

Clinical rationale for cryopreservation of entire embryo cohorts in lieu of fresh transfer

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Recent dramatic increases in success rates with frozen–thawed embryo transfer (FET) are encouraging, as are numerous findings of several reduced risks with FET when compared with fresh transfer. These reduced risks include low birth weight and prematurity, among others. However, FET is also associated with increased risks of macrosomia and large for gestational age. There have been reports of greater implantation and pregnancy rates with FET than with fresh autologous embryo transfer, suggesting superior endometrial receptivity in the absence of ovarian stimulation. As cryo-technology evolves, there is potential for further increase in FET success rates, but for now it may be best to follow an individualized approach, balancing fresh transfer and embryo cohort cryopreservation options while considering patient characteristics, cycle parameters, and clinic success rates. (*Fertil Steril*® 2014;102:3–9. ©2014 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, embryo cryopreservation, frozen embryo transfer, ovarian stimulation, perinatal risk

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From 2006 to 2012, the number of autologous frozen–thawed embryo transfers (FET) reported to the Society for Assisted Reproductive Technology (SART) increased 82.5%, whereas fresh cycle starts increased by 3.1%. There was a clear trend toward increased FET usage relative to fresh cycles in that period (Fig. 1). In 2012 SART’s member clinics reported 17.3% more FETs and 3.2% fewer fresh cycle starts when compared with 2011, suggesting an accelerating trend toward FET.

This increased use of FET corresponded with a more rapid increase in live birth rates with FET than with fresh transfer. In 2006 the reported live birth rates per transfer were 33.1% with FET and 44.9% with fresh transfer in pa-

tients <35 years old, corresponding with a risk ratio (RR) of 0.737 when comparing FET with fresh transfer. By 2012 those respective rates were 42.4% with FET and 47.1% in fresh transfers, so that the RR had increased to 0.900. Over that period, the RR of live birth with FET compared with fresh transfer increased in each SART age group (Fig. 2), and reported birth rates per transfer with FET exceeded those with fresh transfer in four of the five age groups in 2012 (1).

The numbers of live births with FET have therefore also increased more than with fresh transfers (Fig. 3). In 2012, the number of live births with fresh autologous transfer decreased by 2.6% from the prior year, whereas the number of live births from autologous FET

increased by 28.0%. Live births from FET were 31.5% of all reported autologous live births in 2012, compared with just 16.9% in 2006 (1).

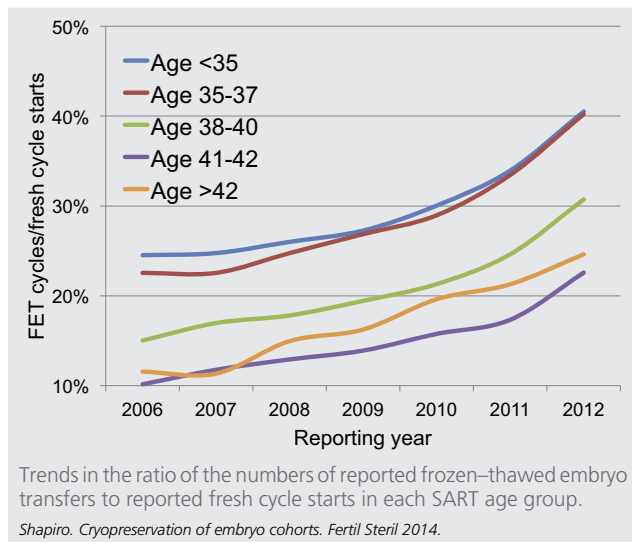
The increase in FET usage and success rates may have resulted from multiple simultaneous causes. Improved cryopreservation techniques may reduce embryo cryo-damage and therefore increase success rates and confidence in cryopreservation and FET. This might encourage more frequent freezing of entire cohorts rather than freezing “second-best” embryos after the morphologically best embryos are transferred in fresh cycles. Cohort banking is also increasingly routine after the use of a GnRH agonist “trigger” to prevent ovarian hyperstimulation syndrome (OHSS) in high responders. The increased use of genetic screening also increases the use of cryopreservation, because embryos are often frozen while awaiting test results, and transfer of confirmed euploid embryos may contribute to increasing FET success rates. Lastly, the steady

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FIGURE 1



decrease in national average numbers of embryos used in each transfer should have left more embryos for potential cryopreservation and FET.

