Over the last decade the use of frozen–thawed embryo transfer has substantially increased, and currently up to one in two embryos transferred has been cryopreserved. To support implantation, endometrial and embryo maturity are required to be synchronized. This can be achieved in various ways. The most commonly applied endometrial preparation methods are the "natural cycle," in which the sequential estrogen and P necessary for endometrial maturation are derived from the developing follicle, and the "artificial" cycle, in which these are sequentially administered. Review of the published data comparing these approaches does not identify a superior approach in terms of clinical outcomes. However, although the "natural cycle" avoids the need for luteal support, the artificial cycle provides more control over timing of ET, and the "modified" natural cycle, in which ovulation is triggered exogenously, may offer both of these advantages. The optimal monitoring strategy for freeze–thaw cycles remains unclear, because only a few studies have addressed this question. Further studies are also required to determine the ideal dosage, method of administration, and duration of estrogen and P supplementation in artificial cycle frozen embryo transfer. (Fertil Steril 2018;109:768–74. ©2018 by American Society for Reproductive Medicine.)

Key Words: Artificial cycle, endometrium preparation, frozen–thawed embryo transfer, modified natural cycle

Discuss: You can discuss this article with its authors and with other readers at https://www.fertstertdialog.com/users/16110-fertility-and-sterility/posts/30466-25539

Over the last decade the proportion of ETs derived from freeze–thaw cycles has increased substantially. According to the report by the US Centers for Disease Control and Preventions summarizing the number of assisted reproductive treatments in 2014, one in two of all embryos transferred in the United States had been cryopreserved (Fig. 1). The European Society of Human Reproduction and Embryology has reported comparable trends (1).

This rise in the number of frozen embryo transfers (FETs) performed can be ascribed to four major developments. First, the introduction of ovarian hyperstimulation at the start of the 1980s resulted in an increased number of embryos available after each treatment cycle. During the same period rapid developments in laboratory techniques increased both the proportion of oocytes that generated viable embryos as well as the live birth rate (LBR) per embryo transferred. This reduced the need for multiple embryo transfer and increased the storage of supernumerary embryos for later use.

The second key development has been the widespread introduction of elective single embryo transfer (eSET). Extended embryo culture was introduced on the premise that only embryos of excellent quality with the highest potential for implantation will reach blastocyst stage selection (2–4). By prolonging the in vitro culture up to 5 days and allowing blastocyst stage embryos to develop, time and natural selection would aid clinical embryo selection. Indeed, the LBR per blastocyst transfer has been demonstrated to be significantly higher compared with cleavage stage ET. The case for multiple embryo transfer was therefore further weakened, and eSET was implemented as standard care in many countries. Given the success of the eSET strategy, it has also been widely adopted for the transfer of cleavage stage embryos.
The third major contributor to the number of FET cycles performed has been the widespread replacement of slow-freeze cryopreservation by vitrification (5, 6). The slow-freeze cryopreservation technique resulted in survival rates varying between 35% and 70%, compared with more than 90% after vitrification. At cleavage stage the low survival rates after slow-freeze cryopreservation may be compensated for by the number of available embryos. During extended culture a greater number of embryos may be lost during the in vitro process, resulting in fewer embryos being available for cryopreservation. As a result, the risk of a failure to transfer blastocysts after slow-freeze cryopreservation is higher than that associated with cleavage stage embryos (odds ratio 2.5, 95% confidence interval 1.76–3.55) (4). Although widespread adoption of vitrification will not necessarily alter this, the overall number of ETs performed can be expected to increase owing to improved survival rates. How cumulative LBRs after vitrification of cleavage stage embryos compare with those after vitrification at the blastocyst stage remains to be elucidated (7, 8).

The latest element to extend the use of FET has been the introduction of “freeze-all” strategies that segment ovarian stimulation in a different cycle from that in which the embryo is transferred (9). This approach is argued to prevent many of the detrimental aspects of ovarian stimulation on the health of the woman and endometrial function. A discussion of the pros and cons of this approach is beyond the scope of this article, but freeze-all protocols may improve outcomes in women at risk of ovarian hyperstimulation syndrome and patients who develop an elevated P level at the time of hCG triggering (10, 11). The results of several ongoing randomized controlled trials will clarify the place of freeze-all protocols in contemporary practice. However, if these studies show improved outcomes from freeze-all strategies compared with fresh transfer, a further rise in the number of FET cycles is to be expected.

