Endometrial preparation before the transfer of single, vitrified-warmed, euploid blastocysts: does the duration of estradiol treatment influence clinical outcome?

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Objective: To investigate whether the duration of estrogen administration before euploid embryo transfer affects clinical outcome.
Design: Retrospective cohort study.
Setting: Private, academic fertility center.
Patient(s): Patients (n = 1,439) undergoing autologous freeze-only in vitro fertilization with preimplantation genetic testing (PGT) followed by endometrial preparation with estrogen and progesterone in a frozen, euploid blastocyst transfer cycle.
Intervention(s): None.
Main Outcome Measure(s): Primary outcome was live birth, and secondary outcomes included implantation, clinical pregnancy, early pregnancy loss, live birth, infant birthweight, low birth weight, infant gestational age at delivery, and preterm birth.
Result(s): The duration of estrogen administration (mean: 17.5 ± 2.9 days; range: 10–36 days) before frozen embryo transfer did not impact implantation (odds ratio [OR] 0.99; 95% confidence interval [CI], 0.95–1.03), clinical pregnancy (OR 0.98; 95% CI, 0.94–1.01), early pregnancy loss (OR 1.03; 95% CI, 0.95–1.12), or live birth (OR 0.99; 95% CI, 0.95–1.03). The duration of estrogen exposure did not affect infant birthweight (in grams) ($\beta = -10.65 \pm 8.91$) or the odds of low birth weight (OR 0.87; 95% CI, 0.68–1.13). For every additional day of estrogen administration, we observed a reduction in gestational age at delivery (in weeks) ($\beta = -0.07 \pm 0.03$), but the odds of preterm delivery were not affected (OR 1.05; 95% CI, 0.95–1.17).
Conclusion(s): Variation in the duration of estradiol supplementation before progesterone initiation does not impact frozen, euploid blastocyst transfer outcome. The duration of estrogen administration was inversely correlated with gestational age at delivery, but this did not translate into an increase in preterm delivery. Further studies are required on the downstream effects of endometrial preparation on the placental–endometrium interface. (Fertil Steril® 2019;111:1177–85. ©2019 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Endometrial preparation, estrogen, FET, perinatal outcomes, pregnancy, vitrification

Advances in vitrification have transformed embryo cryopreservation into a highly efficient, reliable laboratory procedure. Vitrification technology has become a fundamental part of in vitro fertilization (IVF) treatment, playing an instrumental role in the implementation of various forms of assisted reproduction technology (ART) such as preimplantation genetic testing (PGT), fertility preservation, single-embryo transfer, and freeze-only cycles (1). As a result, the proportion of autologous transfer using cryopre-

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served embryos has risen dramatically in the United States over the last decade.

In frozen embryo transfer (FET) cycles, estrogen, and progesterone are sequentially administered to synchronize the embryo transfer with the endometrial window of implantation. Traditionally, endometrial preparation before transfer involved down-regulation with a gonadotropin-releasing hormone (GnRH) agonist to suppress the luteinizing hormone (LH) surge, followed by estrogen administration. Continued administration of estrogen alone, beginning in the early proliferative phase, has also been shown to be sufficient to suppress ovulation by the negative feedback mechanism of the hypothalamic-pituitary axis (2). In the initial estrogen-only phase, the endometrium is thickened and maintained while follicular development is suppressed. Estrogen is continued as daily progesterone administration is initiated 5 days before the scheduled embryo transfer.

The minimum duration of progesterone exposure before FET is known to be critical because the window of implantation is confined to a narrow interval in the luteal phase. Studies using hormone preparations in donor oocyte recipients first characterized the window of implantation, demonstrating that endometrial receptivity greatly diminished when the embryo transfer occurs before or after this critical period (3). In contrast to progesterone, the duration of estrogen supplementation before FET, which determines the length of the proliferative phase, can be artificially varied according to the rate of endometrial thickening, the availability of PGT results, patient preference, and/or coordination of care.

The increased use of FET has allowed investigators to closely examine various aspects of this treatment strategy. Initial studies, focused on comparing various routes of estrogen (4) and progesterone (5) administration, demonstrated a lack of difference in clinical outcomes among patients using intramuscular, oral, transdermal, and vaginal preparations. Systematic reviews and meta-analyses (6, 7) have concluded that there is insufficient evidence to recommend a specific protocol for endometrial preparation in fresh and frozen embryo transfers.

However, these studies did not address whether the duration of the proliferative phase (as determined by the total number of days of unopposed estrogen administration) before FET can affect clinical outcome. The majority of existing data from older studies investigated the effects of varied proliferative phase lengths on donor oocyte recipients' transfer outcomes (3, 8–11). Although some of these studies suggested there is an optimal range of time for uterine lining preparation with unopposed estrogen, the findings conflicted with other studies and reported overall lower success rates, without accounting for variables that could have hindered IVF outcome (i.e., transfer of slow-frozen zygotes).

Despite numerous advancements that have optimized extended embryo culture, PGT, and cryopreservation, there is still much more to be learned about endometrial preparation and synchronization to maximize endometrial function and receptivity. We assessed whether the duration of estrogen administration before progesterone initiation influences FET outcomes in a large, uniform cohort of patients undergoing a euploid, vitrified-warmed, blastocyst transfer.

