Livebirth rate after one frozen embryo transfer in ovulatory women starting with natural, modified natural, or artificial endometrial preparation in Viet Nam: an open-label randomised controlled trial

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Summary

Background Use of frozen embryo transfer (FET) in in-vitro fertilisation (IVF) has increased. However, the best endometrial preparation protocol for FET cycles is unclear. We compared natural and modified natural cycle strategies with an artificial cycle strategy for endometrial preparation before FET.

Methods In this randomised, open-label study, we recruited ovulatory women aged 18–45 years at a hospital in Ho Chi Minh City, Viet Nam, who were randomly allocated (1:1:1) to natural, modified natural, or artificial cycle endometrial preparation using a computer-generated random list and block randomisation. The trial was not masked due to the nature of the study interventions. In natural cycles, no oestrogen, progesterone, or human chorionic gonadotropin (hCG) was used. In modified natural cycles, hCG was used to trigger ovulation. In artificial cycles, oral oestradiol valerate (8 mg/day from day 2–4 of menstruation) and vaginal progesterone (800 mg/day starting when endometrial thickness was ≥7 mm) were used. Embryos were vitrified, and then one or two day-3 embryos or one day-5 embryo were warmed and transferred under ultrasound guidance. If the first FET cycle was cancelled, subsequent cycles were performed with artificial endometrial preparation. The primary endpoint was livebirth after one FET. This trial is registered at ClinicalTrials.gov,

Findings Between March 22, 2021, and March 14, 2023, 4779 women were screened and 1428 were randomly assigned (476 to each group). 99 first FET cycles were cancelled in each of the natural and modified cycle groups, versus none in the artificial cycle group. The livebirth rate after one FET was 174 (37%) of 476 in the natural cycle strategy group, 159 (33%) of 476 in the modified natural cycle strategy group, and 162 (34%) of 476 in the artificial cycle strategy group (relative risk 1·07 [95% CI 0·87–1·33] for natural *vs* artificial cycle strategy, and 0·98 [0·79–1·22] for modified natural *vs* artificial cycle strategy). Maternal and neonatal outcomes did not differ significantly between groups, as the power to detect small differences was low.

Interpretation Although the livebirth rate was similar after natural, modified natural, and artificial cycle endometrial preparation strategies in ovulatory women undergoing FET IVF, no definitive conclusions can be made regarding the comparative safety of the three approaches.

Funding None.

Introduction

In-vitro fertilisation (IVF) is the cornerstone of modern infertility treatment. Advancements in embryo cryopreservation techniques (vitrification) have allowed the wider introduction of frozen embryo transfer (FET) into IVF, while maintaining good pregnancy and neonatal outcomes.[1] Furthermore, for some individuals (for example, women with polycystic ovary syndrome or those with a strong ovarian response), so-called freeze-all IVF cycles are the best approach due to a higher livebirth rate and lower risk of ovarian hyperstimulation syndrome.[2] As a result, the number of IVF cycles that use FET has increased markedly.[3,4]

Successful establishment of a pregnancy in assisted reproductive technology requires synchronisation between embryo transfer and endometrial receptivity. In FET cycles, appropriate endometrial preparation is required to enhance this process. Several endometrial preparation approaches are used during FET in current clinical practice: natural or modified natural cycles with or without luteal phase support; artificial cycles with or without a gonadotropin-releasing hormone agonist; and mild ovarian stimulation using gonadotropins, clomiphene citrate, or letrozole.[5] Of these, natural cycles, modified natural cycles, and artificial cycles are the most commonly used.

In natural FET cycles, exogenous hormones are not used and endometrial preparation relies on the natural menstrual cycle. This approach is commonly used for

Research in context

Evidence before this study

The use of frozen embryo transfer (FET) in in-vitro fertilisation (IVF) has increased. In women with ovulatory cycles undergoing FET, there are at least three different strategies that can be used for endometrial preparation: natural, modified natural, and artificial. Previous studies comparing the effectiveness of these different endometrial preparation protocols for FET have several drawbacks, including selection bias due to retrospective observational design, small sample size, the enrolment of heterogeneous participant populations that have varying baseline characteristics, substantial differences in the endometrial preparation protocols, luteal phase support and methods to detect luteinising hormone surge, and the use of slow freezing rather than vitrification. We did a comprehensive PubMed literature search to identify studies that assessed the effectiveness of the three endometrial preparation protocols in the setting of FET. The search strategy included cohort studies, randomised controlled trials, and meta-analyses up to October, 2020, and was not limited to the English language. The search terms used were "((frozen embryo transfer) OR (endometrial preparation)) AND ((natural cycle) OR (modified natural cycle) OR (artificial cycle) OR (hormonal replacement therapy) OR (programmed cycle)) AND (live birth) AND ((ovulatory) OR (regular menstruation))". We excluded studies that did not evaluate the specified endometrial preparation protocols or did not involve participants with ovulatory cycles. There is little consistency between studies in the assessment and reporting of outcomes. The only previous randomised clinical trial in this field had low embryo survival due to the use of slow freezing rather than vitrification. These limitations lower the reliability of previous study findings and their applicability in clinical practice. As a result, there is no consensus on the optimal endometrial preparation protocol for FET. The results of a Cochrane review did not find sufficient evidence to support the use of one cycle regimen over another for FET in subfertile women with regular ovulatory cycles, but the quality of evidence was low. In addition, there were no direct comparisons between artificial, modified natural, or natural cycles.

Added value of this study

This large, randomised clinical trial compared natural or modified natural endometrial preparation strategies with an artificial strategy in ovulatory women undergoing FET. The large sample size, randomised design, and homogeneous population help to overcome the limitations of previous studies in the field and, therefore, this study addresses a notable gap in the literature regarding the optimal endometrial preparation protocol for FET.

