Reproductive BioMedicine Online (2016) ■■, ■■–■■

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles

Xi Yuan [a,b,c], Sotirios H Saravelos [b], Qiong Wang [a], Yanwen Xu [a], Tin-Chiu Li [b,*], Canquan Zhou [a,**]

[a] *Reproductive Medicine Centre, Department of Obstetric and Gynecology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China;* [b] *Department of Obstetrics and Gynecology, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong;* [c] *MOH Holdings Pte Ltd (MOHH), Singapore*

* Corresponding author. *E-mail address:* tinchiu.li@cuhk.edu.hk (T-C Li).** Corresponding author. *E-mail address:* zhoucanquan@hotmail.com (C Zhou).

Dr. Xi Yuan obtained her MBBS from Sun Yat-sen University in Guangzhou, China in 2013, and later obtained her MD in 2015. She completed her research under the direction of Professor Canquan Zhou in the First Affiliated Hospital of Sun Yat-sen University, and under the direction of Professor TC Li at both the Jessop Wing Hospital, Sheffield, UK, and the Prince of Wales Hospital, The Chinese University of Hong Kong, where the research project was completed.

Abstract This retrospective study assessed the predictive value of endometrial thickness (EMT) on HCG administration day for the clinical outcome of fresh IVF and intracytoplasmic sperm injection (ICSI) cycles. A total of 8690 consecutive women undergoing 10,787 cycles over a 5-year period were included. The 5th, 50th and 95th centiles for EMT were determined as 8, 11 and 15 mm, respectively. Group analysis according to these centiles (Group 1: < 8 mm; Group 2: ≥ 8 and ≤11 mm; Group 3: > 11 and ≤15 mm; Group 4: > 15 mm) demonstrated significant differences ($P < 0.001$) in clinical pregnancy rates (23.0%, 37.2%, 46.2% and 53.3%, respectively), live birth rates per clinical pregnancy (63.3%, 72.0%, 78.1% and 80.3%, respectively), spontaneous abortion rates (26.7%, 23.8%, 19.9% and 17.5%, respectively), and ectopic pregnancy rates (10.0%, 4.3%, 2.1% and 2.2%, respectively). Logistic regression analyses showed EMT as one of the independent variables predictive of clinical pregnancy (OR = 1.097; $P < 0.001$), live birth (OR = 1.078; $P < 0.001$), spontaneous abortion (OR = 0.948; $P < 0.001$), and ectopic pregnancy (OR = 0.851; $P < 0.001$). Future research should aim to understand the underlying mechanisms relating EMT to conception, ectopic implantation and spontaneous abortion.

KEYWORDS: clinical outcome, endometrial thickness, endometrium, IVF–ICSI

http://dx.doi.org/10.1016/j.rbmo.2016.05.002
1472-6483/

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

Introduction

Endometrial assessment has become part of standard monitoring during IVF and intracytoplasmic sperm injection (ICSI) with embryo transfer treatment. The endometrial characteristics, including endometrial pattern, endometrial blood flow, and endometrial thickness (EMT) have been regarded as prognostic factors of IVF–ICSI treatment (De Geyter et al., 2000; Järvelä et al., 2005; Wang et al., 2010). A solid and significant association between these parameters and IVF–ICSI outcome, however, has yet to be found, and controversy in their value still remains ever since the first reports (Alcázar, 2006; Gonen et al., 1989; Ng et al., 2006; Rabinowitz et al., 1986; Schild et al., 2001).

Transvaginal ultrasonography (TVU) is often used to measure EMT on the day of HCG administration as the maximal echogenic distance between the junction of the endometrium and myometrium in the mid-sagittal plane. It has been widely suggested that a thin endometrium is associated with lower IVF–ICSI pregnancy rates, but a consensus is still lacking on what the precise definition of thin endometrium is (Senturk and Erel, 2008), with cut-off values of EMT varying from 7–9 mm in earlier studies (Kasius et al., 2014).

