Risk of ectopic pregnancy is linked to endometrial thickness in a retrospective cohort study of 8120 assisted reproduction technology cycles

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STUDY QUESTION: Is endometrial combined thickness (ECT) measured prior to embryo transfer (ET) associated with ectopic pregnancy (EP)?

SUMMARY ANSWER: Following IVF, the risk of EP is 4-fold increased in women with an ECT of <9 mm compared with women with an ECT of ≥12 mm.

WHAT IS KNOWN ALREADY: Known risk factors for EP include tubal damage, maternal cigarette smoking and endometriosis. EP is also more common following IVF but the underlying causes for this remain unclear.

STUDY DESIGN, SIZE, DURATION: Retrospective cohort study restricted to all IVF cycles leading to a pregnancy (hCG > 50 IU/l) between January 2006 and December 2014. A total of 6465 patients achieved a pregnancy in 8120 cycles. Cycles using preimplantation genetic screening or donor oocytes were excluded.

PARTICIPANTS/MATERIALS, SETTING, METHODS: This cohort consists of 6465 patients achieving a pregnancy in 6920 stimulated cycles with fresh embryo transfers (STIM ET) and 1200 hormone replacement therapy frozen embryo transfers (HRT-FET) cycles at a private IVF unit (Monash IVF, Melbourne, Australia). ECT was the primary independent variable of interest; the primary outcome was a diagnosis of EP. The dataset was analysed using binary logistic general estimating equations (SPSS v22.0) to calculate odds ratio (OR) for EP adjusted for known confounders (aOR). There was no loss to follow-up in the dataset.

MAIN RESULTS AND THE ROLE OF CHANCE: The study groups did not differ significantly prior to IVF treatment. After adjusting for confounders, ECT remained statistically significant as an independent risk factor for EP. Compared with women with an ECT of <9 mm, women with an ECT of 9–12 mm had an aOR of 0.44 (95% CI 0.29–0.69, P < 0.01) and women with an ECT > 12 mm had an aOR of 0.27 (95% CI 0.10–0.77, P = 0.01). These differences remained statistically significant after performing a sensitivity analysis excluding HRT-FET, smokers and patients with tubal infertility.

LIMITATIONS, REASONS FOR CAUTION: The study design is retrospective, and it is possible that not all confounders have been accounted for. Measurement of ECT was performed by highly trained sonographers, but some inconsistency between individuals may be present.

WIDER IMPLICATIONS OF THE FINDINGS: Our group has previously demonstrated an increased risk of placenta praevia with increased ECT. These new findings suggest that the directionality of the uterine peristalsis waves matters more than their frequency or amplitude. Combining the data from both studies we now hypothesize that increased ECT is a marker for increased fundus-to-cervix uterine peristalsis, explaining both the increased placenta praevia risk and the lower EP risk. Further prospective studies are required to confirm these observations.

STUDY FUNDING/COMPETING INTEREST(S): No funding was required for this study.

Key words: ectopic pregnancy / assisted reproductive technology / endometrial combined thickness / uterine peristalsis / IVF / endometrial appearance
Introduction

An ectopic pregnancy (EP), which accounts for roughly 1–2% of all pregnancies, poses a substantial risk of morbidity and mortality to a pregnant woman if it is not recognized and managed appropriately (Farquhar, 2005). EP has therefore been well studied and known risk factors include endometriosis, tubal damage from other causes such as pelvic inflammatory disease, maternal cigarette smoking because of a detrimental effect on tubal motility and assisted reproductive technology (ART), the effect of which is still uncertain (Ankum et al., 1996; Farquhar, 2005; Alkatout et al., 2013).

Research has consistently demonstrated that ART independently increases the risk of EP, despite the embryo being transferred directly into the uterus (Clayton et al., 2006; Perkins et al., 2015). The rates of EP following ART vary widely, with rates as low as 1.6% and as high as 8.6%, over four times those reported for natural conceptions (Clayton et al., 2006; Perkins et al., 2015; Refaat et al., 2015).

The underlying etiology of this specific risk following ART is not understood; however, many associations and risk factors have been identified. A review by Refaat et al. of EP in the setting of in-vitro fertilization-embryo transfer (IVF-ET) identified that maternal characteristics that increase the risk of EP following IVF-ET include all the risk factors for EP following natural conception (Refaat et al., 2015). Tubal pregnancies that occur either naturally or following IVF-ET were identified to share the same tubal risk factors, confirming that tubal damage is a significant contributor to EP. In addition to these maternal factors, Refaat et al. identified that in the setting of IVF-ET, transfer technique did impact risk, with a high volume of transfer medium and multiple embryo transfer (ET) both demonstrating a definitive increase in EP risk (Refaat et al., 2015).