MATERIALS AND METHODS

The study included patients from a single center who underwent a euploid, vitrified-thawed, blastocyst transfer between January 2012 and June 2017. This retrospective cohort study only included patients (n = 1,439) who underwent their first embryo transfer after autologous IVF, PGT, blastocyst vitrification, and warming. The following patients were excluded from the analysis: [1] patients who underwent the transfer of >1 embryo, [2] patients using blastocysts derived from previous stimulation cycles (i.e., cryopreserved oocytes and/or donor oocytes), [3] patients who had prior attempts at conception via IVF and embryo transfer, [4] patients who could not attain an endometrial thickness of ≥7 mm by the 7 to 10 days from initiating estrogen supplementation, and [5] patients who required estrogen administration beyond the standard oral regimen used at the center (intramuscular and/or transdermal). In addition, natural cycle FETs that did not involve estrogen and/or progesterone administration were excluded. The study was approved by the Western Institutional Review Board.

IVF and Laboratory Procedures

Patients underwent ovarian stimulation for IVF as previously described elsewhere (12). When a minimum of two mature follicles measuring ≥18 mm were achieved, oocyte maturation was triggered using recombinant human chorionic gonadotropin (hCG) (Ovidrel; EMD Serono) alone or with 40 IU leuprolide acetate (Lupron; AbbVie Laboratories) in combination with 1,000 IU hCG (Novarel; Ferring Pharmaceuticals). Vaginal oocyte retrieval was performed under transvaginal ultrasound (TVUS) guidance 36 hours after hCG was administered. Approximately 4 hours after retrieval, mature oocytes were fertilized by intracytoplasmic sperm injection (ICSI), as part of routine practice for cases where PGT is planned.

Embryos were cultured to the blastocyst stage and underwent laser-assisted hatching on day 3 of development and trophectoderm biopsy at the blastocyst stage, as previously described elsewhere (12). Blastocyst trophectoderm biopsies were performed from day 5 to 7 of development, depending on the timing of embryo expansion. To meet criteria for trophectoderm biopsy, blastocysts were required to have a discernable inner cell mass with a multicellular trophectoderm herniating from the zona pellucida; using a modified Gardner and Schoolcraft morphologic grade, embryos must have reached a minimum score of 4BC (13). After biopsy, blastocysts were individually vitrified using the modified Cryotop method, as previously described elsewhere (12).

The PGT was performed for chromosomal copy number using either quantitative-Polymerase Chain Reaction (qPCR), array comparative genomic hybridization (aCGH), or targeted next generation sequencing (NGS). Quantitative PCR and aCGH were used before 2016, and NGS was the sole platform used thereafter. We included FETs involving embryos tested with either platform based on unpublished, internal data that has demonstrated no difference in live birth and pregnancy loss rates. A freeze-only treatment approach is recommended for all PGT cases, to facilitate the turnaround of aneuploidy

screening results and coordinate the selection of a euploid embryo for transfer.

Frozen Embryo Transfer Protocol

In a subsequent cycle, patients were administered hormones for endometrial preparation before FET. All patients had formal evaluation of their endometrial cavity via three-dimensional transvaginal sonography and hysterosalpingogram or saline sonogram within the preceding 1–3 months. For scheduling purposes, some patients underwent suppression of their hypothalamic-pituitary-ovarian axis with oral contraceptive pills for a minimum of 14 days. There was no medical indication for pretreatment with oral contraceptives. After the cessation of oral contraceptives for 4 days or on day 3 of spontaneous menses, patients underwent a baseline TVUS and assessment of serum estrogen, progesterone, luteinizing hormone, and β-hCG to confirm that they were in the early proliferative phase of their menstrual cycle and to rule out pregnancy.

Patients then began oral estrogen (Estrace; Teva Pharmaceuticals), 2 mg twice daily for 1 week, then 2 mg three times daily. Oral estrogen was administered with the intention of inducing endometrial proliferation while suppressing the development of a dominant follicle. We performed TVUS every week to assess the recipients' endometrium, with the first ultrasound occurring within 7 to 10 days of initiating estrogen supplementation. Serum progesterone was measured at each visit to rule out premature ovulation, before the initiation of progesterone supplementation.

Once the timing of the FET was determined, progesterone in the form of intramuscular (Watson Pharma) or a combination of oral (Prometrium; Solvay Pharmaceuticals) and vaginal (Endometrin; Ferring Pharmaceuticals) administration was administered daily. The route of progesterone supplementation was based on patient preference. There was no medical indication for the use of a one regimen over the other. Patients received intramuscular progesterone in oil or a combination of oral and vaginal progesterone, starting 5 days before FET.

On the sixth day of progesterone administration, a vitrified blastocyst was selected for transfer based on PGT results and morphology grading according to the center's modified Gardner and Schoolcraft scale (13). Blastocyst warming was performed by the use of a modified Cryotop method as previously described elsewhere (12). Single-embryo transfer was performed via flexible catheter under transabdominal ultrasound guidance.

After FET, daily estrogen and progesterone administration was continued until a negative pregnancy test. If pregnancy was achieved, hormone administration was continued until the expected luteoplacental shift in estrogen and progesterone production, at approximately 8 to 9 weeks' gestation.

Outcome Measures

The independent variable of interest was the duration of estrogen administration, which was defined as the number of days from initiation of estrogen to the day of embryo transfer. The cumulative dose of oral estrogen leading up to FET was also noted. In the evaluation of whether the duration of endometrial preparation with oral estrogen before FET impacted clinical outcome, the primary outcome analyzed was live-birth rate (live birth of an infant $\geq$24 weeks' gestation). Secondary outcomes included the rate of implantation (the number of intrauterine sacs divided by the number of embryos transferred), ongoing pregnancy (the proportion of patients with a fetal heart beat at discharge), and early pregnancy loss (no gestational sac after serum β-hCG $\geq$5 mIU/mL or loss occurring after presence of an intrauterine gestational sac was confirmed), gestational age at delivery, rate of preterm delivery (defined as birth at <37 weeks' gestation), and infant birthweight and the rate of low birthweight (defined as infant birthweight <2,500 g).