The increasing use of FET and the increasing numbers of resulting births compel continuing scrutiny of risks associated with FET, including risk comparisons with the alternative of fresh transfer. Comparisons between FET and fresh transfer are also comparisons of their respective uterine environments, and many have suggested that the reported outcome and risk differences are due to negative effects of controlled ovarian stimulation (COS) on the uterine environment in fresh transfers. This review will therefore start by examining the effects of COS on the uterine environment.

FIGURE 2

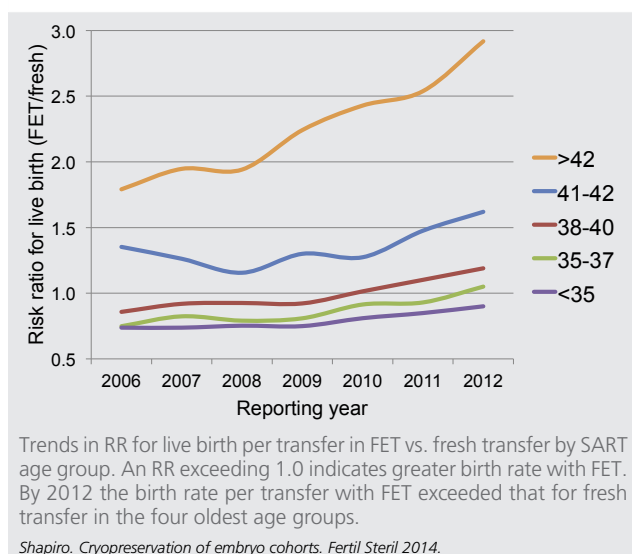
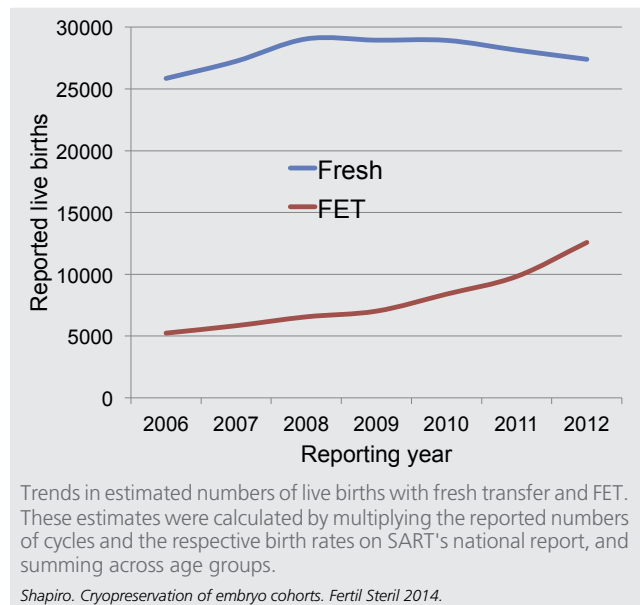


FIGURE 3



EFFECT OF COS ON ENDOMETRIAL DEVELOPMENT AND RECEPTIVITY

Controlled ovarian stimulation with exogenous gonadotropins is routinely used to promote follicular development so that many oocytes may be obtained for cycles of IVF. The developing follicles are typically far more numerous than in natural menstrual cycles and collectively produce supraphysiologic levels of E_2 , P, and other hormones. Estradiol and P are closely linked to endometrial development and maturation.

Two frequently observed features of endometria after COS are advanced histology (2-4) and advanced down-regulation of the P receptor (3, 4), each a suspected indicator of an advanced receptive phase. The degree of histologic advancement correlates with premature P elevation and with implantation failure through an effect of embryo-endometrium asynchrony (2, 5, 6). Nucleolar channel system formation is also advanced after COS (7).

Implantation patterns in cycles with and without COS have shown greater implantation rates of day-5 blastocysts when compared with day-6 blastocysts in cycles with COS exposure, but not in cycles without COS exposure (8, 9), and greater implantation rates of day-6 blastocysts in freeze-thaw cycles than in fresh transfer after COS (9-11). One randomized trial found greater pregnancy and implantation rates with frozen-thawed embryos than with fresh embryos transferred into endometria exposed to COS (12). A comparison of embryos in a shared oocyte donation program found reduced pregnancy rates in donors exposed to COS when compared with recipients without COS exposure using oocytes from the same retrievals (13). Collectively, these findings suggest reduced endometrial receptivity after COS exposure, perhaps through a selection bias against implantation of embryos

that develop slowly. Premature P elevation may exacerbate the effect (14).