More recently identified risk factors for EP associated with ART include ethnicity, fresh non-donor embryo transfer, the number of embryos transferred, volume of transfer fluid and transfer depth (Perkins et al., 2015).

A randomized prospective study evaluated the impact of embryo transfer depth, and determined that there was a 0.4% rate of EP following mid-fundal transfer, as compared with a 1.5% rate following deep-fundal transfer (Nazari et al., 1993). Other potential factors include the transfer day and whether a fresh or frozen embryo impacts on the rate of EP. Smith et al. conducted a retrospective cohort study of 277 EP within 13,654 pregnancies, and found that data were inconclusive as to the impact of transfer day (3 or 5) on the relative EP rate (Smith et al., 2013). The data on the risk of EP in fresh versus frozen embryo transfer are also inconsistent with some studies showing a higher risk in fresh cycles (Ishihara et al., 2011; Shapiro et al., 2012; Huang et al., 2014; Fang et al., 2015; Londra et al., 2015; Perkins et al., 2015) and others showing no difference (Jee et al., 2009; Declerq et al., 2014).

Recently, our group demonstrated that as endometrial combined thickness (ECT) increases, so too does the risk of developing placenta praevia following ART (Rombauts et al., 2014). Increased ECT conferred up to 4-fold greater risk of developing placenta praevia later in the pregnancy, with risk increasing in correlation with the degree of increased ECT. Whilst the cause of this association remains unclear, it is possible that increased ECT may act as a surrogate marker for subendometrial hypercontractility, causing the embryo to be dislodged after replacement (Zhu et al., 2014a,b).

Despite evidence that there is a significant increase in the rate of EP following ART, the underlying cause has not been identified. While several studies have indicated that risk factors associated with EP following ART are similar to those for EP after natural conception, none have been able to account for the higher rates in the ART population. In this study, we hypothesize that, in the same way that ECT is associated with placenta praevia, it may also be associated with the risk of EP.

Materials and Methods

Study design

This was an observational study analysing data from a retrospective cohort of all women who achieved a pregnancy (day 16 serum βHCG > 50 IU/l) resulting from a single or double embryo transfer between January 2006 and December 2014 at Monash IVF (Melbourne, Australia). In this time frame a total of 41,560 IVF cycles occurred, 24,208 fresh and 17,352 frozen. We excluded all non-pregnant cycles and pregnant cycles where embryo biopsy or donor oocytes were used. We also excluded cycles where embryos were transferred on days other than day 2, day 3 or day 5 and those where ECT was not recorded. Natural FET cycles were excluded, as ECT is not measured in our unit for these cycles. This left a total of 8120 cycles, (6920 fresh cycles and 1200 frozen cycles) in a total of 6465 patients (Fig. 1).

The primary outcome measure was EP. The rate of EP was calculated by dividing the number of EP by the total number of pregnancies (βHCG > 50 IU/l). The main exposure was ECT. Demographic data collected from the Monash IVF database included age, body mass index (BMI), parity, smoking data and etiology of infertility as assessed by the patient’s treating clinician. Tubal factor infertility was defined as the presence of any of the following: tubal scarring including occlusion, hydrosalpinx, previous salpingectomy or previous EP. As all IVF outcomes are required to be reported by the Australia and New Zealand Assisted Reproduction Database, there was 100% complete follow-up for the primary outcome in this cohort.

Assessment of primary exposure

The primary exposure measure was ECT (range 3–24 mm), grouped into < 9 mm, 9–12 mm and ≥ 12 mm as reported previously (Rombauts et al., 2014). Highly trained sonographers measured the ECT in a two-dimensional

Figure 1 Patient selection flowchart.
plane on a Voluson E8 or a Voluson 730 Expert using intracavity probes with a frequency range of 5–9 Mhz (GE Healthcare, Australia). The ECT was taken from the last transvaginal ultrasound (TVUS) performed before egg retrieval in fresh cycles, typically on the day before or on the day of the human chonic gonadotrophin (hCG) trigger. In HRT-FET cycles the ECT was taken from the last ultrasound before starting vaginal progesterone.