Even though FET is increasingly considered a safe and (cost) effective element of assisted reproduction, there are some possible safety issues that need to be further elucidated. Frozen embryo transfer has been associated with an increased risk of neonates being born large for gestational age (12–15). This in turn may be associated with a higher incidence of perinatal complications, such as shoulder dystocia and the accompanying neonatal and maternal comorbidity. The cause of the reported increase in incidence of large for gestational age offspring after FET remains unclear. Indeed, follow-up studies have indicated a tendency for in vitro-generated embryos to produce slightly smaller babies compared with those spontaneously conceived (13). It has been proposed that the transfer of embryos into a uterus that has not been subject to the hormonal impact of ovarian stimulation may serve to “unmask” the previously reported tendency of in vitro-generated embryos to produce “large offspring syndrome.” Moreover, there is some suggestion that the endometrial preparation method used in FET cycles can influence birth weight, with one study reporting a tendency to a higher birth weight after modified natural cycle FET (mNC-FET) compared with artificial cycle FET (AC-FET) (16). Placenta accreta is another possible obstetric complication that has been reported to arise more frequently after FET (17, 18). Until further data are available to confirm or refute these observations, a degree of caution in the use of FET as a routine approach in IVF would seem to be merited.

Despite the large number of FETs already performed in many clinics, the best method of endometrial preparation before thawing and transferring continues to be debated. The main aim of endometrial preparation is to optimize pregnancy rates after FET by synchronization of the stage of ET with that of endometrial receptivity. Several methods of achieving this have been described, but they all result in sequential exposure of the endometrium to the proliferative actions of E2 and the secretory changes induced by P. The current paradigm emphasises the importance of transferring the embryo during the “window of implantation,” which opens on the third or fourth day after ovulation and normally remains open for 4 to 5 days. Given that the timing of P is considered to be the key to when this window opens, preparation methods for frozen–thawed embryo transfer are all predicated on detecting either the spontaneous LH surge or on triggering ovulation and then ensuring appropriate exposure to either endogenous or exogenous P. Among the various methods of achieving this, the most widely practiced are the (modified) natural cycle FET (NC-FET) and AC-FET.

**MODIFIED NC-FET**

In NC-FET the sequential exposure to estrogen (E) and P is derived from spontaneous follicle development and ovulation. The timing of embryo thawing and transfer is based on either the observation or triggering of the ovulation. This method of endometrium preparation can, therefore, only be offered to patients with an ovulatory cycle. A distinction should be made between the so-called true natural cycle (tNC-FET) and the modified natural cycle (mNC-FET). In tNC-FET the onset of an LH surge, which causes ovulation of the dominant follicle, is monitored in either blood or urine. As soon as the onset of the LH surge has been determined, thawing and transfer can be planned accordingly. In mNC-FET the development of the dominant follicle is monitored...
by ultrasound. On reaching a follicle size of 16–20 mm, ovulation is triggered by an injection of hCG. Taking into account a 36–38-hour interval between the injection and the moment of ovulation, thawing and transferring is planned (19). Often ultrasound monitoring is supplemented with endocrine monitoring (20). This is primarily justified to detect a premature LH or P rise, both of which may disrupt the appropriate timing of ET. However, there is little evidence that extensive endocrine monitoring modifies outcomes. A small randomized controlled trial showed comparable ongoing pregnancy rates among patients receiving extensive endocrine monitoring vs. ultrasound monitoring alone (21). Elevated P levels at the time of hCG administration are observed in more than one-fourth of all patients undergoing NC-FET cycles (22, 23). Despite the observed negative effect of elevated P levels at the time of hCG administration are observed in more than one-fourth of all patients undergoing NC-FET cycles (22, 23). Despite the observed negative effect of elevated P levels at the time of hCG administration on pregnancy rates after fresh embryo transfer, no such effect was reported in patients undergoing mNC-FET (22). Lee et al. (23) showed comparable results in their study. A subgroup analysis of the study by Lee et al. showed that an extended duration of P elevation did result in lower pregnancy rates (23). So far the results of studies focusing on the effect of elevated LH levels at the designated time of hCG injection have been variable, and cancelling mNC-FET solely on the detection of an LH surge is not supported by the published evidence (22, 24, 25).