Implications of all the available evidence

The findings from this study show that the livebirth rates after natural, modified natural, or artificial endometrial preparation strategies for ovulatory women undergoing FET are similar. However, the study was not adequately powered to detect between-group differences in the rates of key maternal and neonatal complications. Therefore, no definitive recommendations can yet be made regarding the optimal approach for endometrial preparation in FET IVF cycles.

individuals with regular ovulatory cycles, and has the advantage of being less invasive than and avoiding the potential side-effects of hormonal medications. However, natural cycles are less flexible and have a higher cancellation rate. Modified natural cycles are similar to natural cycles but with the addition of a single gonadotropin dose to trigger ovulation. Artificial cycles involve the use of hormonal medications to control and optimise endometrial development, including oestrogen to stimulate endometrial growth and progesterone to mimic the luteal phase of the menstrual cycle. However, higher rates of pregnancy complications (including pre-eclampsia, macrosomia, and postpartum haemorrhage) have been reported after the use of artificial cycle endometrial preparation.[6,7]

The comparative effectiveness of the different types of endometrial support during FET cycles is a subject of debate, and there is insufficient evidence to support the superiority of one method over another with respect to rates of clinical pregnancy or livebirth. Based on data from 18 randomised controlled trials, a 2017 Cochrane systematic review[8] found no significant differences in rates of pregnancy, miscarriage, or livebirth between natural, hormone therapy, and ovulation induction FET cycles, similar to the findings of other literature reviews.[9,10] However, few studies have compared natural or modified natural protocols with an artificial protocol (natural *vs* artificial in four studies[11–14] and artificial *vs* modified natural in two studies[15,16]); two of these studies were only published as conference abstracts, and the overall quality of evidence used in the systematic review was labelled as low or very low.[8,10] Furthermore, all of these studies only considered one cycle of FET, and therefore, cycle cancellation is recorded as unsuccessful livebirth when in fact no FET actually took place. Therefore, the evidence to guide the choice of endometrial preparation for FET cycles in current clinical practice is sparse. We compared the effectiveness and safety of natural versus artificial and modified natural versus artificial approaches for endometrial preparation during FET cycles in ovulatory women undergoing IVF.

Methods

Study design

This single-centre, open-label randomised controlled trial was conducted at IVFMD, My Duc Hospital in

Ho Chi Minh City, Viet Nam. The study protocol was approved by the institutional ethics committee. All study procedures were performed in accordance with Good Clinical Practice and Declaration of Helsinki 2002 principles, including study oversight by an independent data and safety monitoring committee.

This trial is registered at ClinicalTrials.gov,

Participants

Eligible participants were women aged 18–45 years with an indication for FET who had a regular menstrual cycle (cycle length of 24–38 days),[17] and who had undergone no more than three previous IVF or intracytoplasmic sperm injection FET cycles. To be eligible, couples had to agree to have no more than two day-3 embryos or one day-5 embryo (blastocyst) transferred.

Women who had evidence of menopause or anovulation, contraindication for exogenous hormone administration, or uterine abnormalities (eg: adenomyosis; intrauterine adhesions; large leiomyoma [>5 cm in diameter]; unicornuate, bicornuate, or arcuate uterus; unremoved hydrosalpinx; or endometrial polyp), and cycles that included in-vitro maturation, preimplantation genetic testing, or oocyte donation, were not eligible.

Screening for eligibility was performed by treating physicians from the second to the fourth day of the menstrual cycle in the FET cycles. Potentially eligible women were provided with information about the trial when their stimulation cycles or embryo transfer were initiated. Participants were provided with a copy of the informed consent documents, and written informed consent was obtained from all eligible participants before their enrolment in the study.