**ART protocols**

Protocols used were the same as those described elsewhere (Rombauts et al., 2014). Stimulation regimes were used with or without oral contraceptive pill scheduling. Recombinant FSH (rFSH) was used for ovarian stimulation in all regimes. A GnRH agonist or a GnRH antagonist was used for down-regulation. To monitor ovarian stimulation, serum levels of estradiol, progesterone and LH were recorded, followed by TVUS, which was performed by experienced sonographers under the supervision of a subspecialist in obstetrical and gynecological ultrasound.

Thirty eight hours after administration of hCG (either recombinant [rhCG] Ovidrel (Merck Serono, Australia) at 250 μg or urinary [uhCG] Pregnyl (MSD, Australia) at 10 000 IU), oocyte retrievals were performed under IV sedation. To fertilize the oocytes, either standard insemination or ICSI were used, with fertilization results assessed between 16 and 20 h after sperm insemination. Embryos were cultured in single 10 μl droplet in the COOK culture system (Cook Medical, Australia). Day 3 embryos underwent a propanediol slow freezing system and were frozen at the cleavage stage, and blastocysts were vitrified. Throughout the study period there was no change to either culture media or culture conditions.

Fresh embryo transfer of a single embryo was undertaken on day 2 or day 3 (cleavage stage) or day 5 (blastocyst stage). Luteal support was provided vaginally through either progesterone pessaries 200 mg twice daily (Orion, Australia) or once daily Crinone 8% (Merck Serono, Australia). Thawing of all cryopreserved embryos took place on the day prior to transfer, with culture overnight. In this cohort, all frozen-thawed embryo transfer (FET) occurred within a hormone replacement cycle (HRT-FET). HRT-FET cycles were induced with cyclical estradiol and vaginal progesterone and LH were recorded, followed by TVUS, which was performed by experienced sonographers under the supervision of a subspecialist in obstetrical and gynecological ultrasound.

Regulatory approval

This retrospective study was approved by the Human Research and Ethics Committee of the Monash Surgical Private Hospital (P07078, 25 November 2013).

**Results**

Of 8120 embryo transfers, 6920 were from STIM ET and 1200 were from HRT-FET. Within the cohort, 89 women (1.1%) were diagnosed with EP (Table I). The EP rate did not change over the study period. The general characteristics of the patient cohort are detailed in Tables I and II.

After univariate analysis (Table III), tubal infertility showed a borderline significant association with an 80% increase in the risk of EP (OR 2.19, 95% CI 1.32–3.59, P < 0.01). However, the echogenic pattern of the endometrium had no impact on the EP risk. Univariate analysis of the protocol type also revealed that antagonist protocols were associated with a 2-fold increased risk of EP compared with agonist protocols (OR 2.18, 95%, CI 1.60–2.97, P < 0.01). In women undergoing STIM ET or HRT-FET, neither smoking status (OR 0.57, 95% CI 0.46–0.71, P = 0.38) nor day of transfer (OR 0.83, 95% CI 0.60–1.15, P = 0.83) had a statistically significant impact on the risk of EP.

Using predictors identified in the univariate analysis with a P-value of at least 0.15, a multivariate regression was performed (Table IV). When correcting for other factors in the model an ECT of > 12 mm remained associated with a 4-fold reduction in risk of EP (aOR 0.27, 95% CI 0.10–0.77, P = 0.01) compared with an ECT of < 9 mm. Similarly, the antagonist protocol was still associated with a 2-fold increased risk compared with the agonist protocol (aOR 1.94, 95% CI 1.17–3.20, P = 0.01). Compared with older women, women < 30 years of age appeared to have at least a 2-fold lower risk of EP. While we have re-used the ECT cut-offs we reported in our previous study (Rombauts et al., 2014) in the main analysis, we have also reanalyzed the data with different ECT cut-offs that may be more relevant for other IVF units (Supplementary Table S1).