Endometrial thickness measurement is commonly integral to monitoring both NC-FET and AC-FET cycles. The minimal endometrial thickness that should be observed before proceeding to thawing and transferring remains subject to debate. Despite the conflicting evidence with regard to whether thickness alone reflects the histologic, immunologic, and gene-expression adaptation needed for optimal receptivity of the endometrium, an endometrium lining of at least 7 mm is often considered to be desirable before proceeding to thawing and transferring (20, 26, 27). The foundation on which this “cut off” is based is weak because data supporting it are limited to small retrospective studies (28).

Another lively discussion continues concerning the necessity for luteal phase support (LPS) in NC-FET. The available studies that have addressed this reveal conflicting results. This may reflect variations in study design as well as the various methods of LPS, including vaginal suppositories, hCG injections, and IM P. Table 1 provides an overview of studies of LPS in NC-FET (29–35). It is noteworthy that the positive effect of LPS was only observed in those studies using tNC-FET. This raises the suggestion that the hCG administration used in mNC-FET for planning purposes might also represent a form of LPS. Even though the results of the various studies are conflicting and the heterogeneity is large, the routine use of LPS in this context does not seem necessary. Given the treatment burden LPS can represent, this can be considered an advantage of the natural cycle approach.

### ARTIFICIAL CYCLE FET

Natural cycle FET is often considered to have the disadvantage of making prediction of the date of thawing transfer difficult. In this regard, AC-FET is considered advantageous. The ability to predict and plan these procedures, together with the reduced monitoring requirements, are considered by many clinics to justify the requirement (made necessary by the lack of a corpus luteum) for the patient to take luteal support medication for up to 12 weeks in case of a pregnancy. In anovulatory women AC-FET is necessary. To mimic the endocrinology of the follicular phase of the menstrual cycle, E is administered from the first or second day of the menstrual cycle. Adding P to this regimen after several days/weeks imitates the mid-cycle shift to the secretory phase. Planning of thawing and transferring of the embryos depends on the start of P addition. By delaying the moment of P supplementation, thawing and transferring can be delayed, allowing some flexibility.

There is little consensus regarding the necessary minimal duration of E supplementation. Most commonly E is supplemented for 12–14 days before proceeding with

---

**TABLE 1**

<table>
<thead>
<tr>
<th>First author (reference)</th>
<th>Type NC</th>
<th>Design</th>
<th>Intervention</th>
<th>Endpoint</th>
<th>Outcome, n (%)</th>
<th>OR (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bjuresten (29)</td>
<td>tNC</td>
<td>RCT</td>
<td>P vaginal tablets 400 µg/d vs. no luteal phase support</td>
<td>LBR</td>
<td>65/219 (26.7) vs. 44/216 (20.4)</td>
<td>1.4 (0.91–2.1)</td>
</tr>
<tr>
<td>Eftekhari (30)</td>
<td>mNC</td>
<td>RCT</td>
<td>P injections 50 mg 2×/d vs. no luteal phase support</td>
<td>CPR</td>
<td>69/219 (31.5) vs. 54/216 (25.0)</td>
<td>1.6 (1.1–2.5)</td>
</tr>
<tr>
<td>Kim (31)</td>
<td>mNC</td>
<td>Retrospective study</td>
<td>P vaginal 100 µg/d vs. no luteal phase support</td>
<td>CPR</td>
<td>17/51 (33.3) vs. 14/51 (27.4)</td>
<td>1.3 (0.57–3.1)</td>
</tr>
<tr>
<td>Kyrou (32)</td>
<td>mNC</td>
<td>Retrospective study</td>
<td>P vaginal tablets 600 µg/d vs. no luteal phase support</td>
<td>CPR</td>
<td>53/243 (21.8) vs. 44/209 (21.1)</td>
<td>1.1 (0.67–1.6)</td>
</tr>
<tr>
<td>Lee (33)</td>
<td>tNC</td>
<td>Retrospective study</td>
<td>2× hCG 1,500 IU injection vs. no luteal phase support</td>
<td>CPR</td>
<td>53/205 (25.9) vs. 59/203 (29.1)</td>
<td>0.85 (0.55–1.3)</td>
</tr>
<tr>
<td>Lee (34)</td>
<td>tNC</td>
<td>RCT</td>
<td>2× hCG 1,500 IU injection vs. no luteal phase support</td>
<td>OPR</td>
<td>60/225 (26.7) vs. 70/225 (31.3)</td>
<td>1.2 (0.83–1.87)</td>
</tr>
<tr>
<td>Veleva (35)</td>
<td>tNC</td>
<td>Retrospective study</td>
<td>P vaginal 200 µg vs. no luteal phase support</td>
<td>LBR</td>
<td>220/974 (22.6) vs. 48/302 (15.9)</td>
<td>1.5 (1.1–2.2)</td>
</tr>
</tbody>
</table>

