



ARTICLE

Live birth rates with a freeze-only strategy versus fresh embryo transfer: secondary analysis of a randomized clinical trial



BIOGRAPHY

Dr Vuong is Deputy Head of OB/GYN Department, University of Medicine and Pharmacy at HCM City, Vietnam. She was one of the pioneers of IVF in Vietnam, starting in 1997. She has performed about 20,000 ART cycles. Her areas of research activity include ovulation induction, IVM and frozen embryo transfer.

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KEY MESSAGE

Based on randomized clinical trial data, a freeze-only strategy appears to increase live birth rates versus fresh transfer in non-PCOS women with a moderately high progesterone level undergoing IVF. Endometrial thickness does not modify the treatment decisions regarding a freeze-only strategy in women with an endometrial thickness of >8 mm.

ABSTRACT

Research question: What are the roles of serum progesterone and endometrial thickness as biomarkers in the decision between a freeze-only and fresh embryo transfer in IVF for women without polycystic ovary syndrome (PCOS)?

Design: This was a secondary analysis of a randomized controlled trial including 782 couples who were followed up until the end of the first completed cycle. Couples scheduled for their first or second IVF cycle with a FSH/gonadotrophin-releasing hormone antagonist protocol were randomized to a freeze-only ($n = 391$) or fresh embryo transfer ($n = 391$) strategy. The endpoint for this analysis was live birth rate (LBR) after the first embryo transfer.

Results: There was no significant difference in LBR after the first cycle between a freeze-only and fresh transfer strategy. When serum progesterone levels at trigger were in the third quartile (Q3, 1.14–1.53 ng/ml), LBR was significantly higher in the freeze-only versus fresh transfer group ($P = 0.01$); when serum progesterone was ≥ 1.14 ng/ml, LBR was significantly better in the freeze-only group (37.4% versus 23.8% in the fresh transfer group; $P = 0.004$). LBRs in the freeze-only and fresh embryo transfer groups were similar across all quartiles of endometrial thickness, although a small advantage for freeze-only in women with a very thin endometrium could not be excluded.

Conclusions: Serum progesterone level on the day of trigger may have potential as a biomarker on which to base a prospective decision about whether to use a freeze-only or fresh embryo transfer strategy in women undergoing IVF.

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KEYWORDS

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Biomarkers
Endometrial thickness
Freeze-only protocol
Progesterone
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INTRODUCTION

Traditionally, IVF has involved the transfer of fresh embryos. Embryo freezing technology was originally developed to store embryos not transferred in a fresh IVF cycle. However, advances in technology have the potential to increase the attractiveness of freeze-only cycles, with cryopreservation using the vitrification technique improving embryo survival rates compared with conventional slow freezing (Balaban *et al.*, 2008). Cryopreservation of embryos allows the detrimental effects of high-dose hormones used for ovarian stimulation on the endometrium to be avoided and use of a freeze-only strategy is one approach that can be used to prevent ovarian hyperstimulation syndrome (OHSS) (Devroey and Adriaensen, 2011). Therefore, use of freeze-only cycles is becoming increasingly popular (Shapiro *et al.*, 2014).

A number of studies have reported higher clinical and ongoing pregnancy rates after frozen versus fresh transfer (Basirat *et al.*, 2016; Belva *et al.*, 2008; Chen *et al.*, 2016; Healy *et al.*, 2010; Henningsen *et al.*, 2011; Pelkonen *et al.*, 2010; Pinborg *et al.*, 2010; Shi *et al.*, 2018; Shih *et al.*, 2008; Vuong *et al.*, 2018; Wada *et al.*, 1994; Wang *et al.*, 2017; Wennerholm *et al.*, 1997; Wikland *et al.*, 2010). However, results of large randomized controlled clinical studies in this area are conflicting (Chen *et al.*, 2016; Shi *et al.*, 2018; Vuong *et al.*, 2018). In a population of patients with polycystic ovary syndrome (PCOS) from China, the live birth rate (LBR) after a freeze-only strategy was 49% compared with 42% after fresh transfer of two embryos ($P = 0.004$) (Chen *et al.*, 2016). In contrast, two recent trials including only patients without PCOS found no significant differences in the rates of ongoing pregnancy and live birth between the freeze-only and fresh transfer groups (Shi *et al.*, 2018; Vuong *et al.*, 2018).

It has been suggested that higher progesterone levels and a thinner endometrium might be associated with reduced success rates after fresh embryo transfer, while these factors may not influence the success of freeze-only or subsequent frozen cycles (Healy *et al.*, 2016; Wang *et al.*, 2017). We used data from our randomized clinical trial (RCT)

comparing outcomes after freeze-only and fresh embryo transfer cycles in non-PCOS patients (Vuong *et al.*, 2018) to evaluate whether progesterone level and endometrial thickness could be used as biomarkers to guide the choice of a freeze-only strategy or fresh embryo transfer.