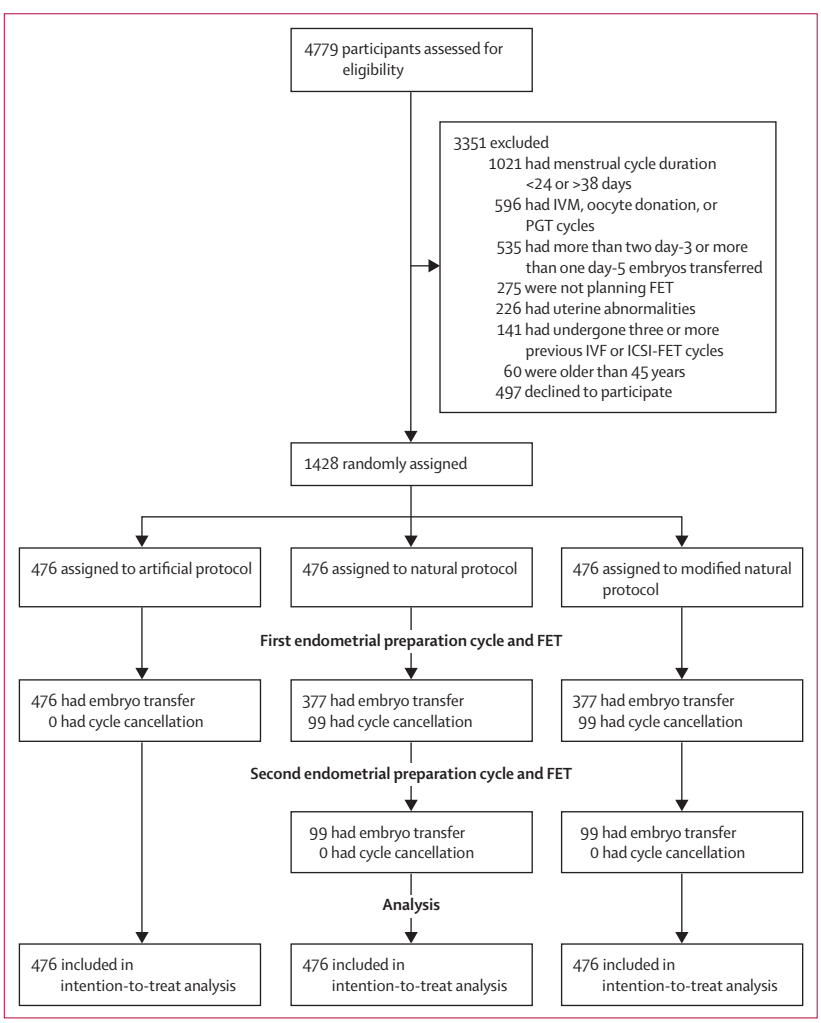

Figure: **Trial profile**
FET=frozen embryo transfer. ICSI=intracytoplasmic sperm injection. IVF=in-vitro fertilisation. IVM=in-vitro maturation. PGT=preimplantation genetic testing.

Randomisation and masking

Participants were randomly assigned (1:1:1) to the natural, modified natural, or artificial protocol group by an independent study coordinator via telephone, using a computer-generated random list and block randomisation with a variable block size of six or nine. The trial was not masked due to the nature of the study interventions.

Procedures

The first endometrial preparation cycle was performed using one of the following endometrial preparation protocols (natural, modified natural, or artificial). In natural cycles, ultrasound was performed on the sixth day of the cycle. Daily ultrasound and determination of serum oestradiol and luteinising hormone (LH) levels were performed when the mean diameter of the dominant follicle was at least 14 mm. LH surge initiation was defined as a concentration that was 180% of the latest serum value available for that individual, which subsequently continued to rise to 20 international units per L or greater as detected by the electrochemiluminescence immunoassay method (cobas e 801, Roche Diagnostics, Mannheim, Germany). Natural cycles were cancelled when no follicle development or onset of LH surge was detected after 21 days. In case of cycle cancellation in the first endometrial preparation, subsequent endometrial preparation was performed using artificial endometrial protocol. Embryo transfer was scheduled for day 4 (cleavage-stage embryos) or day 6 (blastocysts) after the LH surge, as previously suggested.[9]

In modified natural cycles, the first ultrasound scan was performed at the same time as for the natural cycle protocol. Scans were performed daily when there was at least one follicle with a diameter of at least 12 mm. When the mean diameter of the dominant follicle was at least 16 mm, human chorionic gonadotropin (hCG; Ovitrelle 250 µg, Merck, Rahway, NJ, USA) was injected to trigger ovulation. Modified natural cycles were cancelled when there was no follicle development or no dominant follicle with a diameter of at least 16 mm after 21 days, or if there was unexpected spontaneous ovulation before hCG administration. In case of cycle

cancellation in the first endometrial preparation, subsequent endometrial preparation was performed using artificial endometrial protocol. Embryo transfer was scheduled for day 5 (cleavage-stage embryos) or day 7 (blastocysts) after the administration of hCG, as previously suggested.[9]

In artificial cycles, the endometrium was prepared using oral oestradiol valerate (Valiera, Laboratories Recalcine, Santiago, Chile) 8 mg/day, given from the second to the fourth day of menstruation. Endometrial thickness was monitored from day 6 onwards, and vaginal progesterone (Utrogestan, Besins, Paris, France) 800 mg/day was started when endometrial thickness reached at least 7 mm. At least 9 days of oestradiol treatment were required before starting progesterone. In cases where a dominant follicle emerged, serum LH and progesterone were determined to rule out luteinisation; if serum levels of LH were less than 13 international units and those of progesterone were less than 15 nmol/L, luteinisation was deemed not to have occurred and FET was performed. Artificial cycles were cancelled when endometrial thickness was less than 7 mm after at least 21 days of oestradiol administration, or if a dominant follicle emerged.[18] In case of cycle cancellation in the first endometrial preparation due to a thin endometrium (<7 mm) or emerging dominant follicle, subsequent endometrial preparation was performed using again artificial cycle protocol.

Embryos were cryopreserved using the Cryotech (Fort Madison, IA, USA) vitrification method, with a maximum of two embryos per Cryotech. A maximum of two day-3 embryos or one day-5 embryo were warmed on the day of embryo transfer. The timing of embryo transfer was based on the duration of progesterone therapy and the embryo stage(s): on day 4 of progesterone supplementation for day-3 embryos, and on day 6 of progesterone supplementation for day-5 embryos. If no embryos survived after warming, another day-3 or day-5 embryo was warmed. If there were no more embryos left, the cycle was cancelled.

Embryos were graded based on the 2011 Istanbul consensus of the Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology.[19] 2 h after warming, surviving embryos were transferred into the uterus under ultrasound guidance using a soft uterine catheter (Gynétics, Lommel, Belgium). A cycle was cancelled if no embryos had survived after warming. If an embryo was available but there was no transfer due to an unfavourable endometrium, a second endometrial preparation was performed using an artificial cycle. If the endometrium was adequate in the second endometrial preparation (artificial) cycle, FET was performed.

A serum hCG test was performed 10–14 days after embryo transfer. A positive pregnancy test was defined as a serum hCG level of at least 0·025 international units per mL. Transvaginal ultrasound (frequency of 7·5 MHz; Samsung HS30, Samsung Healthcare, Seoul, South Korea) was performed 3 weeks later to confirm fetal viability.

Individuals in the artificial protocol group continued to receive exogenous hormone supplementation with oestradiol valerate (Valiera) 8 mg/day up to 7 weeks of gestation and vaginal micronised progesterone (Utrogestan) 800 mg/day up to 10 weeks of gestation. Women in the natural and modified natural cycle groups did not receive any exogenous hormone supplementation as luteal phase support.