Note: CI = confidence interval; CPR = clinical pregnancy rate; LBR = live birth rate; OPR = ongoing pregnancy rate; OR = odds ratio; RCT = randomized controlled trial.

P supplementation. Evidence supporting a shorter period of E administration is scarce, and there are indications that this might result in higher miscarriage rates (36). Even though AC-FET provides more freedom in the planning of embryo thawing and transfer, some studies suggest caution against extended E supplementation. In non-donor patients a trend toward both lower LBRs and increased pregnancy loss has been reported when E was supplemented for more than 4 weeks (37). The number of (cohort) studies is limited, however, and they often involve patients receiving gamete donation.

As in NC-FET, endometrial thickness is often monitored; however, again the minimal thickness that should be achieved before adding P to the regimen remains unclear. An endometrium greater than 8–9 mm has been reported to be associated with good outcomes (28, 38–40). To reach this minimal endometrial thickness, various E supplementation schedules have been developed. The most commonly reported optimal doses vary between 4 and 12 mg/d. Although there is scant evidence in this regard, increasing the dosage from 4 to 6 mg has been shown to raise serum E levels but without an impact on endometrial thickness (41). Because neither pregnancy rates nor LBRs were an endpoint in this study, randomized trials are necessary to evaluate the effect of increasing dosages on these endpoints. Reducing the first-pass effect by vaginal or transdermal administration of E supplementation to optimize the effect on the endometrium has been suggested by some (42, 43). Even though endometrial thickness seems to be greater after vaginal administration, pregnancy rates have not been reported to be higher (44, 45). Pregnancy rates after AC-FET with transdermal E2 are comparable to those after oral E2 supplementation and might therefore offer a more patient-friendly alternative (46).

The optimal duration and dosage of P supplementation also remains to be elucidated. The route of administration does not seem to influence pregnancy rates and LBRs (47–50). Intramuscular injections of P, despite offering excellent bioavailability, are however often considered less desirable given their associated discomfort side effects and the increased risk of thrombosis (49, 51–53). Vaginal administration is considered to be the administration method of choice from a patient’s point of view, and this route is the first line in most European centers (49, 53). In contrast, US clinics are more ready to advise the IM route. The optimal dose of vaginal P in this context has been the subject of little research. One study has indicated that doubling the standard dosage of 90 mg/d may increase LBRs in anovulatory patients (54). The role of monitoring serum P levels in the luteal phase has received increased interest in recent years, and there is some evidence that outcomes are improved when levels above 100 nmol/L are maintained in the stimulated cycle and above 30 nmol/L in the spontaneous cycle (55). Recently a randomized controlled trial comparing extended P supplementation before embryo thawing and transferring (5 days) with the standard period of supplementation (3 days) in vitrified cleavage stage embryos showed that prolonged administration does not increase pregnancy rates but might lower early pregnancy loss (56).

Even though AC-FET is effective in patients with and without an ovulatory cycle, supplementing E and P to ovulatory patients does not guarantee complete suppression of follicular growth. Reported ovulation rates vary between 0.7% and 8% of the cycles (57–59). A possible explanation for the wide variety in this reported incidence might reside in the various E supplementation protocols prescribed. If the dose of E is low or the duration of the administration is long, escape ovulation might be more likely to occur. The route of E administration may be a further contributory factor worthy of study. Irrespective of the cause of insufficient suppression, if a dominant follicle is observed, signs of luteinization should be ruled out, for example by determination of LH and P levels. Embryo transfer in case of luteinization might result in desynchronization between endometrium and embryo, which can influence pregnancy rates negatively. To avoid ovulation a GnRH agonist can be added to the regimen of E and P. Despite the small but significant benefit with regard to pregnancy rates, supplementation with a GnRH agonist is often declined because of side effects and increased costs (60, 61).