MATERIALS AND METHODS

This is a secondary analysis of a single-centre RCT in Ho Chi Minh City, Vietnam (NCT02471573). The study was approved by the Medical Ethics Committee at My Duc Hospital, Ho Chi Minh City, Vietnam (02/15/ĐĐ-BVMĐ) on 26 May 2015. The trial was conducted according to Good Clinical Practice and Declaration of Helsinki 2002 principles, including oversight by an independent Data Monitoring Committee. All patients provided written informed consent for participation in the RCT.

Study subjects and design

The main study details and results have been reported previously (Vuong *et al.*, 2018). Briefly, couples were eligible if they were infertile, scheduled for IVF and had no more than one previous IVF cycle. Women with PCOS as well as couples undergoing IVF with oocyte donation were not eligible. After informed consent, couples were randomized in a 1:1 ratio to a freeze-only or fresh embryo transfer strategy. Full details of the COS protocol, using a FSH/gonadotrophin-releasing hormone (GnRH) antagonist protocol have been reported in detail previously (Vuong *et al.*, 2018).

Freeze-only and fresh transfer protocols

In the freeze-only group, all grade 1 and 2 embryos (ALPHA Scientists in Reproductive Medicine; ESHRE Special Interest Group Embryology, 2011) were cryopreserved (Cryotech vitrification); grade 3 embryos could also be cryopreserved if requested by the couple. In the next cycle, oral oestradiol valerate (Valiera®; Laboratorios Recalcine SA, Chile) 8 mg/day starting from the second or third day of the menstrual cycle was used to prepare the endometrium. From cycle day 6 onwards endometrial thickness was monitored and vaginal progesterone (Cyclogest®; Actavis Generics, NJ, USA) 800 mg/day started when endometrial thickness was ≥ 8 mm. This is the standard practice at

our clinic based on experience in more than 8000 embryo transfer cycles. A maximum of two grade 1 or 2 embryos were warmed on the day of embryo transfer, 3 days after the start of progesterone. Two hours after warming, surviving embryos were transferred into the uterus under ultrasound guidance. Cryopreserved embryo transfer was not performed when endometrial thickness was < 8 mm.

In the fresh transfer group, a maximum of two grade 1 or 2 embryos were transferred into the uterus under ultrasound guidance on the day of randomization. Any remaining grade 1 or 2 embryos, and grade 3 embryos if the couple requested, were vitrified and transferred in subsequent cycles.

Women in both groups received luteal phase support with oral oestradiol valerate 8 mg/day and vaginal progesterone 800 mg/day (this was continued until 7 weeks' gestation in patients who became pregnant), and serum beta-human chorionic gonadotrophin (HCG) was measured 2 weeks after embryo transfer (Cobas; Roche Diagnostics, Germany). Women with a positive pregnancy test (HCG > 5 mIU/ml) underwent ultrasound scanning of the uterus at 7 and 12 weeks' gestation.

Follow-up

Live birth was defined as the birth of at least one newborn after 24 weeks' gestation that exhibits any sign of life (twins will be a single count). Endometrial thickness was measured as the maximum distance between the endometrial-myometrial interface of the anterior to posterior uterine wall on transvaginal ultrasound (Toshiba, Japan) on the day of randomization (day 3). Serum progesterone (Roche, Germany) was determined on the day of triggering in the stimulated cycle, including the fresh or cryopreserved embryo transfer cycle. The endpoint for this analysis was LBR after the first embryo transfer.

Statistical analysis

The secondary analysis methodology was based on four steps as outlined previously (Little, 2013). The first step, identifying the relevant data set, was achieved by using data from a recent RCT conducted at our study centre. After statisticians had familiarized

themselves with the associated codebooks (Pienta *et al.*, 2011), raw data from the RCT were used to construct a working datafile (Willms, 2011), which was then used to conduct the analyses. All analyses are exploratory and were performed on an intention-to-treat basis by an independent statistician who had access to all data. The protocol for the RCT included planned biomarker analysis for endometrial thickness and serum progesterone level. To do this, patients were divided into four quartiles for each parameter (at the 25th, 50th and 75th percentiles). Subgroup analyses were undertaken in each of the four quartiles and an interaction term between treatment group and the biomarker was added to the regression model and tested for interaction. Next, Subpopulation Treatment Effect Pattern Plot (STEPP) analysis was applied to address the continuous character of the markers (Yip *et al.*, 2016). STEPP constructs overlapping subpopulations along the continuum of the covariate of interest, thereby improving the precision of the estimated treatment effects within the subgroups in a smoothing-by-binning manner. STEPP analysis typically implements the 'sliding window' pattern of subpopulations. In a clinical trial, we consider n patients being assigned randomly to one of the two treatments. A subpopulation of the sliding window pattern is defined by two cut-off values $[Z_{\min}, Z_{\max}]$ cR, so that patients who are randomly assigned to one of the two treatment arms and have the covariate value (Z) between these two cut-off values are included in the subpopulation. Using two smoothing parameters chosen by the investigator – the number of patients per subpopulation (r_2) and the largest number of patients in common between two consecutive subpopulations (r_1), the subpopulations are constructed based on the value of a covariate of interest. The window slides forward by replacing ($r_2 - r_1$) individuals with new individuals having higher covariate values (assume no ties). These two smoothing parameters thus determine the number of subpopulations. As n gets larger, the number of subpopulations grows to $\lceil 1 + (n - r_2)/(r_2 - r_1) \rceil$. All analyses were performed using R for Windows (Version 3.0.1; R Foundation for Statistical Computing, Vienna, Austria). Two-sided P -values < 0.05 were considered to indicate statistical significance.