### Table I  Demographics.

|                      | Intrauterine pregnancy | Ectopic pregnancy |
|----------------------|------------------------|-------------------|
| Age (y); Mean ± SD   | 34.9 ± 4.3             | 35.4 ± 3.8        |
| Treatment cycle; Median (Range) | 2 (1–32)             | 3 (1–15)          |
| BMI (kg/m²); Mean ± SD | 25.0 ± 5.4            | 23.8 ± 4.5        |
| Parity ≥ 1          | 967 (12.0)             | 11 (12)           |
| Smoker              | 156 (1.9)              | 1 (1)             |
| Endometriosis       | 516 (6.4)              | 1 (1)             |
| Tubal               | 553 (6.9)              | 13 (15)           |
| Male                | 3157 (39.3)            | 32 (36)           |
| Unexplained         | 2368 (29.5)            | 28 (32)           |
| Other               | 1213 (15.1)            | 15 (17)           |
| Multiple causes     | 224 (2.8)              | 0 (0)             |

Data are N (%) unless stated otherwise.

*No statistically significant differences between intrauterine pregnancy and ectopic pregnancy groups.
We carried out a sensitivity analysis excluding HRT-FET, smokers and patients with tubal infertility to examine whether the strong association with ECT remained (Supplementary Tables SII and SIII). The exclusion of HRT-FET cycles also allowed us to include the total number of collected oocytes as a further confounder in this analysis. Compared with an ECT of 9 mm, an ECT of 12 mm was still associated with a 4-fold reduction in the risk of EP (aOR 0.24, 95% CI 0.07–0.77, \(P = 0.02\)), and an ECT of 9–12 mm was associated with one-third of the EP risk (aOR 0.38, 95% CI 0.23–0.65, \(P < 0.01\)). The number of oocytes collected, as a proxy for the level of ovarian response, also had an impact on the risk of EP with a 5% increase in the risk for every extra oocyte collected (aOR 1.05, 95% CI 1.01–1.09, \(P = 0.01\)). Compared with agonist cycles, the antagonist protocol was associated with nearly 3-fold increased EP risk (aOR 2.79, 95% CI 1.52–5.12, \(P < 0.01\)).

**Discussion**

This large retrospective cohort study of 6465 women undergoing 8120 embryo transfer cycles has found that a thin ECT is an independent risk factor for developing EP following ART. The number of embryos transferred, the developmental stage of the embryo at transfer, the maximal estradiol serum concentrations and the method of insemination were not associated with an increased risk of EP in this study.

Our findings indicate a strong association between EP and ECT demonstrating that ECT is inversely proportional to the risk of EP. We were expecting to find the opposite as our group has previously established that an increased ECT is positively correlated with an increase in the risk of placenta praevia (Rombauts et al., 2014). In that paper we hypothesized that increased ECT is a marker for uterine peristaltic wave frequency and/or amplitude which may increase the risk of the embryo being dislodged from its original transfer position. However, the fact that increased ECT is associated with a lower risk of EP suggests that it could be the directionality of the uterine peristalsis waves that matters rather than their frequency or amplitude. Combining the data from both studies we now hypothesize that increased ECT is a marker for increased fundus-to-cervix uterine peristalsis, explaining both the increased placenta praevia risk and the lower EP risk.

Our new hypothesis is supported by a number of other observations. First our own observations corroborate those of a recent SART registry based study in which it was shown that EP risk increases with oocyte yield but only for autologous fresh cycles (Acharya et al., 2015). The authors also speculated that the increased EP risk could be due to the supraphysiologic hormonal milieu causing disorganized uterine contractile patterns. Second, Zhu et al. have demonstrated throughout a series of studies that there is a significant relationship between controlled ovarian hyperstimulation (COH), uterine peristaltic wave frequency and clinical pregnancy rates. In a 2012 study they demonstrated that following COH uterine peristaltic wave frequency increased 1.31 times compared with natural cycles (Zhu et al., 2012). Notably, it was also recognized that following COH waves were predominantly in a cervix-to-fundus direction (80–90%), confirming earlier findings by van Gestel et al. (2005). Nevertheless, a subset of patients exhibited peristaltic waves in the opposite direction: from the fundus to the cervix.

