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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Hormone replacement versus natural frozen embryo transfer for euploid embryos

Ange Wang[1] · **Gayathree Murugappan**[1] · **Jonathan Kort**[1] · **Lynn Westphal**[1]

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Abstract

Purpose The goal of this study is to investigate hormone replacement (HR) versus natural frozen embryo transfer outcomes for euploid embryos.

Methods This is a retrospective cohort study at an academic medical center of patients undergoing in vitro fertilization with 24-chromosome day 5/6 preimplantation genetic testing for aneuploidies (PGT-A), from 2014 to 2018 using euploid single embryo frozen transfer. Multivariable logistic regression was used to study the association between transfer outcomes (ongoing pregnancy and miscarriage) with type of frozen euploid embryo transfer (HR versus natural) while controlling for multiple patient and cycle confounders.

Results From a total of 389 cycles, 45.0% utilized HR frozen embryo transfer and 55.0% were natural cycles. We found that when compared to HR frozen embryo transfer, natural cycle frozen embryo transfer had significantly higher ongoing pregnancy rates (aOR 2.05, 1.27–3.31, $p = 0.003$). There was no significant difference in miscarriage rates between the two groups (aOR for natural 0.69, 95% CI 0.37–1.32, $p = 0.27$). When limiting the analysis to only the first transfer at our institution, findings were similar of higher ongoing pregnancy rates and no difference in miscarriage rates.

Conclusions In our multivariate analysis, we found that natural cycle single euploid frozen embryo transfer was associated with significantly higher ongoing pregnancy rates than HR transfer, with no difference in miscarriage rates.

Keywords Preimplantation genetic screening · Preimplantation genetic testing for aneuploidies · Frozen embryo transfer · Hormone replacement frozen embryo transfer · Medicated frozen embryo transfer · Natural frozen embryo transfer

Introduction

The rate of frozen embryo transfer (FET) and preimplantation genetic testing for aneuploidies (PGT-A) is continuing to steadily rise in the United States, due to advances in vitrification, technological improvements in PGT-A, and emerging research about benefits of freeze-only cycles in improving endometrial receptivity and embryo-endometrial synchrony [5, 8, 30, 32, 35]. Given these trends, optimizing protocols for frozen embryo transfer is an important area for research, particularly in the context of euploid embryos. Frozen transfers can be performed using either natural cycle or hormone replacement (HR) protocols, the latter utilizing estrogen supplementation for endometrial preparation. HR protocols can allow the timing of a cycle to be more effectively controlled; this may be beneficial for logistical or medical reasons such as irregular ovulation, though this protocol has the disadvantage of additional medications and cost.

Studies have been mixed on if there is an advantage of one type of protocol over another for untested embryo transfers. Large reviews including two Cochrane reviews and a meta-analysis found no advantage to either type of FET protocol [11, 13, 15]. Smaller studies have reported mixed findings, including no difference between natural versus HR protocols [10, 25, 29, 40], advantage of natural protocols [4, 28], or advantage of HR protocols [18, 41]. There has been very little research on this subject for euploid transfers. A randomized controlled trial (RCT) of 236 patients in

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✉ Ange Wang
angewang31@gmail.com

[1] Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, CA 94305, USA

Italy found no significant difference in implantation, ongoing pregnancy, or miscarriage rates between HR versus natural FET for euploid blastocysts [14]. However, another study found that among 113 euploid FET cycles (65 natural cycles with ovulatory patients and 48 HR with anovulatory patients), live birth/ongoing pregnancy rates were significantly higher in natural FETs after adjustment for maternal age [27].

The goal of our study is to investigate HR versus natural FET outcomes (ongoing pregnancy, miscarriage) for euploid embryos, using a multivariate model with adjustment for confounders across multiple in vitro fertilization (IVF) indications. This is the first study to investigate this question in a multivariate analysis, and the largest study to date on the subject.