Statistical Analysis

Patient demographic and cycle characteristics were compared between clinical outcome groups using Student's *t*-test, Wilcoxon rank sum tests, chi-square, and Fisher's exact tests, as appropriate. Whether binary clinical outcomes were modified by the duration of estrogen supplementation was assessed using multivariable logistic regression models adjusting for major covariates (age, body mass index, endometrial thickness at transfer, embryo day of development at trophectoderm biopsy, whether the embryo had a morphology grade of 4BC or better, and the route of progesterone administration). Adjusted odds ratios (OR) and 95% confidence intervals (95% CI) are reported. The effects of estrogen administration duration on infant birthweight and gestational age were evaluated using multivariable linear regression. All analyses were conducted using SAS version 9.4 (SAS Institute).

A post hoc power analysis was performed and was adjusted based on the inclusion of major covariates in a model with an R^2 of 0.02. A sample size of 1,092 (the number of patients with complete data on all covariates that were included in the multivariable analysis of live birth) provided 80% power (α error = 0.05), to detect a 4.3% absolute reduction in live birth (baseline rate 50%), when the duration of estrogen administration increases by 1 standard deviation, corresponding to an OR of 0.84.

RESULTS

This study included 1,439 patients who underwent an initial embryo transfer of a euploid, vitrified-warmed, blastocyst. At the time of analysis, live birth outcomes were known for the majority of patients (78.9%, n = 1,135), with an unknown live birth outcome occurring only in the case of patients who underwent recent transfer. The mean duration of estrogen administration before FET was 17.5 $\pm$ 2.9 (range: 10–36 days).

Frozen Embryo Transfer Outcome

Patients underwent FET with the following outcomes: 60.1% implantation rate, 55.4% ongoing pregnancy rate, 49.4% live birth rate, and 11.1% early pregnancy loss rate. Baseline demographics and cycle characteristics were compared between patients who did and did not achieve live birth (Table 1). Patients whose transfer resulted in live birth underwent a mean duration of 17.4 $\pm$ 2.8 days (range: 10 to 34 days)

(Supplemental Figure 1, available online) of estrogen administration before FET. Controlling for age, body mass index, endometrial thickness at transfer, embryo day of development at trophectoderm biopsy, whether the embryo had a morphology grade of 4BC or better, and route of progesterone administration, the number of days of estrogen administration did not modify the odds of achieving live birth (OR 0.99; 95% CI, 0.95–1.03; $P=.51$) (Fig. 1). Accounting for the same covariates, the number of days of estrogen administration before FET did not impact the odds of implantation (OR 0.99; 95% CI, 0.95–1.03; $P=.67$) (Supplemental Figure 2, available online), clinical pregnancy (OR 0.98; 95% CI, 0.94–1.01; $P=.19$) (Supplemental Figure 3, available online), or early pregnancy loss (OR 1.03; 95% CI, 0.95–1.12; $P=.43$) (Fig. 2).

Pregnancy Outcome

Patients who had a live birth were stratified by low and normal infant birthweight (4.7% vs. 95.3%, respectively) (Supplemental Table 1, available online) and were found to have similar baseline demographics and cycle characteristics, including a similar mean duration of estrogen administration before FET (17.5 ± 2.4 vs. 17.3 ± 2.8; $P=.72$). No statistically significant modification in infant birthweight (grams) ($\beta=-10.65 \pm 8.91$; $P=.23$) or odds of delivering a low birth weight infant (OR 0.87; 95% CI, 0.68–1.13; $P=.30$) were observed after performing a multivariable regression model that controlled for major covariates, including age, body mass index, endometrial thickness, embryo morphology, route of progesterone administration, gestational age at delivery, and infant sex.

Patients who had a live birth were categorized by experiencing a preterm versus term delivery (8.2% vs. 91.8%, respectively) (Supplemental Table 2, available online). Patients who experienced a preterm delivery had a significantly higher body mass index (25.8 ± 5.6 vs. 23.1 ± 3.7, $P<.001$), and a significantly lower proportion of transfers involving high-quality embryos with a grade A inner cell mass (63.3%

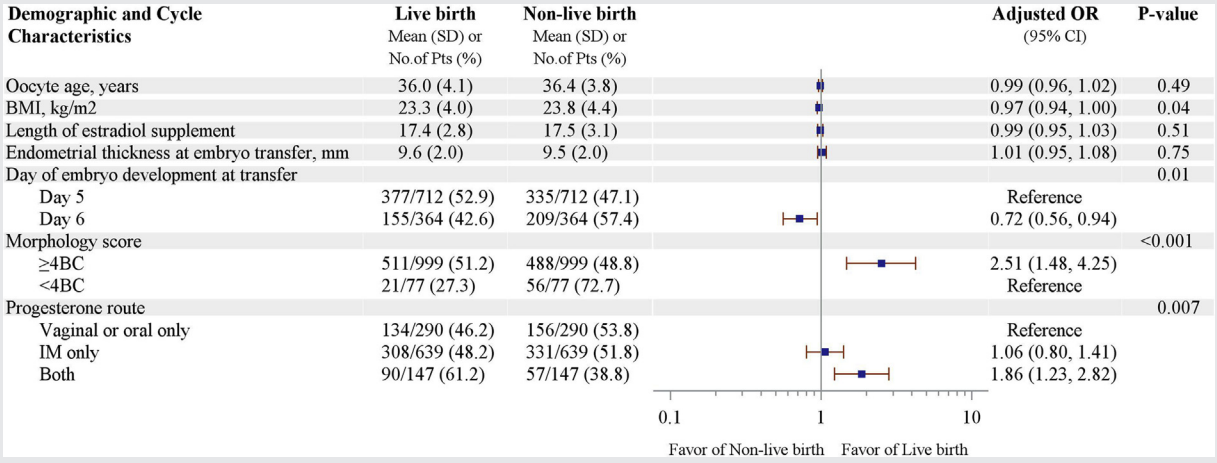
TABLE 1

A comparison of baseline demographic and cycle characteristics according to whether patients achieved a live birth after frozen embryo transfer.