RESULTS

Patients

Between June 2015 and April 2016, a total of 782 couples were randomized, 391 to the freeze-only arm and 391 to the fresh embryo transfer arm (FIGURE 1) (Vuong *et al.*, 2018). The two groups were well matched at baseline and full details of baseline demographic and clinical characteristics have been reported previously (Vuong *et al.*, 2018). Briefly, mean age was 32 ± 4 years, mean duration of infertility was 57 months, approximately two-thirds of patients had primary infertility, and mean body mass index was 20.8 ± 2.2 kg/m² in both groups. In 84% of patients this was the first IVF attempt, approximately two-thirds had primary infertility and the most common indication for IVF was male factor infertility (43% and 40% of patients in the freeze-only and fresh transfer groups, respectively). The total dose of FSH (2731 ± 851 and 2656 ± 869 IU), progesterone level on day of trigger (1.4 ± 0.8 and 1.3 ± 1.1 ng/ml), and oestradiol level on trigger (2019 ± 1470 and 2029 ± 1616 pg/ml) were similar in the freeze-only and fresh transfer groups. There were also no significant between-group differences in the number of oocytes retrieved, number of metaphase II, fertilized (two-pronuclear), cleavage or good day 3 embryos, or the number of embryos transferred. All patients in the freeze-only group returned for embryo transfer, and none were lost to follow-up after the first transfer. At 12 months after randomization, 11 cycles were lost to follow-up after the second embryo transfer (four in the fresh transfer group and seven in the freeze-only group).

Progesterone level

Patient characteristics based on progesterone level quartile are shown in Supplementary Table 1. For LBR after the first embryo transfer, there was a statistically significant interaction between progesterone level and treatment group ($P = 0.0349$). LBRs by quartiles of progesterone level on day of triggering are shown in FIGURE 2. When progesterone levels were < 1.14 ng/ml, LBR tended to be better in the fresh embryo transfer group (38.1% versus 29.4%), but the difference was not statistically significant. In contrast, when serum progesterone was ≥ 1.14 ng/ml the LBR was significantly better in the

freeze-only group (23.8% versus 37.4%, $P = 0.004$). In the STEPP analysis, at median progesterone level points of 1.17 and 1.22 ng/ml, the LBR after first embryo transfer was significantly lower (odds ratio [OR] 4.4; 95% confidence interval [CI] 1.26–15.39 and OR 3.45, 95% CI 1.12–10.63) in the fresh transfer versus freeze-only group (FIGURE 3, Supplementary Table 2).

Endometrial thickness

Patient characteristics based on endometrial thickness quartile are shown in Supplementary Table 3. There was no significant interaction between endometrial thickness and treatment allocation for the LBR after first embryo transfer. In the STEPP analysis, no significant differences in LBR after first embryo transfer were seen between the freeze-only and fresh embryo transfer groups (FIGURE 4, Supplementary Table 4).

DISCUSSION

The results of this analysis of data from a RCT (Vuong *et al.*, 2018) suggest that a freeze-only strategy may be more effective than a fresh embryo transfer strategy in patients with higher serum progesterone levels. On the other hand, endometrial thickness appeared to have limited impact on the relative LBR after fresh versus cryopreserved embryo transfer.

Although the question of whether progesterone level elevation during stimulation decreases IVF success in cycles with fresh transfer has been investigated previously (Venetis *et al.*, 2013), this has not been investigated in the setting of a comparison between fresh transfer and a freeze-only strategy, or in a randomized setting. Therefore, strengths of this study are its randomized design, and the fact that the analysis was prespecified in the study protocol. There was no prior selection or stratification of patients based on serum progesterone or endometrial thickness, allowing the role of these potential biomarkers to be investigated in the original study population.