A recent meta-analysis including 22 studies with a total of 10724 IVF–ICSI treatment cycles suggested that EMT has a limited capacity to identify pregnancy rates after IVF–ICSI (Kasius et al., 2014); the frequently reported cut-off of 7 mm was also considered to have a limited prognostic value for clinical pregnancy. Some of the limitations of this meta-analysis, however, can be acknowledged, including heterogeneity of participants included in various studies; lack of uniform definition of thin endometrium and its cut-off value; and observer variability regarding EMT measurement. In addition, the authors also considered that insufficient data were avaialble to conduct a meta-analysis on the rates of spontaneous abortion, ectopic pregnancy and live birth, which are also of significant clinical interest.

In this study, 10,787 fresh IVF–ICSI cycles were analysed from a single tertiary centre, with the aim of identifying epidemiological cut-off values for EMT, and assessing the predictive value of EMT for clinical outcome, namely intrauterine pregnancy, ectopic pregnancy, spontaneous abortion and live birth.

Materials and methods

Patients

A total of 8690 women undergoing a total of 10,787 fresh IVF–ICSI treatment cycles conducted between 1 January 2009 and 30 September 2013 were included in the study at the Reproductive Medical Center, Department of Obstetrics and Gynecology, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China. In our centre, TVU was carried out routinely on the day of HCG administration.

Fresh IVF–ICSI treatment cycles and embryo transfers within the study period were included, regardless of diagnosis, reproductive history or insemination method. Cycles using donor oocytes, cryopreserved embryos or suspect endometrial abnormalities were excluded. The cause of infertility was categorized as tubal factor, male factor, endometriosis, preimplantation genetic diagnosis (PGD) for thalassemia of both partners, PGD for other monogenic disorders, other or multiple factors (including anovulation and polycystic ovary syndrome) and unexplained. Ovarian stimulation, oocyte retrieval, semen collection and processing, ICSI, PGD and embryo transfer procedures were carried out as previously described (Ding et al., 2014; Khoudja et al., 2013; Shen et al., 2011).

This was a retrospective study of routinely collected clinical data, and therefore did not require prospective ethics approval according to our institutional ethics policy.

Data collection

Patient demographics and characteristics were collected, including maternal and paternal age, height, weight, body mass index, duration of infertility, number of previous treated IVF–ICSI cycles, and basal levels of sex steroid hormones. Serum levels of FSH, LH, oestradiol, progesterone, testosterone and prolactin were measured with particle enzyme immunoassay (Abbott Axsym System, USA). The parameters related to the ovarian stimulation, and IVF–ICSI cycle, included serum level of FSH, LH, oestradiol and progesterone on the day of HCG administration, the EMT and endometrial pattern on day of HCG administration, number of retrieved oocytes and number of transferred embryos. The outcomes measured were clinical pregnancy rate per embryo transfer cycle, live birth rate per embryo transfer cycle, live birth rate per pregnancy, ectopic pregnancy rate per pregnancy and spontaneous abortion rate per pregnancy.

Transvaginal ultrasonography

All the TVU assessments were carriedout by subspecialist clinicians using the same standardized protocols on the same ultrasound machines in our department (ALOKA Color Ultrasound ProSound SSD-3500SV, Hitachi Aloka Medical America Inc., USA). Endometrial thickness and endometrial pattern were measured in the mid-sagittal plane of the uterine body on the day of HCG administration. The maximal thickness from one interface of the endometrial–myometrial junction to the other was measured.

Endometrial pattern was classified as type A (a triple-line pattern consisting of a central hyperechoic line surrounded by two hypoechoic layers); type B (an intermediate isoechogenic pattern with the same reflectivity as the surrounding myometrium and a poorly defined central echogenic line); or type C (homogenous, hyperechogenic endometrium).

All cycles were divided into four groups (group 1 to 4) depending on EMT, using the cut-off by the 5th (8 mm), 50th (11 mm), and 95th (15 mm) percentile of the whole population distribution: group 1 (<8 mm); group 2 (≥8 mm and ≤11 mm); group 3 (>11 mm and ≤15 mm); group 4 (>15 mm).