	Live birth (n = 574)			No live birth (n = 561)			
Variable	**N**	**Mean ± SD or median (Q1, Q3)**	**Range (min–max)**	**N**	**Mean ± SD or median (Q1, Q3)**	**Range (min–max)**	***P* value**
Age at embryo transfer	574	36.7 ± 3.8	22.2–44.6	561	36.3 ± 4.1	22.7–44.6	.08
Age at IVF cycle	572	36.4 ± 3.8	22.1–44.4	559	36.03 ± 4.1	22.6–44.4	.07
Body mass index	561	23.8 ± 4.4	15.7–43	535	23.3 ± 4	14.9–40.2	.05
Gravidity	558	1 (0, 2)	0–7	542	1 (0, 2)	0–7	.77
Parity	555	0 (0, 1)	0–5	543	0 (0, 1)	0–4	.48
Serum estrogen level before transfer	524	285 (208, 385)	61–2,000	514	293.5 (214, 395)	77.7–2,000	.39
Days of oral estrogen administration	574	17.6 ± 3.2	11–36	561	17.4 ± 2.8	10–34	.33
Endometrial thickness at transfer (mm)	574	9 (8, 10.6)	7–19.7	561	9 (8,10.4)	7–20.7	.98
Cumulative dose of oral estrogen (mg)	574	93.8 ± 19.5	20–212	561	92.8 ± 18	36–192	.38
		No. of patients (%)			**No. of patients (%)**		
Day of embryo development at transfer		574 (100)			561 (100)		
5		345 (60.1)			394 (70.2)		<.001
6		219 (38.2)			166 (29.6)		
7		10 (1.7)			1 (0.2)		
Embryo expansion grade at the time of transfer		574 (100)			561 (100)		
3		0 (0)			1 (0.2)		.02
4		189 (32.9)			214 (38.2)		
5		146 (25.4)			158 (28.2)		
6		239 (41.6)			188 (33.5)		
Embryo inner cell mass grade at the time of transfer		555 (100)			552 (100)		
A		345 (62.2)			416 (75.4)		<.001
B		171 (30.8)			125 (22.6)		
C		36 (6.5)			11 (2)		
D		3 (0.5)			0 (0)		
Embryo trophectoderm grade at the time of transfer		555 (100)			552 (100)		
A		170 (30.6)			194 (35.1)		.003
B		252 (45.4)			271 (49.1)		
C		128 (23.1)			86 (15.6)		
D		5 (0.9)			1 (0.2)		
Embryo score		574 (100)			561 (100)		<.001
Better than 4BC		512 (89.2)			539 (96.1)		
Embryo sex		574 (100)			561 (100)		.03
Male		277 (48.3)			307 (54.7)		
Progesterone route		574 (100)			561 (100)		.001
Vaginal/oral progesterone only		163 (28.4)			139 (24.8)		
IM progesterone only		348 (60.6)			330 (58.8)		
Both		58 (10.1)			92 (16.4)		

Sekhon. Duration of estrogen and FET outcome. Fertil Steril 2019.

FIGURE 1



The odds of achieving a live birth after frozen embryo transplant (FET) according to the duration of unopposed estrogen administration.

Sekhon. Duration of estrogen and FET outcome. Fertil Steril 2019.

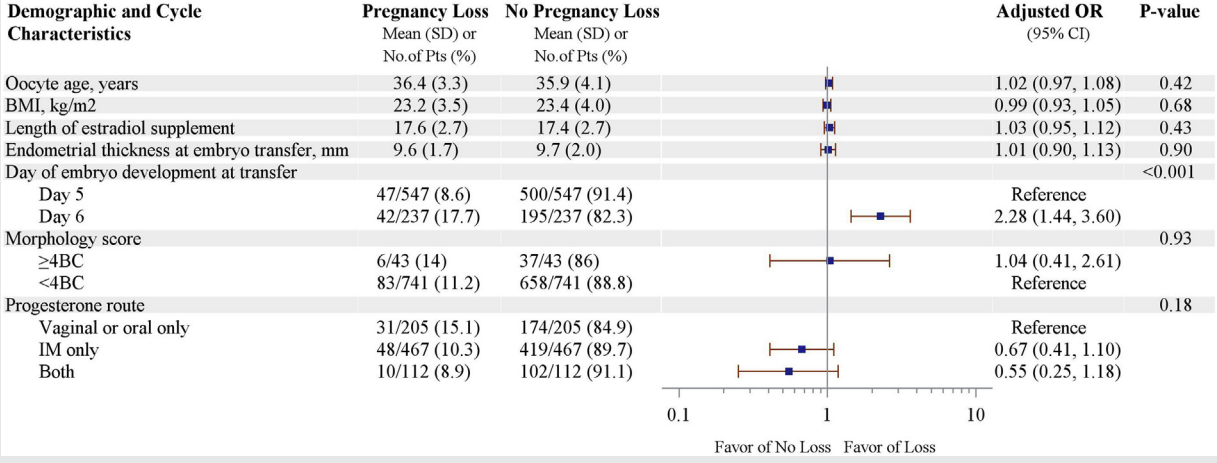
vs. 76.6%, $P=.04$). The number of days of estrogen administration before FET did not significantly modify the odds of preterm delivery (OR 1.05; 95% CI, 0.95–1.17; $P=.35$), as demonstrated by the multivariable linear regression model. However, each additional day of estrogen administration was significantly associated with a reduction in the gestational age at delivery (weeks) ($\beta = -0.07 \pm 0.03$, $P=.01$).