Methods

This analysis of natural versus HR euploid transfers was a retrospective cohort study including patients at our institution from 2014–2018. Our inclusion criteria were as follows: in vitro fertilization (IVF) cycles with euploid embryos based on 24-chromosome preimplantation genetic testing for aneuploidies (PGT-A), day 5/6 trophectoderm biopsy of blastocysts, single embryo transfer, frozen embryo transfer, and cycles with available information on all patient and cycle outcomes and confounders (as detailed below in "Statistical analysis"). We excluded cycles involving donor eggs or cleavage-stage embryos. IRB approval for this study was obtained through the university affiliated with the IVF clinic. Author note: a subset of the cycles in this manuscript was also used to investigate miscarriage history and antimüllerian hormone (AMH) in relation to euploid transfer outcomes in separate studies, with a different set of outcomes reported [37, 39].

The process of IVF stimulation has been previously summarized [22] and includes ovarian stimulation, oocyte retrieval, oocyte and sperm fertilization, blastocyst culture, blastocyst biopsy, and subsequent frozen embryo transfer. One of the three ovarian stimulation protocols was used: long GnRH agonist (leuprolide acetate), antagonist, or microdose flare (also known as the GnRH agonist short flare protocol), with protocol details previously summarized in literature [26]. The protocol was determined based on a variety of factors including ovarian reserve, patient age, basal antral follicle count, and (if applicable) prior response to gonadotropins. Oocyte retrieval was performed 35–36 h after trigger of hCG and/or leuprolide acetate, and either conventional IVF or intracytoplasmic sperm injection (ICSI) was used for fertilization per physician and embryologist discretion. SAGE sequential media and low oxygen conditions were used to culture blastocysts to either day 5 or 6, at which time cells were biopsied from the trophectoderm. The cells were then sent to Ivigen for 24-chromosome screening, with each embryo determined to be euploid or aneuploid. No mosaic embryos were included for transfer in this cohort. On the day of biopsy, embryos were also assigned morphological grades for expansion (1–6), ICM (A, B, or C), and trophectoderm (A, B, or C) based on the Gardner grading system [9]. All embryos were vitrified on the day of biopsy and then transferred in a subsequent cycle after ploidy results were available; hence, all cycles included in this analysis were freeze-only transfers.

The protocol for frozen embryo transfer was either natural or HR. For natural cycles, no medications were given for endometrial preparation, though hCG trigger was used in some cycles to time ovulation, and vaginal progesterone was used for luteal phase support. Most HR cycles used oral estrogen supplementation, though estradiol patches and intramuscular (IM) estrogen were also used in some cycles at the discretion of the physician. HR cycles also used IM progesterone in the luteal phase, in addition to vaginal progesterone. Progesterone supplementation was continued until approximately 10 weeks gestation. In HR cycles, IM progesterone was started after the lining reached an appropriate thickness (usually > 8 mm), and embryo transfer took place on the 6th day of IM progesterone. The type of transfer cycle was determined based on patient preference, medical reasons (such as the absence of ovulatory cycles), and logistical considerations (as the timing of a cycle can be more easily controlled for HR cycles).

Statistical analysis

We retrospectively reviewed all cycle charts to record the type of single embryo transfer (HR versus natural), as well as all variables included in this analysis. These variables included a mixture of patient and cycle characteristics that may potentially affect IVF cycle outcomes: patient characteristics including infertility diagnosis, AMH level, history of pregnancy, history of live birth**,** history of miscarriage, maternal age at retrieval, maternal age at transfer, body mass index (BMI); and cycle characteristics including utilization of ICSI, oocyte number, cohort size, morphology (expansion, inner cell mass, and trophectoderm), and endometrial thickness on day of HCG trigger. Multivariable logistic regression was used to study the association between type of single frozen embryo transfer (natural or HR) and transfer outcomes for euploid single embryo frozen transfers. Our study focused on two outcomes: (1) ongoing pregnancy (defined as viable pregnancy with transfer to obstetrics care at 8 weeks) and (2) miscarriage (loss of a pregnancy after positive beta hCG, including both biochemical pregnancy and clinical miscarriage). For each cycle, we controlled for all confounders based on the values present for the cycle itself (for example, even if a patient was represented twice, we took into account if their age, AMH, embryo quality, etc. varied across cycles). Our analysis was performed using both Python and Microsoft Excel, and all tests were two-sided with significance at the $\alpha = 0.05$ level.