Obstetric care, delivery, and neonatal care were performed according to the routine protocol at the study centre, and all relevant data were collected using an electronic case report form.

	Natural cycle strategy (n=476)	Modified natural cycle strategy (n=476)	Artificial cycle strategy (n=476)
Age at randomisation, years	33·0 (4·6)	33·0 (4·2)	33·4 (4·5)
Age group			
<30 years	103 (22%)	103 (22%)	96 (20%)
30 to <35 years	207 (43%)	204 (43%)	198 (42%)
35 to <40 years	122 (26%)	137 (29%)	133 (28%)
40 to 45 years	44 (9%)	32 (7%)	49 (10%)
BMI, kg/m²	21·1 (2·5)	21·1 (2·4)	21·2 (2·3)
Duration of infertility, years	4 (2–6)	4 (2–6)	4 (2–6)
Type of infertility			
Primary	273 (57%)	248 (52%)	256 (54%)
Secondary	203 (43%)	228 (48%)	220 (46%)
Antral follicle count	13 (9–19)	13 (10–18)	13 (9–18)
Anti-Müllerian hormone, ng/mL	2·7 (1·8–4·1)	2·7 (1·8–3·9)	3·1 (1·8–4·6)
Number of ART attempts			
1	389 (82%)	399 (84%)	372 (78%)
2	68 (14%)	63 (13%)	75 (16%)
3	19 (4%)	14 (3%)	29 (6%)
Total unsuccessful ART cycles*	101	87	130
Indication for IVF or ICSI			
Male factors	148 (31%)	138 (29%)	135 (28%)
Unexplained infertility	109 (23%)	101 (21%)	112 (24%)
Tubal factors	82 (17%)	91 (19%)	75 (16%)
Diminished ovarian reserve†	58 (12%)	60 (13%)	54 (11%)
Female age >38 years	43 (9%)	45 (9%)	65 (14%)
Endometriosis	10 (2%)	9 (2%)	10 (2%)
Others	26 (5%)	32 (7%)	25 (5%)
ICSI	476 (100%)	476 (100%)	476 (100%)
Total cumulus–oocyte complexes	12 (8–17)	11 (8–16)	11 (7–16)
Total metaphase II oocytes	10 (6–14)	9 (6–13)	9 (6–13)
Total fertilised oocytes	7 (4–11)	7 (4–11)	7 (4–10)
Total day-3 embryos	6 (4–9)	6 (4–9)	6 (3–9)
Total day-5 embryos	5 (3–7)	5 (3–7)	5 (3–7)
Total frozen embryos	4 (3–6)	4 (3–6)	4 (3–5)

Data are mean (SD), median (IQR), n (%), or n. ART=assisted reproductive technology. IVF=in-vitro fertilisation. ICSI=intracytoblasmic sperm injection. *An unsuccessful ART attempt was defined as an unsuccessful livebirth. †Defined as an anti-Müllerian hormone level of 1·25 ng/mL or less, or an antral follicle count of five or fewer.

Table 1: **Demographic, baseline, and cycle characteristics for the study population**

Outcomes

The primary outcome was livebirth after one FET. Livebirth was defined as the delivery of at least one newborn after 24 weeks of gestation who exhibits any sign of life (twins where at least one infant was born alive were considered as a single count for that mother).[20] Secondary outcomes included rate of endometrial preparation cycle cancellation, other fertility outcomes (rates of implantation, positive pregnancy test, clinical pregnancy, ongoing pregnancy, multiple pregnancy, and multiple delivery), and early pregnancy complications (ectopic pregnancy or miscarriage). Obstetric and perinatal complications (gestational diabetes, hypertensive disorders in pregnancy, preterm birth, or stillbirth), infant birthweight (for singletons and twins), and neonatal complications (major congenital abnormalities, admission to the neonatal intensive care unit, or mortality [determined using ICD-10 codes]) were recorded for women who had a livebirth. Full details of definitions for gestational diabetes and hypertensive disorders in pregnancy are provided in the appendix (p 1). For women who had prenatal evaluation and delivery at our hospital (nearly 75% of all participants), data were obtained from our electronic database system. Women who had prenatal care or delivery at other hospitals were contacted by telephone and sent their medical records to us. Long-term follow-up of the health of offspring and evaluation of cost-effectiveness will be reported separately when completed, as stated in the protocol. The types of congenital abnormalities were a non-prespecified outcome.

See Online for appendix

	Natural cycle strategy (n=476)	Modified natural cycle strategy (n=476)	Artificial cycle strategy (n=476)	p value	
				Natural *vs* artificial cycle strategy	Modified natural *vs* artificial cycle strategy
Completed first endometrial preparation as randomly assigned	377 (79%)	377 (79%)	476 (100%)	..	..
Endometrial preparation cycle cancellation	99 (21%)	99 (21%)	0	..	..
Second artificial endometrial preparation	99 (21%)	99 (21%)	0	..	..
Embryos transferred	..	..	..	0·37	0·10
1	307 (64%)	293 (62%)	319 (67%)	..	..
2	169 (36%)	183 (38%)	157 (33%)	..	..
Good-quality embryos transferred	..	..	..	0·99	0·99
2	114 (24%)	122 (26%)	113 (24%)	..	..
1	328 (69%)	313 (66%)	326 (68%)	..	..
0	34 (7%)	41 (9%)	37 (8%)	..	..
Stage of embryo(s) transferred	..	..	..	0·62	0·62
Day 3	216 (45%)	215 (45%)	214 (45%)	..	..
Day 5	260 (55%)	261 (55%)	262 (55%)	..	..