DISCUSSION

Hormonal endometrial preparation for FET involves the sequential administration of estrogen and progesterone to precisely time the transfer of a warmed blastocyst into receptive endometrium. The step-up protocol, where the estrogen dose is increased after 4 days, is thought to provide a physiologic stimulation of endometrium that closely mimics the hormone pattern of the proliferative phase of a natural menstrual cycle. The initial administration of unopposed estrogen allows for manipulation of the proliferative phase to maintain the endometrium in its preovulatory state until progesterone administration is initiated to induce transformation of the endometrium to an embryo-receptive state.

The timing of progesterone initiation can vary according to clinical variables (adequate endometrial thickness) and physician preference, which often determine the date of the embryo transfer (14, 15). Timing may also be influenced by external factors, such as the time required for PGT analysis or patient preference. Consequently, estrogen administration for endometrial preparation before FET may artificially extend the proliferative phase from the 1 to 2 weeks

FIGURE 2



The odds of experiencing early pregnancy loss after frozen embryo transplant (FET) according to the duration of unopposed estrogen administration.

Sekhon. Duration of estrogen and FET outcome. Fertil Steril 2019.

duration experienced in a natural 28 day menstrual cycle to up to 4 weeks. Fresh embryo transfers performed in the setting of recent ovarian stimulation have been shown to have reduced implantation rates compared with FET cycles, an effect thought to be mediated by the negative impact of supraphysiologic estrogen levels on embryo-endometrium asynchrony (16).

Supraphysiologic estrogen levels have been shown to alter the expression of genes and implantation factors in the perimplantation endometrium (17). Interestingly, despite the elevated circulating serum estrogen levels associated with artificial endometrial preparation before FET, studies have failed to show that **undergoing** natural cycle FETs leads to improved outcomes (7, 18). Given the dramatic rise in FET cycles over recent years, it is imperative to investigate whether the duration of proliferative-phase estrogen administration can impact endometrial receptivity to embryo implantation and placentation (3).

Using a large retrospective database, we demonstrated that the duration of unopposed estrogen stimulation before FET did not impact implantation, clinical pregnancy, or live birth. The duration of endometrial exposure to estrogen did not modify the FET outcome, demonstrating that endometrial receptivity to an implanting embryo is not affected by prolonged, high-dose estrogen stimulation.

The first studies to demonstrate the feasibility of varying the length of the proliferative phase without compromising implantation and pregnancy rates (3, 19) involved the donor oocyte IVF study model; the recipients of donor oocytes were often maintained on continuous estrogen treatment until donor oocytes became available for insemination. The ability to synchronize oocyte donor and recipient cycles has expanded our understanding of endometrial preparation and embryo-endometrial synchronicity, providing knowledge that can be applied to other treatment protocols such as frozen embryo transfer (20).

Various studies of donor oocyte recipient cycles have yielded conflicting results regarding the limits of endometrial compliance. Some of these studies report shorter durations of estrogen administration to have a negative effect on reproductive potential. Navot et al. (3) reported that donor oocyte recipients that received shorter durations of unopposed estrogen administration (5 to 10 days) experienced increased rates of early pregnancy loss, suggesting that an adequate period of estrogen administration is necessary to achieve a functional endometrium that can successfully maintain an implanted embryo. Similarly, Borini et al. (10) concluded that unopposed estrogen administration for endometrial preparation in donor oocyte recipients within a range of 11 to 40 days resulted in the best reproductive outcomes, and that shorter estrogen replacement periods were correlated with high rates of early pregnancy loss.

There have also been studies that suggest prolonged, unopposed estrogen administration can hinder reproductive outcome in donor oocyte recipients. Michalas et al. (9) reported that pregnancy rate per cycle was comparable when estrogen was administered for 6 to 11 days before progesterone initiation but dropped significantly thereafter. These findings are in contrast with others that demonstrated the feasibility of maintaining donor oocyte recipients on unopposed estrogen for a prolonged period for 3 to 5 weeks before progesterone initiation, without any adverse impact on endometrial morphology (3) or pregnancy rates (21). On the other hand, prolonged unopposed estrogen administration has been associated with a high rate of breakthrough bleeding in donor oocyte recipients, with one study reporting it as a common occurrence in patients continuing administration beyond 40 days (10).

Few studies have investigated the effect of varying durations of estrogen administration before FET on outcomes in patients who underwent transfer of embryos derived from autologous oocytes. Sunkara et al. (22) analyzed the outcome of 1,900 consecutive FET cycles according whether estrogen administration was preceded by GnRH agonist down-regulation and whether estrogen administration duration was <20 or ≥ 20 days. In cycles without prior pituitary suppression, patients in the longer estrogen administration group had a reduced clinical pregnancy rate (25.6% vs. 16.7%, $P=.037$). However, there was no significant difference according to length of estrogen administration in the setting of prior down-regulation with a GnRH agonist (32.6% vs. 31.9%, $P=.825$). The investigators concluded that the prior down regulation was protective against the detrimental effect of prolonged estrogen administration before commencing progesterone supplementation. These results should be interpreted with caution as the study lacks information regarding the methodology of embryo cryopreservation and thawing; along with missing data on embryo developmental stage and number of embryo(s) per transfer.