Results

In our cohort of 389 cycles representing 283 unique patients, 45.0% of cycles were HR and 55.0% of cycles were natural. The average age at retrieval of the cohort was 35.1 years (standard deviation SD 3.5) for the HR group, and 36.1 (SD 3.7) for the natural cycle group (see Table 1). Age at retrieval and transfer were significantly lower for the HR group than the natural group ($p = 0.0083$ and $p = 0.013$, respectively). In the HR group, 22.9% of patients reported ovulatory dysfunction, while 4.7% of patients reported ovulatory dysfunction in the natural cycle group. The leading primary indications for IVF were male factor (26.2%), followed by diminished ovarian reserve (18.8%), and ovulatory dysfunction (11.6%). Diminished Ovarian Reserve is defined as a decrease in the number and quality of the remaining eggs in the ovaries. The DOR definition may be given for several reasons, including a low antral follicle count, low biochemical markers of ovarian reserve such as FSH or AMH, or a poor response to ovarian stimulation. Most baseline patient characteristics were similar between the two groups, including history of prior pregnancy (59.6% overall), history of live birth (30.6%), history of miscarriage (39.6%), and BMI (overall average 23.7 SD 4.5). Primary IVF indications are displayed in Supplementary Table 1.

In terms of cycle characteristics (Table 2), an average of 15.8 oocytes (SD 8.2) were retrieved and the average blastocyst cohort size was 5.7 (SD 3.9). All cycle characteristics were statistically similar between groups, including oocytes retrieved, blastocyst cohort size, use of ICSI, morphology grades (expansion, ICM, and trophectoderm), and endometrial thickness (overall average 9.3 with SD 1.6). Most embryos had good morphological parameters, with the vast majority having expansion grades of 5 and 6, and ICM and trophectoderm grades of A or B.

The unadjusted ongoing pregnancy rates were 42.9% for HR and 60.7% for natural, and unadjusted miscarriage rates

Table 1 Baseline characteristics—patient

	Type of frozen transfer			
	HR	Natural	Overall	*P* value
Total	175	214	389	
Gravidity	104 (59.4%)	128 (59.8%)	232 (59.6%)	0.94
Parity	52 (29.7%)	67 (31.3%)	119 (30.6%)	0.73
History of miscarriage	75 (42.9%)	79 (36.9%)	154 (39.6%)	0.23
Age at retrieval[a]	35.1 (3.5)	36.1 (3.7)	35.7 (3.7)	0.01
Age at transfer[a]	35.6 (3.5)	36.5 (3.7)	36.1 (3.7)	0.01
Body mass index[a]	23.2 (4.1)	24.0 (4.8)	23.7 (4.5)	0.10
AMH category (ng/mL)				
< 1	30 (17.1%)	38 (17.8%)	68 (17.5%)	< 0.001
1 to < 2	34 (19.4%)	51 (23.8%)	85 (27.0%)	
2 to < 5	55 (31.4%)	95 (44.4%)	150 (38.6%)	
5 +	56 (32.0%)	30 (14.0%)	86 (22.1%)	
IVF indication[b]				
DOR	36 (20.6%)	59 (27.6%)	95 (24.4%)	< 0.001
Endo	11 (6.3%)	21 (9.8%)	32 (8.2%)	
Male	68 (38.9%)	72 (33.6%)	140 (36.0%)	
Ovulatory dysfunction	40 (22.9%)	10 (4.7%)	50 (12.9%)	
RPL	17 (9.7%)	22 (10.3%)	39 (10.0%)	
Tubal	9 (5.1%)	19 (8.9%)	28 (7.2%)	
Sex selection	5 (2.9%)	11 (5.1%)	16 (4.1%)	
Single gene	10 (5.7%)	10 (4.7%)	20 (5.1%)	
Uterine	21 (12.0%)	8 (3.7%)	29 (7.5%)	
Unexplained	11 (6.3%)	27 (12.6%)	38 (9.8%)	
Other	7 (4.0%)	9 (4.2%)	16 (4.1%)	