Data are n (%) or p value.

Table 2: **Embryo transfer characteristics after the first frozen embryo transfer cycle**

Statistical analysis

We based the sample size calculation on the primary outcome of livebirth, using multiple comparisons of proportions for treatments versus a control function. We calculated that 428 participants per group were needed to detect an absolute increase in livebirth rate from 30% to 40% (80% power, 5% overall significance level [2·5% for each of the two main treatment comparisons: natural *vs* artificial cycle strategy, and modified natural *vs* artificial cycle strategy]). To allow for a 10% rate of loss to follow-up or withdrawal of consent, we set the recruitment target at 476 participants per group.

We performed the primary analysis on an intention-to-treat basis. The statistical analysis plan allowed for exploratory per-protocol analyses. Baseline data were presented using descriptive statistics (mean [SD] for normally distributed variables or median [IQR] for skewed variables). Categorical data were presented as n (%). We compared the primary outcome between two pairs of intervention groups (natural *vs* artificial, and modified natural *vs* artificial) using Fisher's exact test. We analysed between-group differences in secondary outcome variables using the Student *t* test or Wilcoxon signed-rank test for normally distributed or skewed variables, or Fisher's exact test for categorical variables, and reported these as relative risk (RR) with 95% CIs.

All data on the treatment cycle and the occurrence of pregnancy were collected as part of routine clinical care. Data were collected using Epi Info for Windows (version 2.0). All analyses were done on the intention-to-treat population using R (version 4.0.3). There was a data monitoring committee that had three members.

Role of the funding source

There was no funding source for this study.

Results

4779 women were screened between March 22, 2021, and March 14, 2023, of whom 2854 did not meet the eligibility criteria, 497 declined to participate, and 1428 (476 per group) provided written informed consent (figure). Participant demographics and characteristics were well balanced across the different endometrial preparation strategy groups (table 1). The mean age was 33 years, the mean BMI was 21 kg/m², and the median duration of infertility was 4 years. The most common indications for IVF were male factor, tubal factor, or unexplained infertility, and 1160 (81·2%) women were undergoing their first IVF attempt. Primary infertility was slightly more common than secondary infertility in all groups. 919 (64·4%) study participants had one embryo transferred and the stage of the embryo transferred was relatively evenly split between day 3 (645 [45·2%] participants) and day 5 (783 [54·8%] participants; table 2).

	Natural cycle strategy* (n=476)	Modified natural cycle strategy* (n=476)	Artificial cycle strategy* (n=476)	RR	
				Natural *vs* artificial cycle strategy	Modified natural *vs* artificial cycle strategy
Livebirth					
After the first endometrial preparation	127 (27%)	117 (25%)	162 (34%)	0·78 (0·62–0·99)	0·72 (0·57–0·91)
After the second endometrial preparation	47 (10%)	42 (9%)	0	..	..
Total after one FET†	174 (37%)	159 (33%)	162 (34%)	1·07 (0·87–1·33)	0·98 (0·79–1·22)
After day-3 embryo transfer	56/216 (26%)	53/215 (25%)	43/214 (20%)	1·29 (0·87–1·93)	1·23 (0·82–1·84)
After day-5 embryo transfer	118/260 (45%)	106/261 (41%)	119/262 (45%)	1·00 (0·77–1·29)	0·89 (0·69–1·16)
Positive pregnancy test	228 (48%)	224 (47%)	232 (49%)	0·98 (0·82–1·18)	0·97 (0·80–1·16)
Clinical pregnancy	209 (44%)	198 (42%)	205 (43%)	1·02 (0·84–1·24)	0·97 (0·79–1·17)
Ongoing pregnancy	177 (37%)	164 (34%)	165 (35%)	1·07 (0·87–1·33)	0·99 (0·80–1·23)
Implantation	227/645 (35%)	209/659 (32%)	216/633 (34%)	1·03 (0·89–1·20)	0·93 (0·80–1·09)
Ectopic pregnancy					
Total (intention-to-treat)	5 (1%)	10 (2%)	4 (<1%)	1·25 (0·33–5·05)	2·50 (0·84–9·11)
Per positive beta hCG	5/228 (2%)	10/224 (4%)	4/232 (2%)	1·27 (0·34–5·14)	2·59 (0·87–9·44)
Miscarriage at <12 weeks' gestation					
Total (intention-to-treat)	27 (6%)	24 (5%)	36 (8%)	0·75 (0·45–1·23)	0·67 (0·39–1·11)
Per positive beta hCG	27/228 (12%)	24/224 (11%)	36/232 (16%)	0·76 (0·46–1·25)	0·69 (0·41–1·15)
Miscarriage at 12 to <20 weeks' gestation	3 (<1%)	5 (1%)	3 (<1%)	1·00 (0·19–5·40)	1·67 (0·41–8·13)
Multiple pregnancy	11 (2%)	8 (2%)	8 (2%)	1·37 (0·56–3·55)	1·00 (0·37–2·72)
Multiple delivery					
Total (intention-to-treat)	11 (2%)	8 (2%)	7 (1%)	1·57 (0·62–4·27)	1·14 (0·41–3·26)
Per delivery	11/174 (6%)	8/159 (5%)	7/162 (4%)	1·46 (0·58–3·98)	1·16 (0·42–3·32)

Data are n (%), n/N (%), or RR (95% CI). FET=frozen embryo transfer. hCG=human chorionic gonadotropin. RR=relative risk. *Including cycles with FET after the first endometrial preparation (artificial, modified natural, or natural, as per randomisation) and, if the first cycle was cancelled, the second endometrial preparation (all artificial). †Primary outcome.