Pregnancy

Clinical pregnancy was defined as one in which a positive pregnancy was accompanied later by ultrasonographic evidence

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

of an intrauterine gestational sac or by diagnosing an ectopic pregnancy (ectopic plus clinical intrauterine pregnancy). Ectopic pregnancy was diagnosed by transvaginal ultrasound or by laparoscopic visualization of extrauterine gestation. Spontaneous abortion was defined as clinical intrauterine pregnancy loss before 24 weeks of gestation. Live birth was defined as the delivery of a live baby after 24 weeks gestation.

Data analysis

The demographic and clinical data were stored in a dedicated database of our unit, and extracted for analysis with the Statistical Package for Social Science software (SPSS 20.0, IBM Corp., USA). Normally distributed data were analysed with the use of Student's t-test and one-way analysis of variance. Categorical data were analysed with the use of Pearson's chi-squared test. The prognostic value of the various measurements was determined with the use of forward stepwise binary logistic regression analysis and presented as odds ratios. $P < 0.05$ was considered statistically significant.

Results

Baseline cycle characteristics

A total of 10787 IVF–ICSI fresh embryo transfer cycles were retrospectively investigated in the study, of which 6031 (55.9%) were cycles of IVF and embry transfer, and 4756 (44.1%) were ICSI cycles. Maternal age ranged from 20–45 years. The proportion of patients aged 40 years or older was 9.2% (988/10787), and the mean ± SD age of this group of patients was 41.6 ± 1.6 years. On the day of HCG administartion, EMT ranged from 4–19 mm. The number of embryo transferred per cycle ranged from 1 to a maximum of 3. In total, 24423 embryos were transferred in this cohort, of which 931 (8.6%) were single-embryo transfer cycles. The demographic data are summarized in **Table 1**.

The overall clinical pregnancy rate per embryo transfer cycle was 40.5% (4372/10787) and the overall live birth rate per embryo transfer cycle was 30.3% (3269/10787). The live birth rate per pregnancy was 74.8% (3269/4372), the spontaneous abortion rate per pregnancy was 21.8% (955/4372), and the ectopic pregnancy rate per pregnancy was 3.4% (148/4372).

Among the 931 single-embryo transfer cycles, the clinical pregnancy rate per embryo transfer cycle was 15.1% (141/931) and the live birth rate per embryo transfer cycle was 10.6% (99/931), which were significantly ($P < 0.001$) lower than the rates when two or more embryos were transferred (clinical pregnancy rate per cycle 42.9%; live birth rate per cycle 32.2%).

Endometrial thickness on the day of HCG injection

The overall frequency distribution of EMT (mm) on HCG day of the patient population is shown in **Figure 1**. Among all participants, the mean (±SD) thickness was 11.1 (±2.4) mm, and the 5th, 50th, and 95th percentiles were 8 mm, 11 mm and 15 mm, respectively.

Table 1 Characteristics of women undergoing all fresh cycles with IVF–ICSI ($n = 10787$).

Variable	Mean ± SD, median (range) or number (%)
Maternal age (year)	32.7 ± 4.8
Paternal age (year)	35.2 ± 5.5
Height (cm)	159.0 ± 4.5
Weight (Kg)	53.6 ± 7.2
Body mass index (Kg/m^2)	21.2 ± 2.6
Duration of infertility (year)	4 (1–20)
Number of previous assisted reproduction technique cycles	0 (0–8)
Baseline FSH (IU/L)	6.0 ± 2.0
Baseline LH (IU/L)	3.4 ± 2.2
Baseline PRL (nmol/L)	20.6 ± 18.3
Baseline oestradiol (pg/mL)	37.2 ± 25.7
Baseline T (nmol/L)	0.8 ± 3.6
Endometrial thickness on HCG day (mm)	11.1 ± 2.4
Endometrial morphology	
Type A	9665 (89.6%)
Type B	734 (6.8%)
Type C	388 (3.6)
FSH on HCG day (IU/L)	13.8 ± 6.1
Oestradol on HCG day (pg/mL)	2596.6 ± 1350.3
LH on HCG day (IU/L)	1.3 ± 4.3
Progesterone on HCG day (ng/mL)	0.6 ± 0.4
Number of oocytes retrieved	11.8 ± 6.2
Number of embryos transferred	2 (1–3)
1	931 (8.6%)
2	6076 (56.3%)
3	3780 (35.0%)
Cycle protocols	
Long agonist protocol	9182 (85.1%)
Short agonist protocol	977 (9.1%)
Antagonist protocol	628 (5.8%)
Cause of infertility	
Tubal factor	3394 (31.5%)
Male factor	2313 (21.4%)
Endometriosis	626 (5.8%)
PGD for chromosomal abnormality	182 (1.7%)
PGD for thalassemia of both partners	139 (1.3%)
Multiple factors	3900 (35.2%)
Unexplained infertility	333 (3.1%)