[a]Average (standard deviation)

[b]Indications may overlap, does not sum to 100%

Table 2 Baseline characteristics—cycle

	Type of frozen transfer			
	HR	Natural	Overall	*p* value
Total	175	214	389	0.96
Oocytes retrieved[a]	15.8 (7.4)	15.8 (8.8)	15.8 (8.2)	0.96
Cohort size[a]	5.6 (3.4)	5.7 (4.2)	5.7 (3.9)	0.66
ICSI				
No	40 (22.9%)	56 (26.2%)	96 (24.7%)	0.22
Yes—all ICSI	124 (70.9%)	136 (63.6%)	260 (66.8%)	
ICSI split	11 (6.3%)	22 (10.3%)	33 (8.5%)	
Morphology				
Expansion				
5 to 6	149 (85.1%)	173 (80.8%)	322 (82.8%)	0.45
3–4	25 (14.3%)	38 (17.8%)	63 (16.2%)	
Less than 3	1 (0.6%)	3 (1.4%)	4 (1.0%)	
ICM				
A	85 (48.6%)	113 (52.8%)	198 (50.9%)	0.65
B	86 (49.1%)	95 (44.4%)	181 (46.5%)	
C	3 (1.7%)	3 (1.4%)	6 (1.5%)	
Trophectoderm				
A	77 (44.0%)	89 (41.6%)	166 (42.7%)	0.92
B	90 (51.4%)	113 (52.8%)	203 (52.2%)	
C	7 (4.0%)	9 (4.2%)	16 (4.1%)	
Endometrial thickness[a]	9.3 (1.6)	9.2 (1.6)	9.3 (1.6)	0.53

Four embryos did not have sufficient morphology to receive ICM or trophectoderm grade

[a]Average (standard deviation)

were 21.5% for HR and 15.0% for natural (Table 3). On multivariate analysis controlling for all potential confounders, we found when compared to HR, natural cycle frozen embryo transfer had significantly higher ongoing pregnancy rates (adjusted OR [aOR] 2.05, 95% CI 1.27–3.31, $p=0.003$). There was no significant difference in miscarriage rates between the two groups (aOR for natural 0.69, 95% CI 0.37–1.32, $p=0.27$).

To control for a possible institutional bias of cycle selection, we also performed a secondary analysis including only the first IVF-PGT-A cycle at our institution with similar findings as the main analysis (Supplementary Tables 2, 3). In this secondary analysis, ongoing pregnancy rate was still significantly higher for natural cycles (aOR for natural 2.15, 95% CI 1.18–3.91, $p=0.012$), and miscarriage rates were not significantly different between the two cycles (aOR for natural 0.63, 95% CI 0.29–1.34, p value $=0.23$).

Discussion

In summary, in our cohort, we found that for frozen transfer of euploid embryos, ongoing pregnancy rates were significantly higher for natural transfer when compared to HR transfer. We also found that miscarriage rates did not significantly differ between the two groups. This is the first study to investigate this question in a multivariate analysis and the largest to date on the subject, and adds to the limited literature suggesting possible benefits of natural rather than HR transfer.