The fact that cryopreserved embryo transfer was not performed in patients with thin endometrium (< 8 mm) could limit the ability to determine the influence of endometrial thickness

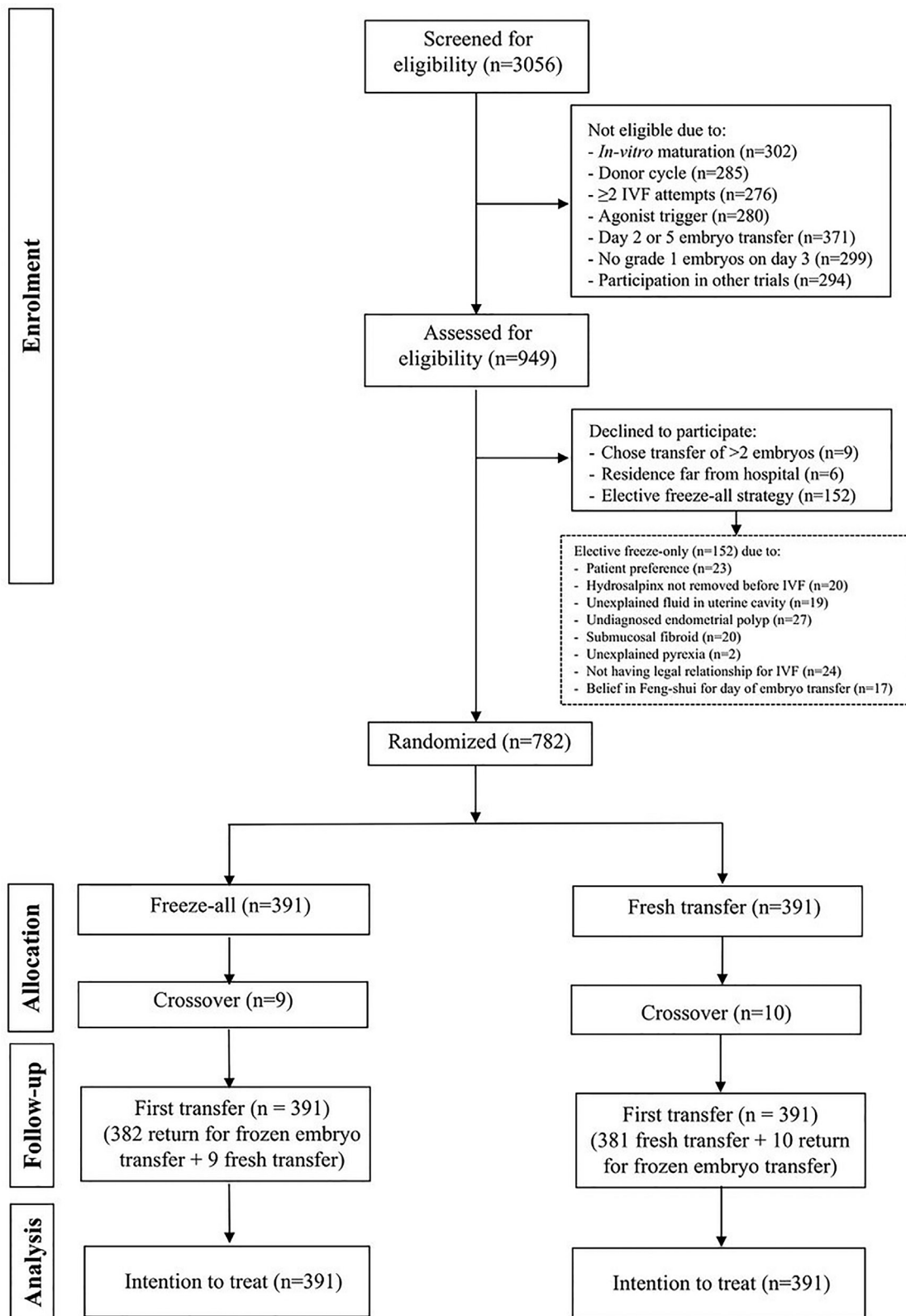


FIGURE 1 CONSORT flow chart. Data from *Vuong et al. (2018)*.

on ongoing pregnancy rate. Another limitation is that this is a secondary analysis of data, and smaller numbers in

patient subgroups mean that the analysis is relatively underpowered. The fact that between-group differences in LBR based

on serum progesterone levels in Q4 failed to achieve statistical significance while those in Q3 did might be related to