| **Table II Cycle characteristics.** | Intrauterine pregnancy | Ectopic pregnancy | \(P\)-value |
|-----------------------------------|------------------------|-------------------|------------|
|                                   | \(N = 8031\)           | \(N = 89\)        |            |
| Maximum estradiol (pmol/l) Mean ± SD | 3552 ± 3267            | 3419 ± 3164       | NS         |
| Number of oocytes Median (Range)  | 9 (1–54)               | 10 (1–34)         | NS         |
| Protocol                          |                        |                   | 0.006\(^a\) |
| Agonist                           | 3281 (40.9)            | 22 (25)           |            |
| Antagonist                        | 3565 (44.4)            | 52 (58)           |            |
| Hormone replacement therapy       | 1185 (14.8)            | 15 (17)           |            |
| ICSI                              | 5688 (70.8)            | 64 (72)           | NS         |
| Blastocyst transfer               | 5677 (70.7)            | 62 (70)           | NS         |
| Single embryo transfer            | 6102 (76.0)            | 75 (84)           | NS         |
| Endometrial combined thickness Mean ± SD | 9.6 ± 2.5             | 8.5 ± 2.6         | \(<0.001\) |
| Endometrial combined thickness    |                        |                   |            |
| \(<9\) mm                         | 2946 (36.7)            | 53 (60)           |            |
| 9–12 mm                           | 4181 (52.1)            | 32 (36)           |            |
| \(>12\) mm                        | 904 (11.3)             | 4 (5)             |            |
| Endometrial appearance            |                        |                   | NS         |
| Proliferative                     | 6686 (86.8)            | 72 (85)           |            |
| Secretory                         | 45 (0.6)               | 1 (1)             |            |
| Non-specific                       | 975 (12.7)             | 12 (14)           |            |

Data are \(N\) (%) unless stated otherwise.

NS, not significant.

\(^a\)Calculated for stimulated cycles.

\(^b\)Fisher’s exact test.

\(^c\)Student’s \(t\)-test.

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Subsequent to this, they investigated the impact of the increased wave frequency with mock embryo transfer, and determined that fluid movement within the uterine cavity was positively correlated with increased wave frequency both before and after embryo transfer in cases where fluid was extruded from the uterus (Zhu et al., 2014a, b). Their most recent study evaluated the relationship between uterine peristaltic wave frequency before embryo transfer, and clinical pregnancy rates. Analysis confirmed that the association is independently significant, and inversely proportional (OR 0.49, 95% CI 0.34–0.70, P < 0.001), so that decreased wave frequency leads to a higher likelihood of clinical pregnancy (Zhu et al., 2014a, b). This supports the previous belief that decreased uterine peristalsis facilitated embryo implantation (IJland et al., 1997). Zhu et al. further hypothesized that intense uterine peristalsis adversely affects embryo migration after embryo transfer and extrudes it out of the uterus, leading to implantation failure or EP. Within their analysis they determined that the EP rate was 2.7%, which is in line with previous assessments following ART (Refaat et al., 2015). They did note that uterine peristaltic wave frequency was slightly increased in the EP group as compared with those with intrauterine pregnancy, but the difference was not significant, possibly as a consequence of uneven distribution of sample size. The authors were not able to comment on the direction of these waves.

Another possible explanation may be that when embryo transfer is taking place, there is higher placement of the catheter in patients with a thinner endometrium. A retrospective cohort study conducted in Massachusetts evaluated the impact of embryo transfer depth on IVF and embryo transfer outcomes and determined that clinical pregnancy rate was significantly influenced by the embryo transfer distance from the fundus. For every additional millimeter distance away from the fundus, the odds of a clinical pregnancy were increased by 11% (OR 1.11, 95% CI 1.07–1.14), and as distance increased, the rate of EP decreased (Pope et al., 2004). This is supported by an earlier study comparing deep-fundal embryo transfer with mid-fundal embryo transfer, which found EP rates following deep-fundal transfer accounted for 12.2% of all pregnancies in this group, compared with 3.0% following mid-fundal transfer (Nazari et al., 1993). However, in our study the transfer technique was standardized and carried out under trans-abdominal ultrasound guidance but it is possible that small variations amongst clinicians exist.