Plausible biological mechanisms

Existing studies have suggested that supraphysiologic hormone levels may adversely affect endometrial receptivity and synchrony with the embryo, though mostly in the context of fresh versus frozen cycles. Literature has found that genes involved in endometrial receptivity and angiogenesis may be adversely impacted by controlled ovarian stimulation [17, 19, 24]. As a result, though hormone levels are usually closer to physiologic levels for HR transfer than fresh cycles, exogenous estrogen supplementation during HR cycles still results in higher hormone levels when compared to natural cycles. It is theorized that these elevated hormone levels may still cause molecular changes negatively impacting pregnancy outcomes. In addition, patient populations choosing HR versus natural transfer may have underlying differences in ovulation, embryo quality, or other biological aspects that may affect their FET success rates. Multiple studies have also shown a correlation between lining thickness and pregnancy outcomes in frozen embryo transfer cycles [3, 21], though research is unclear on if one type of protocol is better for endometrial thickness.

The presence of a corpus luteum in natural cycles also may also contribute to physiologic differences that affect success rates of HR versus natural transfer. Progesterone produced by the corpus luteum is essential for sustaining a pregnancy, and the absence of the corpus luteum in HR cycles means that the initial pregnancy is entirely reliant on

Table 3 Euploid transfer outcomes by type of frozen transfer

Type of transfer	HR	Natural cycle	*p* value
Unadjusted ongoing pregnancy (OP) rate	42.9%	60.7%	
Adjusted odds ratio OP, OR (95% CI)	Reference	2.05 (1.27–3.31)	0.003
Unadjusted miscarriage rate	17.7%	15.0%	
Adjusted odds ratio miscarriage	Reference	0.69 (0.37–1.32)	0.27

progesterone replacement. Some studies have suggested that intramuscular progesterone supplementation or higher doses of vaginal progesterone can increase FET success rates in HR cycles [2, 16, 20]; therefore, if the progesterone replacement protocol were inadequate or followed incorrectly, this may adversely impact pregnancy outcomes. Furthermore, one study reported that in a cohort of 47 women, HR cycles were associated with significantly lower endothelial function when compared to natural cycles [7]. This was hypothesized to possibly be due to the absence of corpus luteum products in HR cycles, and it is possible that resulting biological differences may be a contributor to disparities in pregnancy outcomes between the two types of protocols.

Comparison with existing literature

As the number of frozen embryo transfers and PGT-A continues to increase in the United States, the subject of optimizing frozen embryo transfer is an important area for research. Frozen embryo transfers have risen 82.5% from 2006–2012, due to multiple reasons including advances in vitrification, increased use of PGT-A, and emerging research about benefits of freeze-only cycles [32]. PGT-A has also steadily increased in usage among ART cycles due to advancements in the field including next generation sequencing [30], as well as studies suggesting that PGT-A may improve pregnancy outcomes in certain populations [5, 8, 35]. In addition, PGT-A often necessitates frozen embryo transfer as biopsy results are not commonly available within 24 h for current testing platforms.

Multiple studies have suggested that freeze-only transfer may be associated with improved pregnancy outcomes compared to fresh transfer [1, 6, 33, 34, 38]. A subset of frozen transfer, freeze-only transfer occurs when all embryos in a cohort are cryopreserved for transfer in a subsequent cycle. In contrast, frozen transfer may occur after the highest quality embryo has been transferred in a fresh cycle and supernumerary embryos are cryopreserved for later transfer. It is believed that freeze-only transfer may be more beneficial than fresh transfer due to a variety of factors, including more physiologic hormone levels and avoidance of a premature progesterone elevation, thereby improving endometrial receptivity and embryo-endometrium synchrony [17, 19, 24]. Of note, all cycles in this analysis were freeze-only transfer cycles.