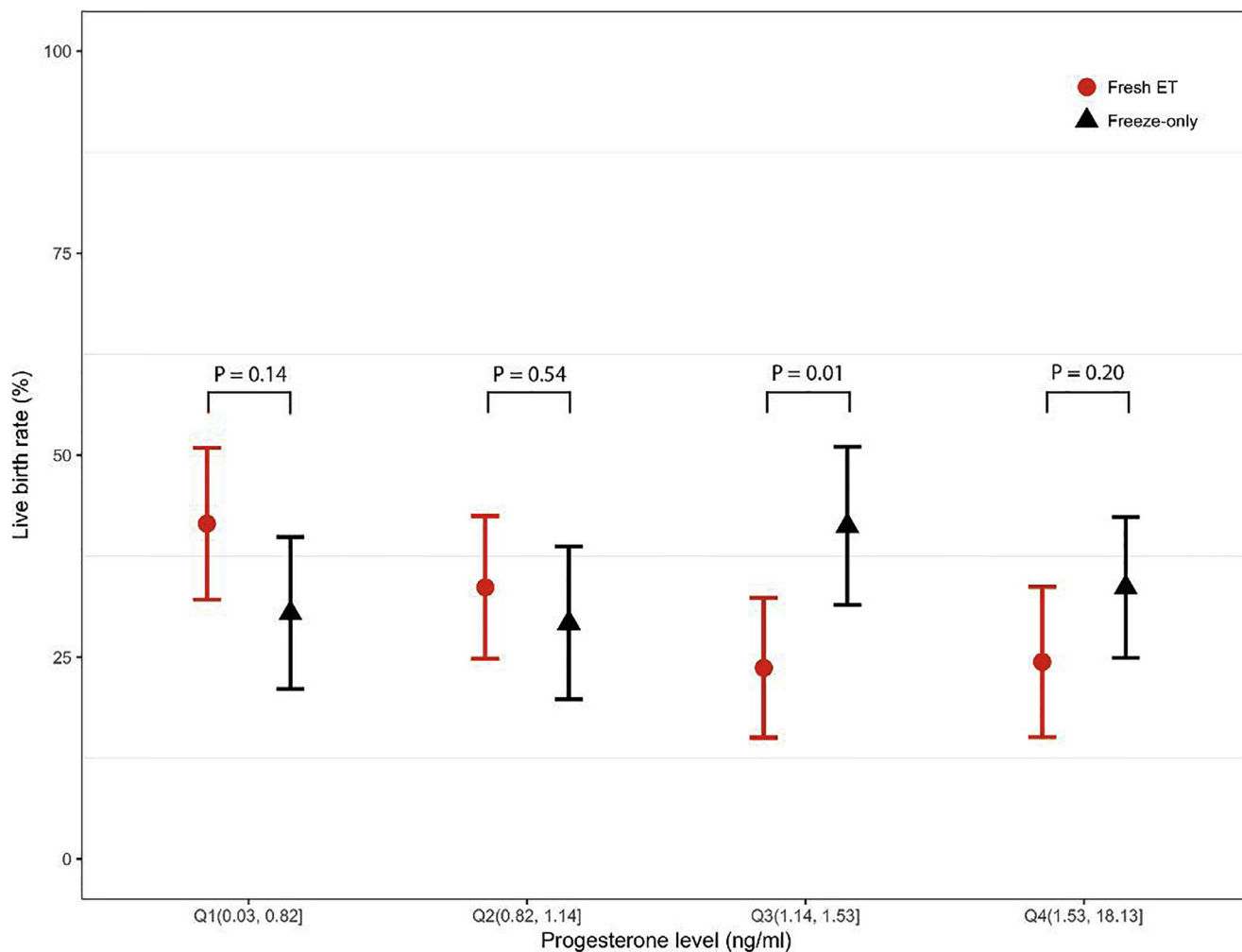


FIGURE 2 Live birth rate after first embryo transfer (ET) in the freeze-only and fresh embryo transfer groups by quartiles of progesterone level on the day of triggering.

limited power. Individual participant data meta-analysis of our data combined with that from the other large RCT (*Shi et al., 2018*) comparing fresh embryo transfer with a freeze-only strategy would help overcome this problem.

The serum progesterone level on the day of GnRH agonist or HCG trigger has been identified as a potential predictor of fertility outcomes in previous studies (*Huang et al., 2012; Nayak et al., 2014; Ochsenkuhn et al., 2012*). In addition, a recent retrospective cohort study has reported differential effects of serum progesterone on implantation and ongoing pregnancy rates in freeze-only versus fresh transfer cycles (*Wang et al., 2017*). In that study, cycles with serum progesterone level at trigger >1.0 ng/ml had significantly higher implantation and ongoing pregnancy rates when a freeze-only versus fresh transfer strategy was used (52% versus 45.3%; OR 1.31,

95% CI 1.13–1.51). This was particularly the case in women aged >35 years (OR 1.73, 95% CI 1.34–2.24). Conversely, when trigger day progesterone level was ≤ 1.0 ng/ml there were no significant differences in the outcomes studied between patients managed using a freeze-only compared with fresh transfer strategy. In addition, data from retrospective studies have reported a negative effect of elevated serum progesterone levels on the day of trigger on LBR in fresh embryo transfer cycles but not in subsequent frozen cycles, suggesting that elevated progesterone levels were not detrimental in frozen embryo transfer cycles (*Healy et al., 2016; Lahoud et al., 2012*). This is consistent with our study finding that LBRs in the freeze-only versus fresh embryo transfer group were significantly higher in the STEPP analysis when median serum progesterone was 1.17 or 1.22 ng/ml and when serum

progesterone was 1.14–1.53 ng/ml in the quartile-based analysis. Although numerical between-group differences in LBR were seen when progesterone levels were higher, these failed to reach statistical significance. Therefore, it is unclear whether our finding represents a clear trend in outcomes after fresh embryo transfer as serum progesterone level increases.