**COMPARING mNC-FET WITH AC-FET**

A meta-analysis of eight retrospective trials comparing NC-FET with AC-FET included four that used NC-FET and four that used mNC-FET (61). The conclusion of this systematic review was that neither NC-FET nor AC-FET was superior in terms of clinical outcomes.

Subgroup analyses separating true NC-FET and mNC-FET did not alter these conclusions. However, the heterogeneity of the included studies with regard to the primary outcomes reported, cancellation, and inclusion criteria was substantial. Even though most studies reported comparable pregnancy rates and LBRs between the two methods of endometrium preparation, one suggests a possible increase in miscarriage rates after AC-FET (62). These findings have not thus far been confirmed by other studies. More recently Yarali et al. (63) performed a systematic review including both retrospective and prospective studies. In line with the 2013 review by Groenewoud et al. (64), it was concluded that the means of endometrial preparation did not impact on pregnancy rates or LBRs. However, miscarriage, cancellation rates, and cost-effectiveness were not described in any of the included trials. The first large randomized controlled trial addressing LBRs, cancellation rate, and cost was published in 2016 (64). The ANTARCTICA trial was performed between February 2009 and April 2014 and included 995 patients. Patients were allocated to endometrium preparation with either mNC-FET or AC-FET. The primary outcome of this study was LBR. Live birth rates after mNC-FET (11.5%) were comparable to those after AC-FET (8.8%). On the basis of the absolute difference of −0.027 (95% confidence interval −0.065 to −0.012), non-inferiority of AC-FET over mNC-FET was concluded. Despite comparable live birth and pregnancy rates, cancellation rates were significantly higher in the AC-FET arm (AC-FET 26.7% vs. mNC-FET 20.4%; odds ratio 1.4, 95% confidence interval 1.1–1.9, P = .02). This was mainly due to the strict cancellation criteria with regard to endometrial thickness. Costs were
comparable between both treatment entities (mNC-FET €617.50 vs. AC-FET €625.73, P = .54). On the basis of the results of the ANTARCTICA trial, one could conclude that to ovulatory patients both methods of endometrial preparation can be offered without compromising pregnancy rates and/or LBRs. Some concerns were raised after the publication of the ANTARCTICA trial; both pregnancy rates and LBRs were deemed to be low, and there was a high miscarriage rate (65). These may reflect the method of presenting these rates (rates per started cycle) and the use of slow-freeze procedures (64). Since the publication of the ANTARCTICA trial, a further retrospective study comparing mNC-FET with AC-FET has been reported. Orvieto et al. (66) concluded that mNC-FET with luteal phase support was to be preferred over mNC-FET without LPS or AC-FET. Ghobara et al. (60) recently updated their Cochrane systematic review. No additional randomized controlled trials comparing mNC-FET with AC-FET could be identified. Ghobara et al. therefore concluded that there is no difference in LBRs between the different methods of FET preparation. Figure 2 shows an update of the 2013 meta-analyses by Groenewoud et al., to which the results of the ANTARCTICA trial have been added. The conclusion remains that no method can be shown to be superior with regard to LBRs. Figure 3 provides a summary of the comparison of NC-FET versus AC-FET.

In conclusion, despite the wide experience with the above-mentioned protocols for endometrium preparation, there is a lack of evidence to support the superiority of one method over the other. Additional studies resulting in more detailed information on essential monitoring and luteal phase support in NC-FET as well as optimal E/P regimens in AC-FET are still required. For now, both mNC-FET and AC-FET can be offered to ovulatory patients without compromising pregnancy rates and LBRs. At this time, patient preference together with logistical aspects for the treating clinic would seem to be the key factors governing selection of the optimal means of preparing the endometrium for FET. However, as the use of FET continues to increase and further refinements in endometrial programming protocols arise, it is possible that individualized approaches will be shown to be beneficial.

REFERENCES


TABLE 2

Summary, NC-FET vs. AC-FET.


FIGURE 3


43. Laliberte F, Dea K, Duh MS, Kahler KH, Rolli M, Lefebvre P. Does the route of administration for estrogen hormone therapy impact the risk of venous thromboembolism? Estradiol transdermal system versus oral estrogen-only hormone therapy. Menopause 2011;18:1052–9.