It cannot be precisely known in advance which patients or cycles will have low preovulatory P and rapidly developing embryos. Furthermore, only a minority of cycles have such ideal synchrony (15). Therefore, cohort cryopreservation might convey an a priori greater chance of success (16). Alternatively, a flexible protocol that keeps open the options of fresh transfer or cohort cryopreservation, while mindful of embryo pace and P levels, may offer an excellent alternative. Indeed, one study found an implantation rate of 79.8% with fresh euploid day-5 blastocysts transferred on day 6 (17).

Previously, real-time decisions to freeze entire cohorts in lieu of fresh transfer were counted as failed fresh cycles in the reports of SART. Forthcoming revisions will effectively remove this artificial penalty from SART's reports (18) and might therefore encourage increased use of cohort cryopreservation and subsequent FET.

Frozen embryo transfer has been associated with reduced risk of implantation failure when compared with fresh transfer in a randomized trial (12). This randomized trial involved conventional slow freezing of entire cohorts of bipronuclear oocytes, with subsequent thaw of the entire cohorts, postthaw extended culture to the blastocyst stage, and transfer of the two best blastocysts from each cohort. Postthaw culture was used to ensure transferred embryos were free of cryo-damage, as demonstrated by their resumed development to morphologically acceptable blastocysts.

Resumed development is a more rigorous indicator of postthaw viability than is immediate postthaw survival assessment alone. For example, one study found that 100% of vitrified-warmed blastocysts survived, but only 45% resumed development (19). Another study of thawed conventionally slow-frozen bipronuclear oocytes found an 85.5% survival rate, but only 53.5% as many blastocysts formed from thawed bipronuclear oocytes as in matched fresh cycles (20). These findings suggest frequent, latent cryopreservation damage that is unnoticed in typical postthaw survival assessment, and that such damage may place an upper limit on implantation rates with transfer of merely "survived" embryos. It is reported that postthaw extended culture of thawed bipronuclear oocytes prevents transfer of significantly cryo-damaged embryos (21).

A retrospective matched-cohort comparison (11) of single embryo transfers found significantly greater ongoing pregnancy rates with day-6 blastocysts in FET when compared with fresh day-6 blastocyst transfer, but no significant difference between FET and fresh transfer with day-5 blastocysts. Another retrospective study also found greater implantation rates with vitrified-warmed blastocysts than with fresh blastocyst transfer (22).

RISKS ASSOCIATED WITH COS, FRESH TRANSFER, AND FET

There is an established causal relationship between COS and OHSS in high responders who receive a "trigger" of hCG (23). Frozen embryo transfer may be used to prevent continuation of early-onset OHSS or to eliminate the risk

of late-onset OHSS. Although the risk of significant OHSS is virtually eliminated with the use of a GnRH agonist "trigger" for final oocyte maturation, the use of agonist trigger has been associated with abrupt termination of the luteal phase, complete and irreversible luteolysis, and reduced live birth rates (24, 25). Therefore, cohort cryopreservation is often used after agonist trigger to improve the chance of live birth (26).

Ectopic pregnancy risk is greater in IVF pregnancies than in spontaneous pregnancies. It is hypothesized that this increased risk may result from COS exposure and resulting supraphysiologic hormone levels, such as through effects of E₂ on uterine contractions (27) or the effect of elevated P on cilia (28). There are some reports that FET has a reduced risk of ectopic pregnancy (both visualized ectopic pregnancies and pregnancies of unknown location) when compared with fresh transfer (29–31), although others finding no significant difference (32–34).

Other risks that have been linked to fresh transfer after COS exposure include pre-eclampsia, low birth weight (LBW), small for gestational age (SGA), prematurity, preterm LBW, antepartum hemorrhage, placental abruption, and perinatal death (35–53). It is hypothesized that some of these risks are increased through altered placentation due to supraphysiologic hormone levels after COS exposure and a resulting adverse uterine effect (44).

A comprehensive meta-analysis reported that, when compared with fresh-transfer pregnancies, FET pregnancies were associated with significantly reduced risks of preterm birth (RR 0.84), SGA (RR 0.45), LBW (RR 0.69), perinatal mortality (RR 0.68), placental abruption (RR 0.44), and placenta previa (RR 0.71). Risks of very preterm birth, very LBW, congenital anomalies, and neonatal intensive care did not differ significantly. Increased risk of cesarean section delivery was observed with FET (RR 1.10) (35). Since that meta-analysis was published in 2012, several additional reports have emerged.

Another recent meta-analysis (51) found FET was associated with reduced risk of preterm birth.