Treatment protocols for frozen embryo transfer are generally classified as HR (which use estrogen for endometrial preparation) or natural (which do not use medications for endometrial preparation). Both types of transfers may include luteal phase progesterone support, though this is more common in HR protocols (particularly the use of intramuscular progesterone). Existing literature on outcomes between the two types of transfer protocols for non-PGT-A screened embryos is somewhat variable, though large reviews have suggested equivalent outcomes. A Cochrane review including 18 RCTs with 3,815 women found that there was insufficient evidence to recommend natural versus HR FET for ovulatory women, though evidence was of low quality [11]. Another Cochrane review of 22 RCTs on FET endometrial preparation with embryos from donor oocytes also found that there was insufficient evidence to recommend one type of FET protocol [13]. A systematic review and meta-analysis on the subject including 20 articles also found no significant differences in clinical pregnancy rate, ongoing pregnancy rate, or live birth rate between the two transfer protocols [15]. For smaller studies, the evidence is more mixed, with several studies reporting no difference between natural versus HR protocols [10, 25, 29, 40], and some studies reporting an advantage of either natural [4, 28] or HR protocols [18, 41]. However, heterogeneity in protocols, limited sample size, multiple time periods, and unclear report of methods may account for differences between study findings.

Despite the existing data on frozen transfer protocols, studies on comparisons of these protocols for euploid embryos are extremely limited. An RCT of 236 patients found no significant difference in implantation, ongoing pregnancy, or miscarriage rates between HR versus modified natural FET for euploid blastocysts [14]. In contrast, a separate cohort study found that among 113 euploid FET cycles (65 natural cycles with ovulatory patients, 48 HR with anovulatory patients), live birth/ongoing pregnancy rates were significantly higher in natural FETs with adjusted OR 2.68 (95% CI 1.22–5.87) after adjusting for maternal age [27]. There were no significant differences in miscarriage rates in the analysis. The findings of this second study are very similar to our analysis, which we also found higher ongoing pregnancy rates for natural cycles, with similar miscarriage rates.

However, the Melnick et al. cohort study does have several differences compared to our analysis. We used a multivariate regression which controlled for multiple potential patient and cycle confounders, while the original analysis controlled for age only. Our findings are also more generalizable, as we did not restrict HR cycles to anovulatory patients. In addition, we had a larger sample size with over three times the number of patients. However, the findings of both this study and our study differed from the prior RCT. This may have been due to several reasons, including subtle differences in protocol within the classification of natural or HR. In the RCT, intramuscular progesterone was used for both natural and HR cycles, while this was mostly limited to HR cycles for our study and the prior cohort study. In addition, all HR cycles in the RCT received a GnRH agonist and oral estradiol, while in our study and the prior cohort study, there was more variation in protocol including IM estrogen and estradiol patches for some cycles. Some HR cycles in the prior cohort study also used GnRH antagonists. In addition, the RCT was conducted in Italy, while both the cohort studies were conducted in the United States, and there may have been more patient population heterogeneity in the US studies. Previous studies have suggested that FET success may differ by ethnicity for certain situations [31]. As there are very few studies on this subject in the context of euploid embryos, further study in larger cohorts is warranted, including larger RCTs, and further stratification by subgroups in a heterogeneous population.

Future areas for research

In addition to the need for additional larger studies in heterogeneous populations and RCTs as above, future areas for research also include specific protocols within HR and natural transfer. Current literature has mostly stratified cycles as either natural or HR, but there are many possible distinctions within these categories including the type of estrogen supplementation, usage of GnRH agonist or antagonist, and type of luteal phase progesterone support. In addition, some studies have also studied the use of letrozole as endometrial preparation for FET [36], which also warrants investigation for euploid embryos. Given this wide variation in protocols, it is important to further study individual distinctions in protocols and their contribution to success rates, ideally in the context of euploid embryos which lessens consideration of aneuploidy as a cause for failure. Cost effectiveness is another area for future study, though the Greco et al. RCT found that HR and natural FETs were similar in terms of cost effectiveness [14]. However, in that study all cycles used IM progesterone, and cost effectiveness will also likely vary by geography and corresponding insurance coverage patterns. There is also emerging research that pregnancy outcomes such as hypertensive disease may vary for natural versus HR transfer, through mechanisms such as absence of corpus luteum contributing to endothelial dysfunction [7]. Therefore, it will become increasingly important to investigate this question of frozen transfer protocols as it pertains to optimizing not only ART outcomes, but also measures of maternal health in pregnancy.