ICSI, intracytoplasmic sperm injection; PGD, preimplantation genetic diagnosis; PRL, prolactin.

The mean EMT (±SD) in patients who achieved a clinical pregnancy was 11.5 (±2.4) mm, which was significantly thicker ($P < 0.001$) than the mean (±SD) EMT of 10.8 (±2.4) mm in women who did not conceive (**Figure 2**). No pregnancy occurred when the EMT was below 4 mm. Among those who conceived, the mean (±SD) EMT of the three outcome groups were as follows: live birth (11.6 ± 2.4 mm); spontaneous abortion (11.2 ± 2.3 mm); and ectopic pregnancy (10.6 ± 2.4 mm). Significant differences were reported among the three groups ($P < 0.001$, analysis of variance). The mean (±SD) EMT in ectopic pregnancy group, however, was not significantly thinner than that of the non-pregnant group (analysis of variance) (**Figure 2**).

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

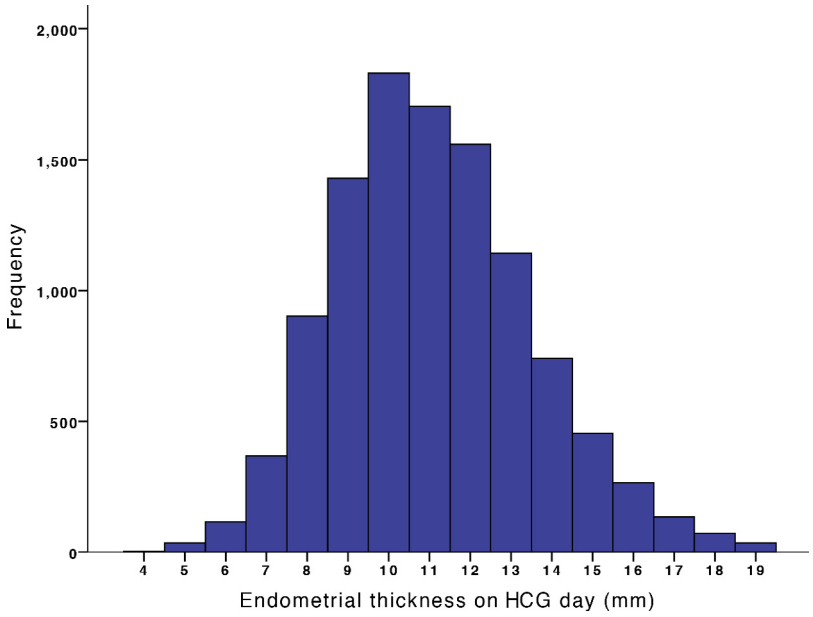

Figure 1 The frequency distribution of the endometrial thickness on HCG day: (1) 1st and 99th percentile: 6 and 17 mm; (2) 3rd and 97th percentile: 7 and 16 mm; (3) 5th and 95th percentile: 8 and 15 mm; (4) 50th percentile: 11 mm.