### Table III Univariate analysis of factors associated with the risk of an ectopic pregnancy.

| Predictor variable | EP/IUP | OR      | 95% CI       | P   |
|--------------------|--------|---------|--------------|-----|
| All Patients       |        |         |              |     |
| Previous births    | 0      | 78/7064 | 1            | –   |
|                    | 1      | 6/745   | 0.73         | 0.32–1.65 | 0.46 |
|                    | > 1    | 5/222   | 2.04         | 0.69–5.84 | 0.19 |
| Age                | <30    | 8/1514  | 1            | –   |
|                    | 30–35  | 43/3174 | 2.56         | 1.21–5.45 | 0.01 |
|                    | 35–40  | 30/2739 | 2.07         | 0.95–4.52 | 0.07 |
|                    | >40    | 8/604   | 2.51         | 0.94–6.70 | 0.07 |
| BMI kg/m²          |        | 0.95    | 0.91–1.00    | 0.05 |
| Smoking            | No     | 88/7875 | 1            | –   |
|                    | Yes    | 1/156   | 0.57         | 0.08–4.11 | 0.58 |
| Tubal infertility  | No     | 76/7331 | 1            | –   |
|                    | Yes    | 13/700  | 1.79         | 0.99–3.24 | 0.05 |
| Endometriosis      | No     | 88/7515 | 1            | –   |
|                    | Yes    | 1/516   | 1.65         | 0.02–1.19 | 0.07 |
| Protocol           | Agonist| 22/3281 | 1            | –   |
|                    | Antagonist| 52/3565 | 2.18         | 1.32–3.59 | <0.01 |
|                    | HRT    | 15/1185 | 1.89         | 0.98–3.64 | 0.06 |
| ICSI               | Yes    | 64/5688 | 1            | –   |
|                    | No     | 25/2343 | 0.95         | 0.60–1.51 | 0.82 |
| Embryo stage at ET | Cleavage| 27/2354 | 1            | –   |
|                    | Blastocyst| 62/5677 | 0.83         | 0.60–1.50 | 0.83 |
| Embryos transferred | 1     | 75/6102 | 1            | –   |
|                    | 2     | 14/1929 | 0.59         | 0.33–1.05 | 0.07 |
| ECT                | <9 mm  | 53/2946 | 1            | –   |
|                    | 9–12 mm| 32/4181 | 0.43         | 0.28–0.66 | <0.01 |
|                    | >12 mm | 4/904   | 0.25         | 0.09–0.68 | <0.01 |
| STIM ET only       | Number of oocytes | 1.03 | 1.00–1.06 | 0.08 |
|                    | Maximum E2 (pmol/L) | 1.00 | 1.00–1.00 | 0.88 |

EP/IUP, ratio of ectopic pregnancies over intrauterine pregnancies; OR, odds ratio; CI, confidence interval; ET, embryo transfer; STIM ET, stimulated cycles with fresh embryo transfers; HRT-FET, hormone modulated frozen embryo transfers; ECT, Endometrial combined thickness; E2, Estradiol.
Smoking did not confer an increase in risk of EP. However, we account for this due to the very small number of smokers within the study population (smoking was self-reported in only 1.9% of all cycles). Additionally, following multivariate analysis, BMI was not a significant contributing factor, which is in line with current knowledge (Koning et al., 2012). As expected, the univariate analysis identified tubal infertility as an independent risk factor for EP in line with existing knowledge, which identifies it as a risk factor for EP in both natural conceptions and following ART (Refaat et al., 2015). However, in our data set it did not remain significant in the multivariate analysis.

One unexpected finding from our study was that antagonist protocols were associated with a 2-fold increased risk compared with agonist cycles, whereas HRT-FET showed no increased risk in our study. This finding is in contrast to the recent study of Londra et al. (2015) and it further highlights the conflicting results from different studies on the impact of cryopreservation of embryos on subsequent EP risk. Londra et al. demonstrated that the likelihood of EP was 65% lower in women who had undergone a FET irrespective of whether an agonist or antagonist protocol was used in the stimulated cycle. They also found a lower risk for fresh or frozen donor oocytes and thus recognized that the increased risk was therefore in women who had undergone ovarian hyperstimulation in the transfer cycle, suggesting that a difference in the tubal-uterine environment impacts on the implantation of the embryo. Another possibility is that the ovarian stimulation causes a change in the type or frequency of uterine contractions as suggested earlier. Similar findings have been reported by several other recent studies (Ishihara et al., 2011; Shapiro et al., 2012; Huang et al., 2014; Fang et al., 2015); however, a large meta-analysis in 2009 based on 13 059 pregnancies from seven studies, identified that there was no statistically significant difference between fresh and frozen embryo transfer on the risk of EP (OR 1.66, 95% CI 0.62–4.41) (Jee et al., 2009). A large retrospective cohort study published in 2014 by Declerq at al. also found no significant difference in EP risk between fresh or frozen cycles (1.92% fresh versus 1.28% frozen, P = 0.23) (Declerq et al., 2014).