One potential mechanism for a negative effect of higher serum progesterone levels on clinical and ongoing pregnancy rates after fresh embryo transfer is the influence of this hormone on the endometrium, which influences embryo implantation. Such an association has been reported in a number of studies (*Castillo et al., 2015; Chetkowski et al., 1997; Huang et al., 2012; Labarta et al., 2011; Li et al., 2011; Melo et al., 2006*). More specifically, it has been suggested that higher serum progesterone levels

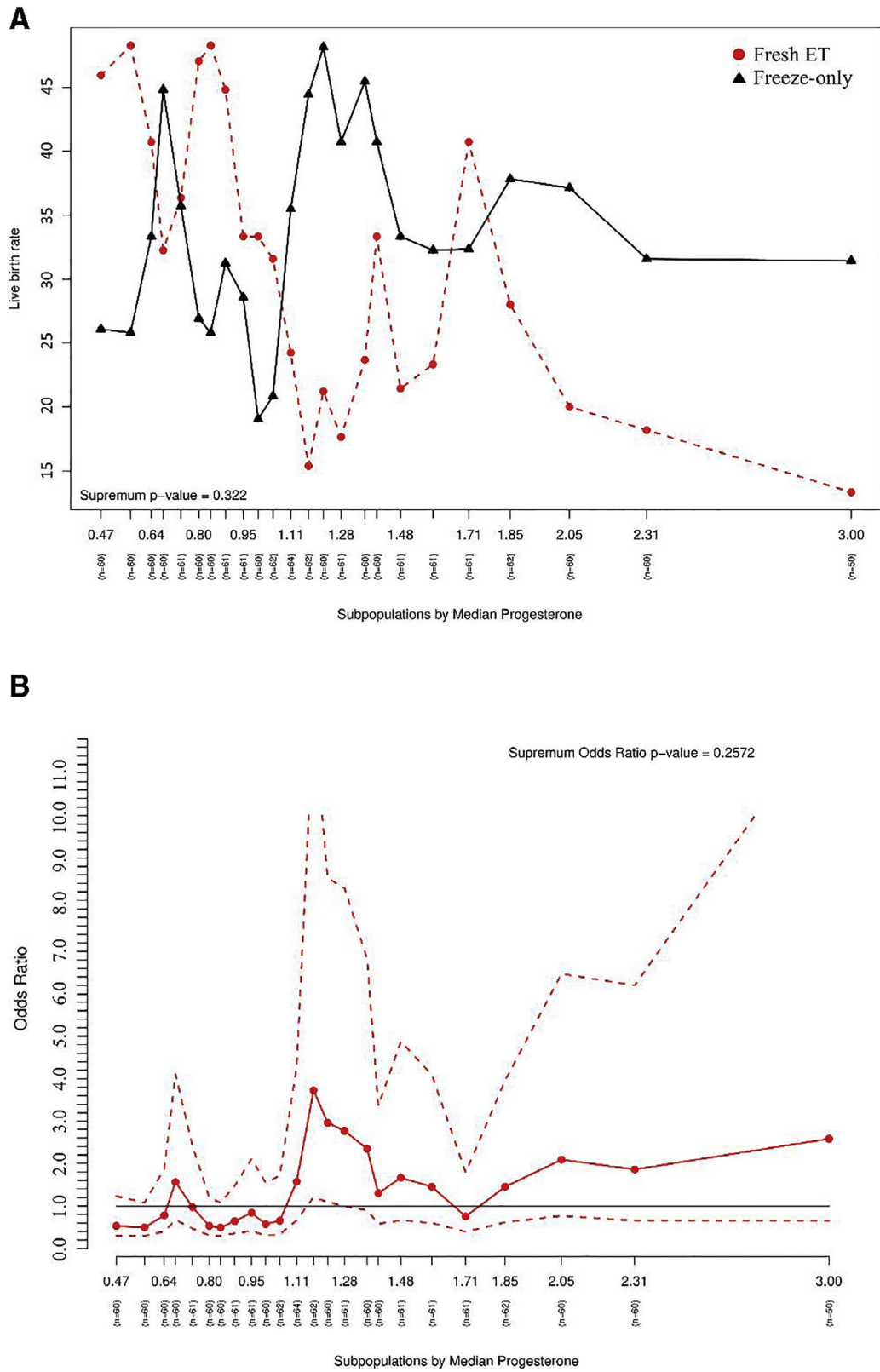


FIGURE 3 Subpopulation Treatment Effect Pattern Plot (STEPP) analysis (A) for live birth rate after first embryo transfer (ET) at different median values of progesterone level on the day of triggering. (B) Odds ratio values for live birth rate after first embryo transfer in the freeze-only versus fresh embryo transfer group based on different median values of progesterone level on day of triggering (solid line = odds ratio; dotted lines = 95% confidence intervals).

