiple births	3/36 (8.3%)	4/63 (6.3%)	11/140 (7.9%)	17/152 (11.2%)	0.7

Data are presented as mean ± SD unless otherwise stated and analysed using chi-square test.

non-top quality embryos were frozen. Transfer of a top quality embryo in the fresh cycle was not associated with an improved PR in subsequent FET. This result underlines the importance of eSET in the fresh cycle, as the procedure maximizes the number of top quality embryos to be frozen.

Hormone treatment in the FET cycle was also found to affect the probability of live birth. In the present analysis, luteal support with progesterone after spontaneous ovulation was associated with the best LBR compared with natural and hormonally substituted cycles. This is in contrast with a previous smaller study that found similar implantation, pregnancy and LBRs in spontaneous and estrogen + progesterone hormonally substituted cycles (Gelbaya *et al.*, 2006). On the other hand, a recent randomized study revealed that luteal phase support with progesterone increased the PR in natural cycles (Bjuresten *et al.*, 2011), suggesting a beneficial effect of exogenous progesterone. To date, the mechanism through which exogenous progesterone exercises its beneficial action after a spontaneous ovulation remains unclear.

The present results extend our previous findings of elevated miscarriage rate in patients with hormonal substitution (Veleva *et al.*, 2008). The need for hormonal substitution was also strongly associated with anovulation in the present study. Anovulatory patients have higher rates of insulin resistance (Diamanti-Kandarakis, 2006), which in turn

Table IV Multivariate logistic regression analysis for live birth using the final adjusted model.

	P-value	OR (95.0% CI)
Embryo quality		
No top quality embryos frozen		Reference group
Top quality embryo(s)		
Frozen	0.02	1.85 (1.10–3.14)
Thawed	0.007	1.93 (1.20–3.11)
Transferred	<0.0001	3.41 (2.12–5.49)
Type of FET cycle		
Spontaneous, luteal support		Reference group
Spontaneous	0.003	0.58 (0.40–0.83)
Hormonal substitution	<0.0001	0.46 (0.31–0.69)
BMI	0.02	0.96 (0.92–0.99)
Two embryos versus one transferred	0.01	1.45 (1.08–1.94)
Overnight culture	0.07	1.37 (0.98–1.93)

Hosmer–Lemeshow test $P = 0.4$.
OR, odds ratio; CI, confidence interval.

may lead to an unfavourable uterine milieu (Sobaleva and El-Toukhy, 2011). As in our previous study on miscarriage, an increase in BMI had an independent effect on outcome; however, in contrast to its marked effect on miscarriage, it was associated with only a minimal decrease in LBR.

The present analysis also provides evidence that endometrial function in FET might be similar to that in the fresh cycles since LBRs after the transfer of a top quality embryo were similar after ovum retrieval and in FET. The same was also true for all studied FET and fresh cycles. A more detailed comparison of the effects of different endometrial preparation protocols was not possible since all single top quality embryo transfers were carried out in patients with spontaneous ovulation receiving luteal progesterone. However, the present results do not support the idea of freezing all embryos for transfer in FET in order to improve outcomes (Shapiro *et al.*, 2012; Maheshwari and Bhattacharya, 2013).

As expected, the present analysis found that the transfer of two embryos in FET cycles resulted in a higher PR. However, the transfer of two embryos should always be weighed against the risk of multiple pregnancy. In line with the eSET strategy used in the fresh cycles, in Finland only one high-quality frozen–thawed embryo is usually transferred at a time. In our unit, transfer of a single top quality embryo has shown good results during the study period (PR 69/225, 30.7%, multiple birth rate 0%), confirming the results of a previous investigation (Hydén-Granskog *et al.*, 2005). During the study period there were only 58 FET cycles in which two top quality embryos were transferred (10.7% of all top quality embryo transfers), resulting in four multiple pregnancies (multiple birth rate 20.0%).

In the present study, LBR in the group with no top quality embryos frozen was quite low (10.4%). This raises the question of whether a re-evaluation of freezing criteria is necessary in order to avoid costly treatments with a low success rate. In our centres, the practice is that an expected LBR of >10% is sufficient for freezing and thawing.

The present analysis did not show an independent effect of overnight culture on LBR. This can be explained by the fact that by itself, overnight culture does not improve embryo quality but rather facilitates the selection of the best embryos for transfer. However, it is possible that Day 3 criteria do not explain fully the embryo's implantation potential, as after the transfer of a top quality embryo the LBR was higher after overnight culture (Day 3: 32.6 versus Day 2: 19.5%, $P = 0.001$). Such an increase was not noted in non-top quality embryos (Day 3: 16.5% versus Day 2: 14.5%, $P = 0.6$) and more research with larger study groups is needed to clarify this issue.

In conclusion, in the present study we found that in 78% of cycles in which top quality embryos were frozen, the morphological characteristics were retained after thawing. The longer that high-quality characteristics persist before FET, the greater is the probability of pregnancy. Therefore, an eSET policy improves the outcome of FET by leading to an increased number of good-quality embryos to be frozen. Spontaneous cycles with luteal support are the optimal treatment protocol in FET and frozen–thawed top quality embryos transferred in such a cycle may result in a similar LBR as in the fresh cycles.

Authors' roles

Z.V. is responsible for conception and design, data collection, analysis and interpretation, manuscript preparation and critical discussion. H.M. contributed with interpretation of data and critical revision of the manuscript, as did M.O., S.N.-H. and J.S.T. All authors gave final approval of the submitted version.

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Conflict of interest

None declared.

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