We recognize that this study has some limitations. The study design is retrospective, and it is possible that not all confounders have been accounted for. In particular, it would have been interesting to investigate whether EP risk is correlated with progesterone serum concentrations on or prior to the day of hCG trigger. Measurement of ECT was performed by highly trained sonographers, but some inconsistency between individuals may be present. The notes section in our database suggests no cervical or caesarean scar EPs were diagnosed in the timeframe analysed. Even though we cannot rule out misclassification, those EP locations are rare and the likelihood of misclassification in a set of 90 EPs is remote and unlikely to have significantly altered the 4-fold increased EP rate in women with an ECT of <9 mm compared with women with an ECT of >12 mm. In addition, our dataset did not allow us to correct for recently identified risk factors for EP, including ethnicity, volume of transfer fluid and transfer depth (Perkins et al., 2015).

The study is strengthened by the large cohort size and the 100% follow-up. Additionally, there was no change in the method of embryo culture throughout the study period. As most risk factors for EP are well defined, most of these important confounders were accounted for in our study.

The findings are significant as EP is a high-risk outcome of early pregnancy, associated with significant maternal morbidity and mortality if it is not recognized and managed (Farquhar, 2005). If our hypothesis is true, it would be worthwhile considering whether oxytocin antagonists can ameliorate the risk of both EP and placenta praevia by promoting uterine quiescence.

While there have been studies assessing the use of ECT as a diagnostic tool in the differentiation of EP from other forms of pregnancy of unknown location in the first trimester (Moschos and Twickler, 2008; Ellaithy et al., 2013), we have not found any studies reporting an association between ECT and EP in patients undergoing ART where ECT is measured before embryo transfer. Hence, this study is the first to demonstrate that pre-ET ECT is indirectly proportional to the risk of EP following ART. We have demonstrated that this risk is independent of other significant risk factors, including tubal factor infertility. We suggest that this finding may be due to a proportional increase of waves in the fundocervical direction, as ECT increases. This also provides support for the increased risk of placenta praevia with increased ECT, which our group has demonstrated previously. Further studies are underway to explore the impact of ECT on not only the frequency but also the direction of uterine peristaltic waves in women undergoing ART.

### Supplementary data

Supplementary data are available at [http://humrep.oxfordjournals.org/](http://humrep.oxfordjournals.org/).

### Authors’ roles

L.R. and C.M. were involved in the study design, implementation, data collection and analysis. L.R., R.M. and S.F. contributed to the preparation of the manuscript. All authors approved the final draft.

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**Table IV Multivariate analysis of factors associated with the risk of an ectopic pregnancy.**

| Predictor variable                  | aOR   | 95% CI          | P   |
|------------------------------------|-------|-----------------|-----|
| Endometrial combined thickness     |       |                 |     |
| <9 mm                              | I     | –               | –   |
| 9 – 12 mm                          | 0.44  | 0.29–0.69       | <0.01|
| >12 mm                             | 0.27  | 0.10–0.77       | 0.01|
| Protocol                           |       |                 |     |
| Agonist                            | I     | –               | –   |
| Antagonist                         | 1.94  | 1.17–3.20       | 0.01|
| HRT                                | 1.32  | 0.67–2.61       | 0.42|
| Embryos transferred                |       |                 |     |
| 1                                 | I     | –               | –   |
| 2                                 | 0.61  | 0.34–1.11       | 0.10|
| Tubal infertility                  |       |                 |     |
| No                                 | I     | –               | –   |
| Yes                                | 1.72  | 0.95–3.11       | 0.07|
| Endometriosis                      |       |                 |     |
| No                                 | I     | –               | –   |
| Yes                                | 1.59  | 0.92–1.14       | 0.07|
| Age                                |       |                 |     |
| <30 year                           | I     | –               | –   |
| 30 – 35 year                       | 2.58  | 1.21–5.50       | 0.01|
| 35 – 40 year                       | 2.20  | 1.00–4.82       | 0.05|
| >40 year                           | 2.68  | 0.99–7.20       | 0.05|
| BMI kg/m²                          | 0.96  | 0.91–1.00       | 0.07|

All variables entered in regression model listed.

aOR, adjusted odds ratio; CI, confidence interval; ECT, endometrial combined thickness; HRT, hormone replacement therapy.
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Conflict of interest
S.F., C.M. and R.M. have no conflict of interest to declare. L.R. has a minority shareholding in Monash IVF and has received unconditional research and educational grants from MSD®, Merck-Serono® and Ferring®. He serves on an advisory board for MSD® and Ferring®.

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