A study published by Liao et al. (23) compared oral and combined oral and vaginal estrogen administration in patients with inadequate endometrial thickness (<8 mm on cycle day 13). The cohort who received oral estrogen followed by vaginal administration had a significantly increased duration of estrogen administration before FET compared with patients who received oral estrogen alone (20.4 ± 2.0 vs. 23.9 ± 3.4, $P<.01$). Regardless of the differing regimens and length of administration, both groups had a similar implantation and clinical pregnancy rate. It should be noted that all patients were administered estrogen for an extended duration due to having inadequate midcycle endometrial thickness. In contrast, our study confirmed that FET outcome is unaffected by the duration of estrogen administration in patients, with adequate endometrial thickness before FET.

More recently, Bourdon et al. (24) performed a large retrospective analysis ($n = 1,377$ frozen blastocyst transfers from autologous IVF) that demonstrated that extending estrogen for endometrial preparation beyond 28 days before FET was associated with a significant reduction in the live-birth rate. The discrepancy between the findings of this study with our results may be attributable to major differences in study design: the investigators included cases involving the transfer of multiple, unscreened embryos, the days of estrogen supplementation were treated as a categorical metric, and endometrial thickness at time of transfer varied from 6 to 18 mm.

No prior studies have assessed whether the duration of estrogen administration before FET could have downstream consequences on the quality of placentation and perinatal

outcome. A statistically significant inverse relationship between longer duration of estrogen administration before FET and reduced gestational age at delivery was noted. Prolonged exposure to unopposed estrogen could result in an endometrial environment that, even with sufficient for embryo attachment and early invasion, may impact placentation. The fact that estrogen administration duration did not significantly modify the odds of preterm delivery or low birthweight suggests that any negative effect of prolonged proliferative-phase estrogen exposure on placentation is likely to be mild.

Possible explanatory mechanisms include supraphysiologic exposure to estrogen resulting in placentation defects with resulting downstream obstetric complications, warranting delivery at an earlier gestational age. The obstetric factors that could have influenced the timing of delivery could not be assessed because the patients were discharged to various practices at 9 weeks' gestation. Another possible explanation is that the patients maintained on prolonged estrogen supplementation before FET are more likely to possess inherent uterine defects that may also predispose them to earlier delivery. Our analysis attempted to minimize any effects related to uterine factor infertility (i.e., Asherman's syndrome, history of multiple uterine surgeries) by excluding patients who were unable to achieve an endometrial thickness of at least 7 mm or who required alternative routes of estrogen administration in addition to the standard, oral step-up regimen that is routinely used.

To date, our study provides the largest comprehensive, well-controlled analysis of the relationship between duration of endometrial preparation and successful embryo transfer and perinatal outcome. There is scant literature available regarding the ideal duration of endometrial preparation for FET, with most of the early literature based on donor oocyte recipients undergoing the transfer of multiple zygote or cleavage stage unscreened embryos (2, 8, 11). Differences in methodology, patient populations, and treatment protocols among these limit the ability to compare the findings of various studies. Including only patients who underwent transfer of vitrified-warmed, single, euploid blastocysts allowed the study to mitigate the potential negative effect of maternal age and ovarian stimulation, and the influence of the number, stage, and quality of the embryos transferred, which have confounded the interpretation of results from prior studies. Furthermore, the study group represented a homogenous group of patients restricted to those undergoing their first FET cycle after freeze-only IVF who achieved adequate endometrial thickness after receiving the standard oral estrogen administration regimen.

The endometrial thickness threshold of 7 mm is used clinically in our practice based on internal data that suggests optimal FET outcomes in patients with an endometrial lining thickness measuring 7 mm or greater. We excluded patients with endometrial thickness less than 7 mm on their first ultrasound (7 to 10 days from initiating estrogen supplementation) to avoid the inclusion of cases where an extended duration of estrogen supplementation was medically indicated. Based on this rationale, the patients who received alternative routes of estrogen supplementation (intramuscular, vaginal, and transdermal) were also excluded.

A study design that includes only the first transfers could potentially minimize the inclusion of patients with underlying issues associated with recurrent FET failure. Also, by including freeze-only cycles and first FETs, bias associated with the best embryo being selected for fresh transfer and lesser quality supranumerary embryos being cryopreserved for future use was avoided. Finally, the stringent exclusion criteria ensured that the duration of estrogen exposure before FET was not related to patient or cycle characteristics, depending only on patient and physician availability or preference.

Due to the retrospective nature of this analysis, we cannot formally reconstruct and perform an analysis of the rationale behind the timing of FET scheduling and duration of unopposed estrogen administration. However, by excluding patients with $<$7 mm endometrial thickness and those who required supplemental routes of estrogen administration, we theoretically excluded uterine factor cases. The exclusion of patients using alternative routes of administration of estrogen (intramuscular, transdermal) may limit the generalizability of our findings. Even though the route of estrogen administration has not been shown to influence pregnancy rates (25, 26), we excluded these patients because the use of transdermal and intramuscular formulations are often used in patients with known risk factors for a thin lining or prior insufficient endometrial response to oral estrogen. We did not account for the endometrial pattern in the multivariate analysis because all patients had a homogenous endometrium on ultrasounds performed the day before and the day of FET.

The patients' route of progesterone supplementation varied, as intramuscular, oral, or vaginal, or a combination of regimens. Although the use of either regimen is considered the standard of care for endometrial preparation, having been widely reported to have comparable pregnancy rates (27–29), we included the route of progesterone supplementation as a covariate in the multivariate regression model. Only cycles in which FET was performed were included in the analysis, so we could not assess whether the duration of estrogen administration impacted the odds of cycle cancelation due to breakthrough bleeding or premature ovulation.