Table 3: **Fertility outcomes after one FET**

The first FET cycle was cancelled in 99 (21%) women in the natural cycle group and 99 (21%) women in the modified natural cycle group; there were no cycle cancellations in the artificial cycle strategy group. There were also no cancellations in the second round of artificial cycle endometrial preparation for women who had their first natural or modified natural cycle cancelled. The most common reason for cancellation of the first FET cycle was no development of follicles (66 [67%] cancellations in the modified natural cycle strategy group and 67 [68%] in the natural cycle strategy group; appendix p 1).

The livebirth rate after one FET (primary outcome) was 174 (37%) of 476 in the natural cycle strategy group, 159 (33%) of 476 in the modified natural cycle strategy group, and 162 (34%) of 476 in the artificial cycle strategy group (RR 1·07 [0·87–1·33] for natural *vs* artificial cycle strategy and 0·98 [0·79–1·22] for modified natural *vs* artificial cycle strategy; table 3). All other fertility outcomes (rates of implantation, positive pregnancy, clinical pregnancy, ongoing pregnancy, ectopic pregnancy, miscarriage, and multiple pregnancy; table 3) and obstetric outcomes (table 4) were also similar across the three endometrial preparation strategy groups.

Apart from the 21% cancellation rate for the first FET cycle in the modified natural and natural protocol groups (compared with a 0% cancellation rate in the artificial cycle strategy group), there were no significant between-group differences in fertility outcomes (appendix p 1) and obstetric outcomes (appendix p 2). In the second endometrial preparation cycle (artificial protocol in all groups), the livebirth rate was 47 (47%) of 99 in women who had a natural cycle cancelled and 42 (42%) of 99 in women who had a modified natural cycle cancelled; all other fertility and obstetric outcomes were also similar in these two groups (appendix p 3).

Discussion

In this large randomised clinical trial, we compared livebirth rates after the first successful endometrial preparation and FET in groups that were defined based on the type of initial endometrial preparation: natural versus artificial, or modified natural versus artificial. We found that the three strategies were similar with respect to livebirth rate and other fertility and obstetric outcomes after one FET, but that the cancellation rate of the first endometrial preparation cycle was significantly higher in the natural and modified natural versus artificial strategy

	Natural cycle strategy* (n=476)	Modified natural cycle strategy* (n=476)	Artificial cycle strategy* (n=476)	RR	
				Natural then artificial cycle *vs* artificial cycle	Modified natural *vs* artificial cycle
Obstetric outcomes					
Preterm delivery 20 to <28 weeks' gestation, singletons	0	0	1 (<1%)	..	..
Preterm delivery 28 to <32 weeks' gestation, singletons	0	2 (<1%)	0	..	..
Preterm delivery 32 to <37 weeks' gestation, singletons	15 (3%)	11 (2%)	7 (1%)	2·14 (0·90 to 5·61)	1·57 (0·62 to 4·27)
Preterm delivery 20 to <28 weeks' gestation, twins	1 (<1%)	0	0	..	..
Preterm delivery 28 to <32 weeks' gestation, twins	1 (<1%)	0	0	..	..
Preterm delivery 32 to <37 weeks' gestation, twins	4 (<1%)	4 (<1%)	2 (<1%)	2·00 (0·39 to 14·40)	2·00 (0·39 to 14·40)
Gestational diabetes	44 (9%)	37 (8%)	32 (7%)	1·37 (0·88 to 2·18)	1·16 (0·72 to 1·87)
Hypertensive disorders in pregnancy	10 (2%)	8 (2%)	12 (3%)	0·83 (0·35 to 1·93)	0·67 (0·26 to 1·61)
Hypertensive disorders in pregnancy per ongoing pregnancy	10/177 (6%)	8/164 (5%)	12/165 (7%)	0·78 (0·33 to 1·80)	0·67 (0·26 to 1·62)
Perinatal outcomes					
Singleton birthweight, g (469 babies)	3178·4 (463·2)	3147·1 (515·2)	3245·9 (450·3)	−67·5 (−170·1 to 35·1)†	−98·8 (−210·3 to 12·7)†
Twin birthweight, g (52 babies‡)	2370·0 (444·7)	2598·9 (342·2)	2300·0 (434·8)	70·0 (−260·7 to 400·7)†	298·9 (−24·2 to 622·1)†
Singleton birthweight, very low	0	1 (<1%)	1 (<1%)	..	1·00 (0·04 to 25·30)
Singleton birthweight, low	6 (1%)	8 (2%)	5 (1%)	1·20 (0·36 to 4·16)	1·60 (0·53 to 5·30)
Singleton birthweight, high	5 (1%)	5 (1%)	4 (<1%)	1·25 (0·33 to 5·05)	1·25 (0·33 to 5·05)
Singleton birthweight, very high	1 (<1%)	0	0	..	..
Major congenital abnormalities§	1 (<1%)	2 (<1%)	1 (<1%)	1·00 (0·04 to 25·3)	2·00 (0·19 to 43·00)
Admission to NICU	15 (3%)	8 (2%)	18 (4%)	0·83 (0·41 to 1·65)	0·44 (0·18 to 0·99)
Stillbirth	0	0	0	..	..
Neonatal mortality	1 (<1%)	0	1 (<1%)	1·00 (0·04 to 25·30)	..
Obstetric outcomes per livebirth					
Preterm delivery 20 to <28 weeks' gestation, singletons	0/163	0/151	1/155 (<1%)	..	..
Preterm delivery 28 to <32 weeks' gestation, singletons	0/163	2/151 (1%)	0/155	..	..
Preterm delivery 32 to <37 weeks' gestation, singletons	15/163 (9%)	11/151 (7%)	7/155 (5%)	2·04 (0·86 to 5·34)	1·61 (0·64 to 4·38)
Preterm delivery 28 to <32 weeks' gestation, twins	1/11 (9%)	0/8	0/7	..	..
Preterm delivery 32 to <37 weeks' gestation, twins	1/11 (9%)	0/8	0/7	..	..
Preterm delivery 20 to <28 weeks' gestation, singletons	4/11 (36%)	4/8 (50%)	2/7 (29%)	1·27 (0·25 to 9·18)	1·75 (0·34 to 12·6)
Gestational diabetes	44/174 (25%)	37/159 (23%)	32/162 (20%)	1·28 (0·82 to 2·03)	1·18 (0·73 to 1·90)
Hypertensive disorders in pregnancy	10/174 (6%)	8/159 (5%)	12/162 (7%)	0·78 (0·33 to 1·80)	0·68 (0·27 to 1·64)
Perinatal outcomes per livebirth					
Singleton birthweight, g (469 babies)	3178·4 (463·2)	3147·1 (515·2)	3245·9 (450·3)	−67·5 (−170·1 to 35·1)†	−98·8 (−210·3 to 12·7)†
Twin birthweight, g (52 babies‡)	2370·0 (444·7)	2598·9 (342·2)	2300·0 (434·8)	70·0 (−260·7 to 400·7)†	298·9 (−24·2 to 622·1)†
Singleton birthweight, very low	0/163	1/151 (<1%)	1/155 (<1%)	..	1·03 (0·04 to 26·00)
Singleton birthweight, low	6/163 (4%)	8/151 (5%)	5/155 (3%)	1·14 (0·34 to 3·96)	1·64 (0·55 to 5·44)
Singleton birthweight, high	5/163 (3%)	5/151 (3%)	4/155 (3%)	1·19 (0·31 to 4·80)	1·28 (0·34 to 5·18)
Singleton birthweight, very high	1/163 (<1%)	0/151	0/155	..	..
Major congenital abnormalities§	1/185 (<1%)	0/167	1/169 (<1%)	..	..
Admission to NICU	15/185 (8%)	8/167 (5%)	18/169 (11%)	0·76 (0·40 to 1·46)	0·45 (0·20 to 1·01)
Stillbirth	0/185	0/167	0/169	..	..
Neonatal mortality	1/185 (<1%)	0/167	1/169 (<1%)	0·91 (0·06 to 14·50)	..