An Australian registry study compared birth defects after fresh transfers and FET with spontaneous pregnancies in fertile controls, and found fresh transfer, particularly fresh transfer after intracytoplasmic sperm injection, had increased risk for birth defects when compared with fertile controls, but found no significantly elevated risks with FET vs. fertile controls (45). Another Australian study of 6,946 birth outcomes found that the risk of blastogenesis birth defects was significantly greater after fresh transfer when compared with spontaneous pregnancies, but that births after FET did not exhibit this increased risk (46). Two explanations for the difference between FET and fresh transfers were hypothesized, including an embryo-screening effect through cryo-survival and an endometrial effect through hormone levels altered by COS.

A large Japanese registry study compared 48,158 deliveries after fresh transfer or FET, all with single embryo transfer (36). In that study, FET was associated with increased incidence of large for gestational age, placenta accreta, and pregnancy-induced hypertension, but reduced incidence of

SGA, LBW, and prematurity when compared with fresh transfer. The study found no significant differences between FET and fresh transfer with respect to rates of placenta previa, placenta abruption, or macrosomia.

A relatively small clinical study comparing fresh transfer and elective cohort cryopreservation followed by FET in high responders found reduced risk of pre-eclampsia with FET (52).

An American registry study compared singleton births resulting from fresh autologous cycles, autologous FET, fresh oocyte donation cycles, and donor FET and found increased risk of LBW in fresh autologous cycles when compared with autologous FET. However, when fresh donor cycles were compared with donor FET, no such differences were observed (44). This suggested a cause of increased LBW risk was isolated to the fresh autologous cycle, the only cycle type with uterine exposure to COS.

One retrospective clinical study compared singleton births resulting from 2,531 fresh transfers and 4,092 FETs after elective primary freezing with vitrification (47). This study used minimal stimulation (clomiphene citrate in combination with low-dose gonadotropins) and single embryo transfer. No significant differences in prematurity, total birth defects, or perinatal mortality were found, but greater birth weight and reduced incidences of LBW and SGA with FET were reported. This study is interesting because, historically, most risk information came from registry studies that were dominated by FET cycles that used supernumerary “second-best” embryos subject to conventional slow freezing or an unknown mix of methodologies. Additionally, the use of minimal stimulation in this study may have altered or mitigated some uterine effects of COS exposure when compared with other studies that relied mainly on conventional stimulation.

SUMMARY

In 2006–2012 there was a clear shift toward increased use of FET in the United States, coincident with live birth rates increasing more rapidly in FET than in fresh transfers. Evidence suggests the trend toward increased use of FET was accelerating. The effectiveness of embryo cryopreservation for preventing late-onset OHSS, for remedying a defective luteal phase after GnRH agonist trigger, and in allowing time for genetic test results should perpetuate these trends for some time to come. In combination with safe ovulatory trigger protocols, it is possible to have a virtually OHSS-free center without compromising success rates (54).

It seems likely that COS exposure and the resulting altered hormone levels advance and otherwise alter endometrial development so that endometrial receptivity is impaired, especially for relatively slow embryos, and may be exacerbated by premature P elevation. Both the ability to implant and the quality of implantation may be affected. Other factors implicated in endometrial function include inhibin, activin, prostaglandin, vascular endothelial growth factor, human leukocyte antigen G, relaxin, cytokines, chemokines, selected homeobox gene activity, integrin, and more.

There is growing evidence that the endometrium is less receptive in fresh transfers after COS than in FET, and that

certain perinatal and maternal risks are reduced with FET when compared with fresh autologous transfer. The maternal risk of late-onset OHSS is effectively eliminated with FET. Among perinatal risks, the risks of LBW and preterm birth are clearly reduced in FET. Many reports have found singletons born after FET weigh more than singletons resulting from fresh transfer. Altered endometrial development and an altered uterine environment after COS are increasingly suspected as contributors to these observed differences. If the effect of COS on endometrial development is indeed the cause of these risk differences, then it may be that other techniques to improve endometrial receptivity, such as endometrial scratching or mild stimulation, might also be associated with similarly reduced perinatal risks.

According to the US Centers for Disease Control and Prevention, assisted reproductive technology accounted for 1.5% of infants born in the United States in 2010, while accounting for 5.6% of infants born with LBW and 4.4% of preterm infants (55). Of course, much of these increased risks result from transfer of multiple embryos, resulting in increased frequencies of multiple pregnancy. However, the results of numerous studies suggest that, even for singleton deliveries, these risks are enhanced by uterine COS exposure. Reducing these risks is an important goal because prematurity and LBW are, in turn, associated with numerous increased risks in the offspring (48, 49).

However, there is increased risk of macrosomia in infants from FET when compared with fresh transfer, and this increased risk cannot be explained by maternal factors alone (56). Overall, there are numerically more risks reportedly decreased than increased with FET when compared to fresh transfer (Table 1).