At each patient visit before FET, the serum progesterone levels were measured to rule out premature ovulation. The most significant limitation to this study's findings was patient discharge at 9 weeks' gestation to various obstetric practices, limiting our ability to assess various pregnancy-related factors that may have contributed to the timing of delivery. Furthermore, due to the retrospective nature of the analysis we were not able to account for obstetric risk factors such as a history of prior preterm delivery. We also could not account for other patient factors, such as tobacco exposure, that could contribute to risk of treatment failure or adverse obstetric outcomes.

This is the largest study to address the effect of estrogen supplementation duration before FET in patients with adequate endometrial thickness. Based on our findings, the duration of estrogen administration before FET can vary widely without compromising FET outcome. Although an

inverse relationship between the number of days of estrogen administration and the gestational age at delivery was observed, increasing cumulative estrogen exposure did not increase patients' risk of delivery premature and/or low birth weight infants. We conclude that once adequate endometrial thickness is achieved, FET can be scheduled in a flexible manner, according to patient and/or clinic preference, without compromising clinical outcome.

Well-designed, prospective, clinical trials are needed to further assess the effect of cumulative estrogen exposure before FET and to optimize the timing of the embryo transfer date. Prospective studies involving more detailed obstetric follow up will allow for a better understanding of the association between duration of exposure to estrogen before FET and gestational age at delivery. Studying the endometrial transcriptome may provide a better understanding of how increasing the duration of estrogen administration in the proliferative phase of FET cycles may affect endometrial function. In this era of precision and genomic medicine, identification of gene pathways that are modulated by extended estrogen exposure may reveal the molecular mechanisms underlying pregnancy establishment, maintenance, and placentation.

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Preparación endometrial antes de transferencia única de blastocistos euploides vitrificados: ¿la duración del tratamiento con estradiol influye en el resultado clínico?

Objetivo: Investigar si la duración de la administración de estrógenos antes de la transferencia de un embrión euploide afecta el resultado clínico.

Diseño: Estudio de cohorte retrospectivo.

Ámbito: Privado, centro de fertilidad universitario.

Paciente(s): Pacientes (n=1,439) sometiéndose a fecundación in vitro autóloga para congelación con test genético preimplantacional (PGT) seguido de preparación endometrial con estrógeno y progesterona en un ciclo de transferencia de blastocisto euploide criopresevado.

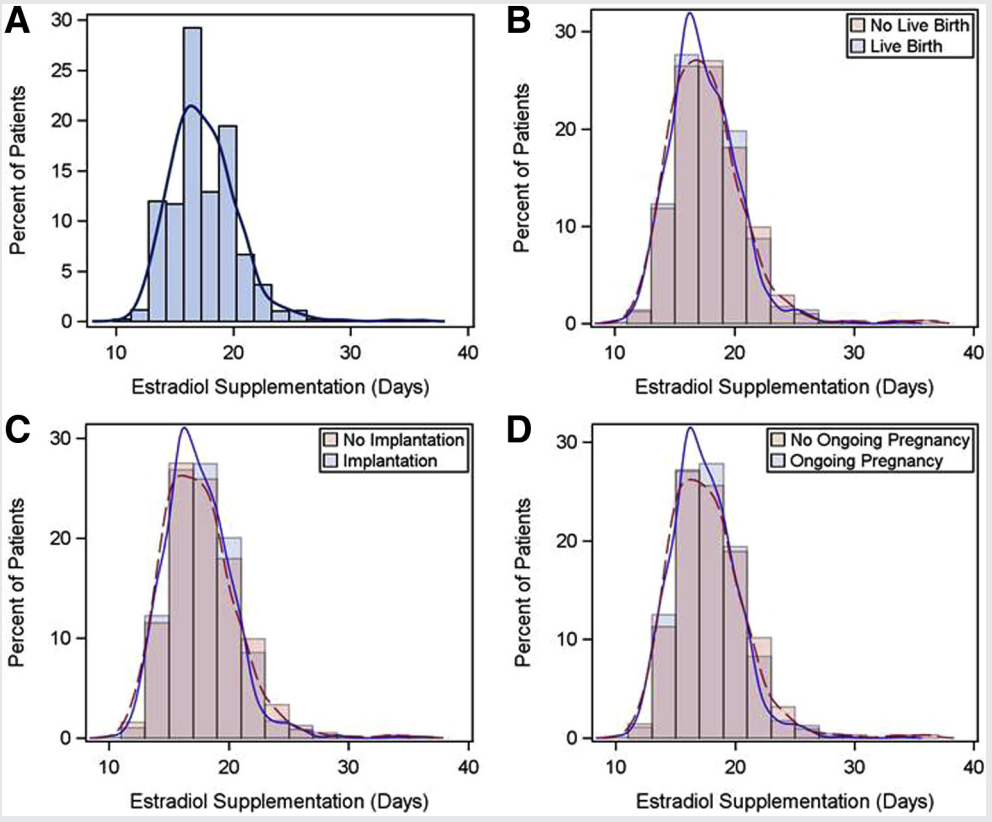
Intervención(es): Ninguna.

Principal(es) medida(s) de resultado: El resultado principal fue nacido vivo, y los resultados secundarios incluyeron implantación, embarazo clínico, pérdida precoz de embarazo, nacido vivo, peso infantil al nacer, bajo peso al nacer, edad gestacional infantil al parto, y parto pretérmino.