Strengths and limitations

This study's strengths include the large sample size in the context of a relatively novel subject, multivariate analysis including multiple cycle and patient confounders, and the use of euploid embryos which allows us to focus the investigation on the uterine environment (as aneuploidy is established as a major cause of FET failure). The use of a multivariate analysis in this subject matter is particularly important as multiple other factors may affect a transfer's likelihood of success, even in the context of euploid embryos; furthermore, these factors may be linked together (for example, embryo quality and ovarian reserve). This is the largest study to date on the subject of natural versus HR transfer for euploid embryos, as most previous literature in this area has focused on untested embryos. As transfers are increasingly moving towards frozen cycles given improved outcomes with freeze-only cycles and the use of PGT-A, our study has widespread clinical applicability in terms of choosing a transfer protocol.

This study does have several limitations. First, this study is a retrospective cohort study and is, therefore subject to selection bias. In addition, as detailed above there are distinctions between natural and HR cycle protocols, and the protocols were not completely standardized for all cycles due to this study's retrospective nature. Though this is the largest study to date on the subject of transfer protocols for euploid frozen embryo transfer with 389 cycles, further study is warranted in larger cohorts. Our cohort may have had a better than average prognosis based on the ability to produce at least one euploid embryo for transfer, as well as the relatively high average age and AMH. In addition, though our study is designed to focus on the uterine environment given the use of euploid embryos, we cannot discount that PGT-A has a small false negative and false positive rate that may have affected outcomes [12, 23]. We also did not have ethnicity data available for all patients and were not able to include this as a confounder in the multivariate analysis. Finally, we did not analyze the data separately for natural cycles with and without hCG trigger. However, our main hypothesis was to investigate natural versus HRT cycles, as literature suggests that supraphysiologic hormone levels and the number of corpus luteum present may affect the uterine environment in complex ways, and the presence of hCG trigger is not expected to affect these parameters.

Finally, there may be regional or clinic variation in preference for natural or HR cycles. For clinics that have a strong preference towards one type of cycle, there may be a selection bias in that one type of cycle may almost always be chosen initially, and the second type of cycle may be chosen as a secondary option if the first one is unsuccessful. To fully address this concern, we performed a secondary analysis of only the first FET cycle at our institution and found that overall results were similar to the main analysis. Our clinic does not have a default preference for natural or HR cycles and the selection is based on mostly patient preference or medical/logistical considerations, as seen by the fact that cycles were almost evenly distributed between natural and HR cycles.

Conclusion

After adjustment for potential confounders including ovulatory dysfunction, we found that natural cycle single euploid frozen embryo transfer was associated with significantly higher ongoing pregnancy rates than hormone replacement transfer. There was no significant difference in adjusted miscarriage rate between the two groups. This is the first study to investigate this question in a multivariate analysis and the largest study on the subject to date. Our results add to the limited body of literature on this subject in the context of euploid embryo transfer, suggesting a possible advantage to natural cycles. Further study in larger and more heterogeneous cohorts is warranted, including on the biological differences underlying natural versus HR transfer.

Author contributions AW: protocol/project development, data collection or management, data analysis, and manuscript writing/editing. GM: protocol/project development and manuscript editing. JK: protocol/project development and manuscript editing. LW: protocol/project development and data manuscript editing.

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Compliance with ethical standards

Conflict of interest LW has sponsored research through Celmatix Inc. (which does not pertain to this study). No other conflicts of interest to disclose for AW, GM, JK, or LM. The study itself was not funded by any sources.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. IRB approval was obtained through Stanford University.

Informed consent As this was a retrospective chart review using de-identified data, informed consent was not obtained from individual patients, but IRB approval was obtained for the overall study as above. Retrospective chart reviews are exempt from needing informed consent from individual participants per the IRB at our academic institution.

Animal studies This article does not contain any studies with animals performed by any of the authors.

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