TABLE 1

Comparison of fresh transfer and FET with respect to maternal and fetal risks.

- Reduced risks in FET
 - OHSS
 - LBW (<2,500 g)
 - SGA
 - Preterm LBW
 - Preterm delivery (<37 wk)
 - Placenta previa
 - Placental abruption
 - Antepartum hemorrhage
 - Perinatal mortality
- Increased risk with FET
 - Placenta accreta
 - Macrosomia (>4,500 g)
 - Large for gestational age
 - Cesarean section delivery
- Risks without a clear difference
 - Implantation failure^a
 - Ectopic pregnancy^a
 - Pre-eclampsia
 - Very low birth weight (<1,500 g)
 - Very preterm delivery (<32 wk)
 - Neonatal intensive care unit admission
 - Congenital abnormalities

^a May depend on the FET protocol, patient population, and cycle parameters. Shapiro. Cryopreservation of embryo cohorts. *Fertil Steril* 2014.

The use of cryopreservation and subsequent FET might increase procedure costs relative to a fresh transfer, depending on how a center elects to price IVF services. Greater expense may also result from the medications and monitoring required in some FET protocols. Another potential cost to consider includes that associated with extra patient time required for FET. Additionally, in the near absence of OHSS risk, especially after agonist trigger, it may be tempting to increase gonadotropin dose to maximize oocyte yield and quality, further increasing expense. However, FET might reduce the overall cost when considering the reduced risk of relatively expensive OHSS treatments and perhaps also reduced perinatal risks with their associated costs.

Implantation rates of up to 70% can be attained when undamaged embryos are transferred in first-time IVF patients 18–40 years of age without genetic screening (12, 57). Programs that do not achieve similar rates in similar patients may be unknowingly transferring cryo-damaged embryos. Such damage is not unusual and can affect half of “surviving” embryos, even with vitrification (19), potentially imposing an artificial ceiling on success rates of FET protocols that eschew postthaw culture.

Those trials that achieved implantation rates near 70% used bipronuclear oocyte cohort cryopreservation followed by cohort thaw and culture to the blastocyst stage before transfer of the morphologically best blastocysts. This method differs from typical FET with supernumerary embryos because it allocates the cohort’s best embryos to FET, and also because it precludes the transfer of cryo-damaged embryos that cannot resume development (21).

The oocyte donation cycle is an excellent comparator for assessing the quality of transferred embryos in FET in young autologous patients (21). Frozen embryo transfer and donor cycles typically have similar endometrial preparation, lacking uterine COS exposure. In contrast, comparison of fresh autologous transfer and FET, particularly FET lacking significant postthaw culture, would find no significant difference in success rates whenever the degree of latent cryo-damage in FET approximates the impairment of endometrial receptivity in fresh transfer.

Of course, the success of FET relies on effective endometrial preparation and luteal support. However, it seems these techniques are largely mature at this point. A recent meta-analysis (58) found the success rates were not significantly different when comparing natural cycle FET vs. modified natural cycle FET, natural cycle vs. artificial (hormone replacement) cycles, artificial cycles with and without GnRH agonist down-regulation, and natural cycle vs. artificial cycles with GnRH agonist.

Further research should seek to resolve the discordant findings with respect to ectopic pregnancy and confirm the underlying cause(s). Further examination of preimplantation embryo–endometrial interaction would be beneficial. In addition, a comparison of fresh transfer and FET with blastocyst vitrification coupled with genetic screening may help to optimize IVF outcomes.

The era of embryo-centric dogma in IVF may be passing, because there is increasing recognition of the critical role of an uncompromised endometrium in facilitating implantation

and determining implantation quality. Although cohort cryopreservation exacts an embryonic cost through the risk of embryo cryo-damage, fresh autologous transfer exacts its own cost through the risk of transferring the best embryos into a uterine environment impaired by ovarian stimulation. Years ago, cryopreservation techniques were relatively poor, and this balance leaned heavily in favor of fresh transfer. As cryopreservation techniques improve, that balance shifts toward cryopreservation.

Whether fresh transfer or FET would maximize the chance of success in a given patient depends on the a priori expected outcome for that patient (considering, e.g., age, diagnosis, and history) at her center, and also depends on dynamic cycle parameters, such as preovulatory P levels, trigger agent, and embryo developmental pace. Although the trends have been steadily shifting in favor of cohort cryopreservation and FET on the basis of success rates, there is not yet any clear choice that maximizes success rates for all patients at all centers, and therefore individualized approaches remain appropriate.

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