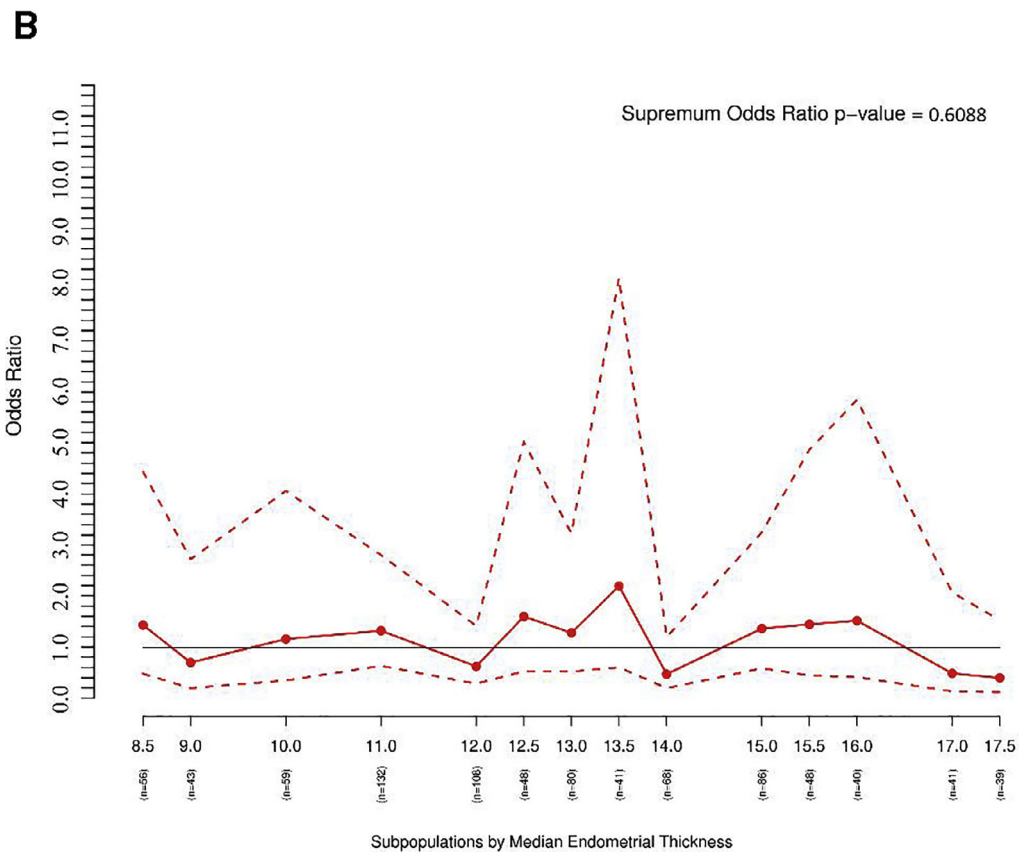
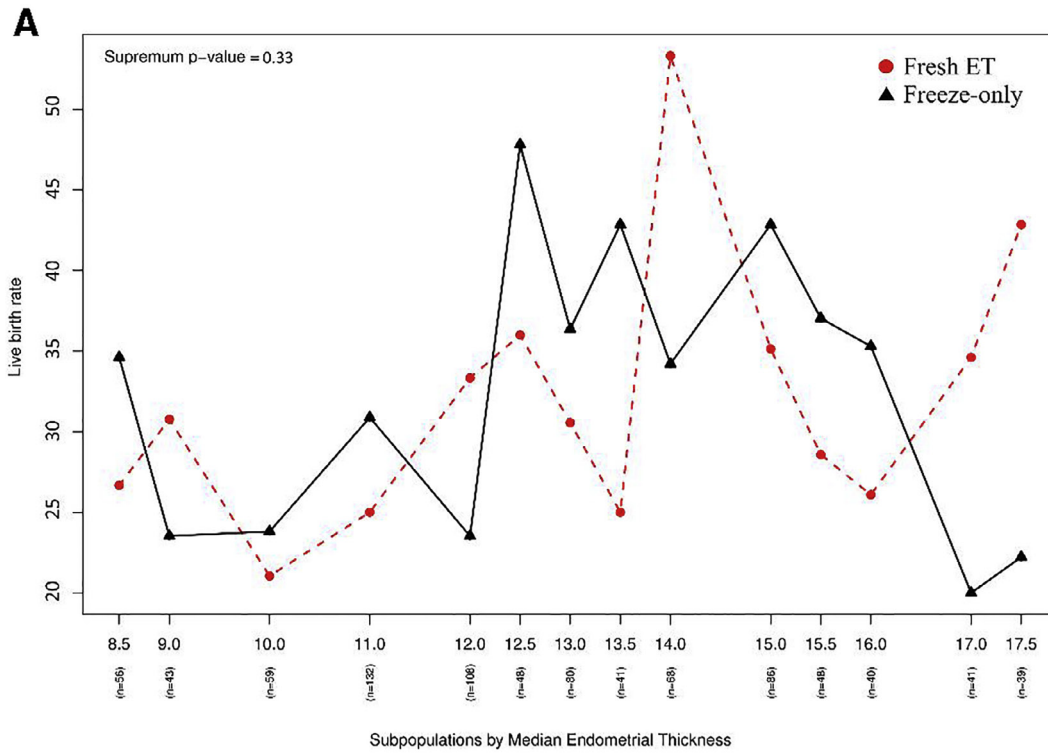