Resultado(s): La duración de la administración de estrógenos (media: 17.5 ± 2.9 días; rango: 10-36 días) antes de transferencia de embriones criopreservados no impactó en la implantación (odds ratio [OR] 0.99; intervalo de confianza 95% [IC], 0.95-1.03), embarazo clínico (OR 0.98; IC 95%, 0.94-1.01), pérdida precoz de embarazo (OR 1.03; IC 95%, 0.95-1.12), o nacido vivo (OR 0.9; IC 95% 0.95-1.03). La duración de la exposición a estrógeno no afectó el peso infantil al nacer (en gramos) (β= -10.65 ± 8.91) o la posibilidad de bajo peso al nacer (OR 0.87; IC 95%, 0.68-1.13). Por cada día adicional de administración de estrógeno, observamos una disminución en la edad gestacional al parto (en semanas) (β= -0.07 ± 0.03), pero la posibilidad de parto pretérmino no fue afectada (OR 1.05; IC 95%, 0.95-1.17).

Conclusión(es): La variación en la duración de la suplementación de estradiol antes del inicio de progesterona no impacta en el resultado de transferencia de blastocisto euploide criopreservado. La duración de la administración de estrógeno se correlacionó inversamente con la edad gestacional al parto, pero esto no se trasladó en un aumento en parto pretérmino. Se requieren estudios adicionales sobre los efectos posteriores de la preparación endometrial sobre la interface placentaria-endometrial.

SUPPLEMENTAL FIGURE 1

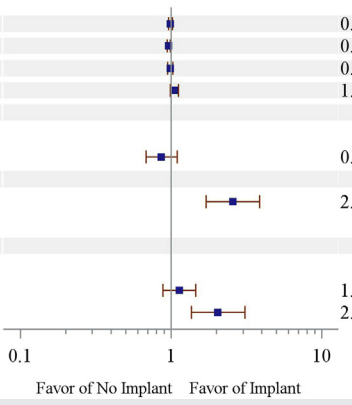


Histogram displaying the distribution of the sample according to the duration of estrogen administration (days): **(A)** all patients, **(B)** patients stratified according to whether they had a live birth, **(C)** implantation, and **(D)** ongoing pregnancy.

Sekhon. Duration of estrogen and FET outcome. Fertil Steril 2019.

SUPPLEMENTAL FIGURE 2

Demographic and Cycle Characteristics	Implant Mean (SD) or No.of Pts (%)	No Implant Mean (SD) or No.of Pts (%)	Adjusted OR (95% CI)	P-value
Oocyte age, years	36.0 (4.0)	36.3 (3.8)	0.99 (0.96, 1.02)	0.35
BMI, kg/m2	23.4 (4.0)	24.1 (4.6)	0.96 (0.94, 0.99)	0.002
Length of estradiol supplement	17.4 (2.7)	17.5 (3.1)	0.99 (0.95, 1.03)	0.67
Endometrial thickness at embryo transfer, mm	9.7 (2.0)	9.4 (2.0)	1.05 (0.99, 1.12)	0.08
Day of embryo development at transfer				0.23
Day 5	569/909 (62.6)	340/909 (37.4)	Reference	
Day 6	244/434 (56.2)	190/434 (43.8)	0.86 (0.68, 1.10)	
Morphology score				<0.001
≥4BC	770/1230 (62.6)	460/1230 (37.4)	2.57 (1.71, 3.86)	
<4BC	43/113 (38.1)	70/113 (61.9)	Reference	
Progesterone route				0.003
Vaginal or oral only	211/374 (56.4)	163/374 (43.6)	Reference	
IM only	484/806 (60.0)	322/806 (40.0)	1.13 (0.88, 1.46)	
Both	118/163 (72.4)	45/163 (27.6)	2.04 (1.36, 3.08)	

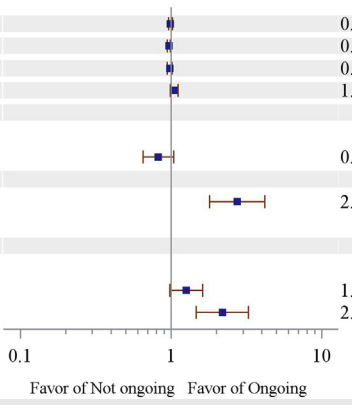


The odds of achieving implantation after frozen embryo transfer (FET) according to the duration of unopposed estrogen administration.

Sekhon. Duration of estrogen and FET outcome. Fertil Steril 2019.

SUPPLEMENTAL FIGURE 3

Demographic and Cycle Characteristics	Ongoing Mean (SD) or No.of Pts (%)	Not ongoing Mean (SD) or No.of Pts (%)	Adjusted OR (95% CI)	P-value
Oocyte age, years	36.0 (4.1)	36.3 (3.8)	0.99 (0.96, 1.02)	0.47
BMI, kg/m2	23.4 (4.0)	24.0 (4.5)	0.97 (0.94, 0.99)	0.008
Length of estradiol supplement	17.4 (2.7)	17.6 (3.1)	0.98 (0.94, 1.01)	0.19
Endometrial thickness at embryo transfer, mm	9.7 (2.0)	9.4 (1.9)	1.04 (0.99, 1.11)	0.14
Day of embryo development at transfer				0.10
Day 5	529/909 (58.2)	380/909 (41.8)	Reference	
Day 6	219/434 (50.5)	215/434 (49.5)	0.82 (0.65, 1.04)	
Morphology score				<0.001
≥4BC	712/1230 (57.9)	518/1230 (42.1)	2.74 (1.80, 4.18)	
<4BC	36/113 (31.9)	77/113 (68.1)	Reference	
Progesterone route				<0.001
Vaginal or oral only	186/374 (49.7)	188/374 (50.3)	Reference	
IM only	451/806 (56.0)	355/806 (44.0)	1.26 (0.98, 1.62)	
Both	111/163 (68.1)	52/163 (31.9)	2.19 (1.47, 3.25)	



The odds of achieving ongoing pregnancy after frozen embryo transfer (FET) according to the duration of unopposed estrogen administration.

Sekhon. Duration of estrogen and FET outcome. Fertil Steril 2019.