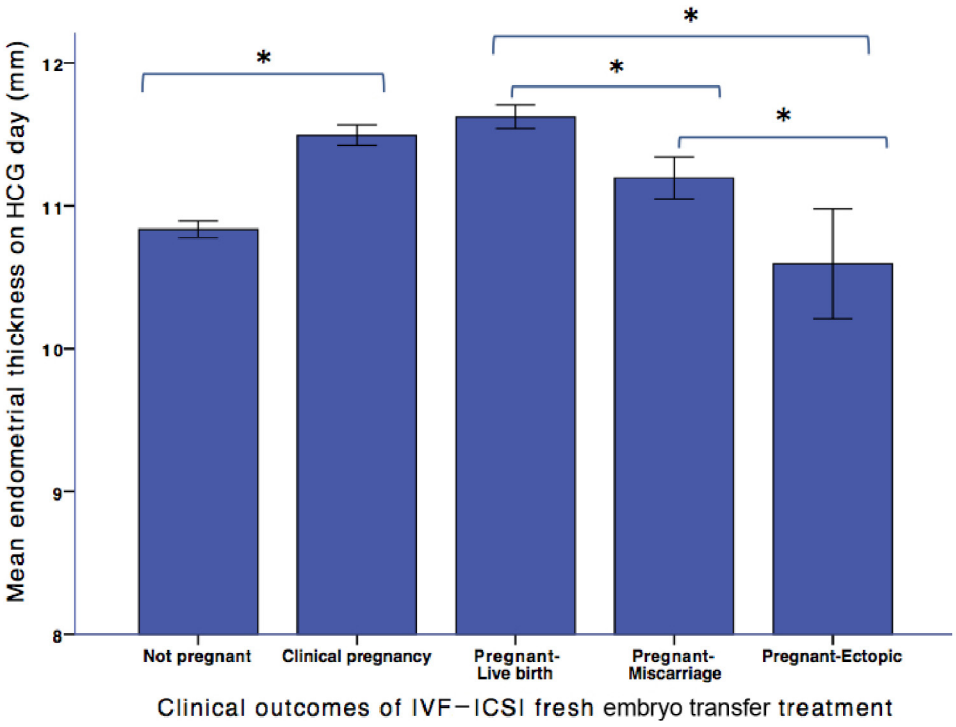

Figure 2 A comparison of endometrial thickness on HCG day among different groups of patients. Results shown are mean and 95% CI. *Comparisons of mean endometrial thickness between each two groups and among three groups: not pregnant versus clinical pregnancy: $P < 0.001$; live birth versus spontaneous abortion: $P < 0.001$; live birth versus ectopic pregnancy: $P < 0.001$; spontaneous abortion versus ectopic pregnancy: $P < 0.001$; live birth versus spontaneous abortion versus ectopic pregnancy: $P < 0.001$. ICSI, intracytoplasmic sperm injection.

When comparing the clinical outcome of the four groups with different EMT: group 1 (<8 mm); group 2 (≥8 mm and ≤11 mm); group 3 (>11 mm and ≤15 mm); group 4 (>15 mm), significant differences were reported in clinical pregnancy rate per embryo transfer cycle: group 1 (23.0%); group 2 (37.2%); group 3 (46.2%); and group 4 (53.3%) ($P < 0.001$ among the four groups) (Figure 3a). Reported live birth rate per clinical pregnancy were as follows: group 1 (63.3%); group 2