Data are mean (SD), n (%), n/N (%), or RR (95% CI). RR=relative risk. FET=frozen embryo transfer. NICU=neonatal intensive care unit. *Including cycles with FET after the first endometrial preparation (artificial, modified natural, or natural, as per randomisation) and the second endometrial preparation (all artificial). †These values are absolute between-group differences and 95% CIs. ‡26 pairs of twins. §These were ventricular septal defect in the natural cycle group and congenital muscular torticollis in the artificial cycle group; in the modified natural cycle group there were two congenital anomalies that resulted in termination of pregnancy (one Down syndrome with pregnancy terminated at 16 weeks' gestation and one cystic hygroma with pregnancy terminated at 21 weeks' gestation, ie, no livebirths with congenital abnormalities in this group).

Table 4: **Obstetric and perinatal outcomes after one FET**

groups. No artificial cycles were cancelled, probably because we excluded women with endometrial cavity or uterine abnormalities, and because we started using oestradiol 8 mg/day early in the cycle (meaning that no follicle could escape to become dominant).

The effectiveness of endometrial preparations protocols in ovulatory women is a topic of interest and ongoing research. A 2017 Cochrane review[8] did not find sufficient evidence to support the use of one cycle regimen over another for FET in subfertile women with regular ovulatory cycles, noting that the quality of evidence was low to very low. However, none of the included studies directly compared artificial, modified natural, and natural cycles. In addition, previous trials[16,21–23] considered cancellation cycles to be the same as unsuccessful cycles after FET. This approach could make the analysis more straightforward by including only one cycle, but might not reflect the reality of clinical practice. For individuals with cancelled cycles, frozen embryos remain unused and their chance of having a livebirth is unchanged. Therefore, we believe it is important to take a subsequent FET into account for those whose first cycle was cancelled. Although randomly assigning participants to endometrial preparation with a natural or modified natural cycle was ethical, practical considerations and individual preference meant that it was not feasible for us to schedule women who had a cancelled cycle to use the same protocol for a second round of FET. Despite the different approach taken to the analysis, our results are consistent with existing meta-analysis conclusions[6,8,24] and data from previous retrospective studies,[21–23] which reported no significant difference in the livebirth rate between different strategies for endometrial preparation in FET cycles.

Compared with the current trial, the largest randomised trial comparing modified natural versus artificial cycle (the ANTARCTICA trial)[16] reported lower livebirth rates and a higher cycle cancellation rate in the artificial endometrial preparation group. The cycle cancellation rates for natural and modified natural cycles in our study were similar but, in contrast to the ANTARCTICA trial, no artificial cycles were cancelled. Possible explanations for the differences in data between the two trials include between-centre differences in artificial cycle implementation versus consistent single-centre procedures, and use of slow freezing versus vitrification for embryo cryopreservation. Inclusion and exclusion criteria might also have played a role, because women with cavity adhesion were excluded from our study but it is not clear whether this was the case in the ANTARCTICA trial. Other differences between our study and the ANTARCTICA trial included the dosage of oestradiol used (8 mg/day *vs* 6 mg/day), the endometrial cutoff thickness for initiation of progesterone (7 mm *vs* 8 mm), and an inadequate embryo survival of zero *vs* 58·1%. The higher livebirth rates in our study compared with the ANTARCTICA trial could be due to differences in the embryo freezing method, or might have been influenced by the geographical origin of the study populations. The mean age of the included women was almost identical (33–34 years), and in both studies women had normal menstrual cycles.