FIGURE 4 Subpopulation Treatment Effect Pattern Plot (STEPP) analysis (A) for live birth rate after first embryo transfer at different median values of endometrial thickness on day of randomization. (B) Odds ratio values for live birth rate after first embryo transfer in the freeze-only versus fresh embryo transfer group based on different median values of endometrial thickness on day of randomization (solid line = odds ratio; dotted lines = 95% confidence intervals).

could accelerate maturation of the endometrium and promote secretory transformation, resulting in a smaller window for implantation, which in turn decreases pregnancy rates in fresh transfer cycles where this occurs (*Castillo et al., 2015; Chetkowski et al., 1997*). A comprehensive meta-analysis of studies in this area, which included more than 60,000 IVF cycles, found that there was a significant negative correlation between serum progesterone level on the day of trigger ≥ 0.8 ng/ml and decreased pregnancy rates in fresh transfer, but not frozen-thawed, cycles (*Venetis et al., 2013*). In our study, the negative effect of high serum progesterone levels on the LBR after fresh embryo transfer was seen at slightly higher levels than reported in the meta-analysis (1.17–1.22 versus ≥ 0.8 ng/ml).

Although the data highlighted above suggest that if the serum progesterone level on the day of GnRHa trigger is high then frozen embryo transfer should be preferred over fresh embryo transfer, this study does not provide conclusive support for an unacceptable LBR. Nevertheless, we did find a stronger impact of serum progesterone on the LBR in the fresh transfer than in the freeze-only group, which supports a role for progesterone as a biomarker that could be useful in guiding an overall clinical decision about whether to undertake fresh embryo transfer or to freeze all embryos for transfer in subsequent cycles.

For women with an endometrial thickness above 8 mm, we did not identify a statistically significant influence of endometrial thickness on LBRs in either freeze-only or fresh embryo transfer IVF cycles. Existing literature about the effects of endometrial thickness on pregnancy outcome is inconsistent, with some studies suggesting that greater endometrial thickness is associated with increased likelihood of pregnancy (*Al-Ghamdi et al., 2008; Aydin et al., 2013; Check et al., 1991, 1993; Dickey et al., 1992; Kovacs et al., 2003; Kumbak et al., 2009; Noyes et al., 1995; Rinaldi et al., 1996; Wu et al., 2014*), and others showing no effect (*Bassil, 2001; De Geyter et al., 2000; Schild et al., 2001; Yuval et al., 1999*). Despite the association between thicker endometrium and pregnancy rates

reported in many studies, the authors of a meta-analysis of data from 22 studies concluded that although endometrial thickness of <7 mm was associated with a significantly lower probability of clinical pregnancy, the number of cases where endometrial thickness is in this range is low, and that the use of endometrial thickness as a tool to decide whether to cancel the cycle, freeze all embryos or stop additional IVF treatment was not justified based on their results (*Kasius et al., 2014*). This is consistent with our data showing that no pregnancy occurred when endometrial thickness was <7 mm (three fresh embryo transfers were undertaken below this threshold). Although we did not find either a strong clinical difference or a statistically significant difference, the possibility that women with a very thin endometrium benefit from a freeze-only strategy cannot be excluded. Nevertheless, when endometrial thickness is over 8 mm there does not appear to be any reason to use this parameter as a biomarker for decisions about whether to use a freeze-only or fresh transfer strategy. However, if a decision was made to cancel a fresh IVF cycle due to low endometrial thickness, then a freeze-only cycle would be an alternative treatment approach.

The results of analysis based on RCT data suggest that serum progesterone level on the day of trigger may have potential as a biomarker on which to base a prospective decision about whether to use a freeze-only or fresh embryo transfer strategy in women undergoing IVF. Based on our results and from literature review (*Venetis et al., 2013*), we recommend the use of freeze-only and subsequent frozen embryo transfer in cycles that show increased progesterone levels on day of trigger.

While we suggest that high progesterone levels might be a useful biomarker to identify women who would benefit from a freeze-only strategy, we also found lower LBRs when using a freeze-only strategy in women with a low progesterone level. Given that the overall effectiveness of the freeze-only and fresh transfer strategies in our study were comparable (*Vuong et al., 2018*), this could suggest that fresh embryo transfer should be the preferred strategy in women with low progesterone. However, this needs to be investigated further.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi: [10.1016/j.rbmo.2018.12.012](https://doi.org/10.1016/j.rbmo.2018.12.012).

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