There is ongoing debate about whether FET performed in the presence of more physiological and natural hormonal profiles results in fewer maternal and fetal complications compared with artificial cycles. We found no statistically significant between-group differences in the rate of other maternal and neonatal complications in our study. Existing data include a 2023 systematic review and meta-analysis,[7,25] which found significantly higher risks for several adverse obstetric and neonatal outcomes in artificial versus natural FET cycles. The overall quality of evidence used in the latest systematic review and meta-analysis was very low to moderate (moderate for key maternal outcomes such as hypertensive disorders in pregnancy and postpartum haemorrhage), which prevents us from making strong conclusions.

The debate about the safety of different endometrial preparation protocols is driven by the observation that ovulatory cycles are characterised by the presence of the corpus luteum and its secretion of relaxin, nitrous oxide, and endothelial growth factor. The absence of the corpus luteum in artificial cycles for endometrial preparation before FET is a serious concern that has been evaluated and discussed previously.[25,26] Endometrial preparation with artificial cycle FET has been shown to be associated with higher rates of hypertensive disorders in pregnancy, postpartum haemorrhage, and preterm birth compared with normal cycle FET.[27,28] The rate of miscarriage at less than 12 weeks duration with the artificial cycle FET strategy in this study was slightly, but not significantly, higher than those in the modified natural and natural strategy groups (8% *vs* 5% *vs* 6%). Potential contributors to the minimal between-group differences in the rate of preeclampsia could have been a lack of power due to the small number of events, the characteristics of the study population (young, ovulatory, and lean), or the effect of the routine pre-eclampsia prevention strategy used at our centre (aspirin 162 mg/day until 36 weeks' gestation if the calculated risk at 11–14 weeks' gestation was >1/100 using the Fetal Maternal Medicine model). Nevertheless, the rate of hypertensive disorders in pregnancy based on the number of ongoing pregnancies was 8% for all the artificial cycles performed in this study, compared with 4% in the natural and modified natural cycles that resulted in an ongoing pregnancy. This represents an approximate doubling of the rate of hypertensive disorders in pregnancy during artificial cycles. However, it is important to note that natural and modified natural cycles can only be used in women with a regular menstrual cycle (ovulatory cycle), whereas artificial cycles can be used in all women presenting for infertility treatment, including those with higher risk of obstetric complications, such as women with polycystic ovary syndrome or menopausal women.[23]

Strengths of our study are that it was conducted at a large single centre and that procedures were performed

in a highly uniform manner, including the use of vitrification for embryo cryopreservation in all participants to maximise embryo survival and quality after warming. A limitation of the study is its open-label design. However, the nature of the treatment interventions meant that it was not possible for physicians and participants to be unaware of treatment allocation, and the primary outcome of livebirth was objective and is not subject to detection bias, as was the case for all other outcomes. Performance bias is also unlikely to have occurred because the stage and number of embryo transfers were similar between all the study groups. Our use of a design with three interventional groups could have resulted in lower statistical power compared with a two-group design comparing a combination of natural and modified natural cycles with artificial cycles. Although all participants in this study provided informed consent to be randomly assigned to one of the three initial strategies for endometrial preparation, it is possible that the acceptability of these different approaches could vary in clinical practice. The absence of acceptability data is a limitation of this study and is an important area for future research. Another key limitation is that the study was not powered to detect between-group differences in the rate of maternal and neonatal complications. Therefore, although the differences in rates of events such as pre-eclampsia did not differ significantly between groups, this might have been due to insufficient power rather than no relevant difference. In addition, the power calculation was based on a 10% between-group difference in the livebirth rate, which is a large difference in current assisted reproductive technology practice. This difference implies that a small benefit of one of the strategies, in particular the natural cycle strategy, cannot be excluded. The external generalisability of the study findings to a diverse group of women managed in a range of different health-care settings might be limited by the nature of the study population. We only included ovulatory women from Viet Nam from a single centre. A quarter of eligible women declined to participate, the upper age limit for enrolment was 45 years, and 35% of participants had double embryo transfer, all of which might affect generalisability. Some of this information should come from ongoing randomised controlled trials that are comparing different endometrial preparation protocols for FET, such as NatPro[29] and COMPETE.[30]

In conclusion, for ovulatory women undergoing FET, the livebirth rate did not differ significantly between endometrial preparation strategies starting with a natural, modified natural, or artificial cycle, followed by a subsequent artificial cycle in those with a cancelled first cycle. No definitive conclusions can be made regarding the comparative safety of the three approaches.

Contributors

VNAH, TDP, NTN, RW, RJN, BWM, TMH, and LNV designed the study and monitored data collection. TDP conducted data analysis and verification. VNAH, TDP, and LNV wrote the statistical analysis plan, planned the first draft of the manuscript with the assistance of a professional medical writer, and wrote the first draft of the manuscript. All authors had full access to the data and were involved in editing the manuscript, critical revisions of the manuscript, and the final decision to submit for publication.

Declaration of interests

LNV reports speaker and conference fees from Merck, MSD, and Ferring; grants from MSD; and scientific board fees from Ferring. TMH reports speaker fees from Merck, MSD, and Ferring. RW reports grant funding from the National Health and Medical Research Council of Australia (NHMRC). RJN reports grant funding from the NHMRC. BWM reports acting as a paid consultant to Merck, ObsEva, and Guerbet; owning stock in ObsEva; and holding an NHMRC Investigator Grant. All other authors declare no competing interests.

Data sharing

Access to relevant anonymised participant-level data will be considered on reasonable request submitted to the corresponding author.

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