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

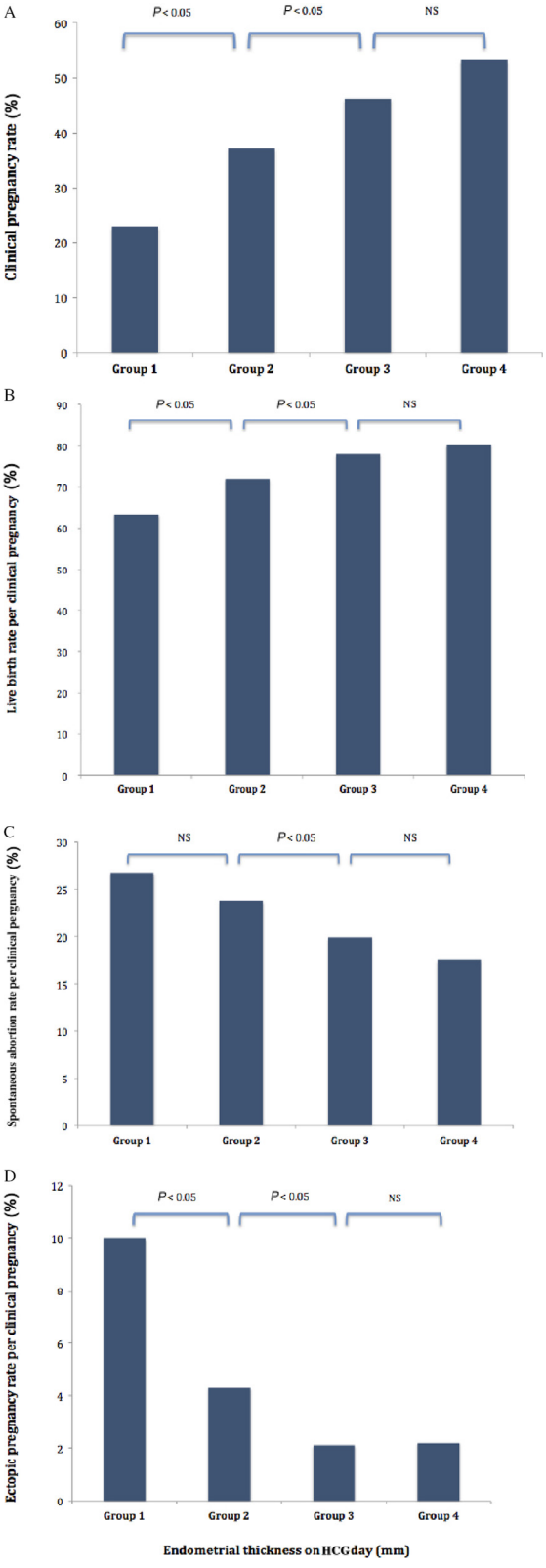

Figure 3 (a–d): The clinical outcomes among four different endometrial thickness groups based on cut-offs of 8, 11 and 15 mm (the 5th, 50th and 95th percentile): Group 1: EMT <8 mm; group 2: 8 mm ≤ EMT ≤ 11 mm; group 3: 11 mm <EMT ≤15 mm; group 4: EMT >15 mm. *P*-values between each two groups are shown in the figures. 2 x 4 contingency tables for all graphs demonstrates $P < 0.001$.

(72.0%); group 3 (78.1%); and group 4 (80.3%) ($P < 0.001$ among the four groups) (Figure 3b). The spontaneous abortion rate per clinical pregnancy was as follows: group 1 (26.7%); group 2 (23.8%); group 3 (19.9%); and group 4 (17.5%) ($P < 0.001$ among the four groups) (Figure 3c). Ectopic pregnancy rates per clinical pregnancy were as follows: group 1 (10.0%); group 2 (4.3%); group 3 (2.1%); and group 4 (2.2%); $P < 0.001$ among the four groups (Figure 3d).

No significant difference were found in pregnancy rates based on endometrial morphology. The clinical pregnancy rates per embryo transfer cycle were 40.4% (3905/9665) for type A, 43.9% (322/734) for type B and 37.4% (145/388) for type C, respectively.

In the subset of patients aged younger than 40 years, with basal FSH less than 10 IU/L, who received long agonist protocol and who underwent single-embryo transfer ($n = 507$), the mean (±SD) EMT in patients who achieved a clinical pregnancy was 11.4 (±2.3) mm, which was significantly thicker ($P < 0.05$) than the mean (±SD) EMT of 10.8 (±2.3) mm in women who did not conceive. The clinical outcome of the four groups with different EMT in this subset were compared: group 1 (<8 mm); group 2 (≥8 mm and ≤11 mm); group 3 (>11 mm and ≤15 mm); group 4 (>15 mm); a trend approaching but not reaching statistical significance was observed: clinical pregnancy rate per embryo transfer cycle: group 1: 3/24 (12.5%); group 2, (43/289 (14.9%); group 3, 37/173 (21.4%); and group 4, 4/21 (19.0%). Live birth rate per clinical pregnancy was as follows: group 1: 1/3 (33.3%); group 2: 32/43 (74.4%); group 3: 25/37 (67.6%); and group 4: 2/4 (50.0%). Spontaneous abortion rate per clinical pregnancy was as follows: group 1: 2/3 (66.7%); group 2: 10/43 (23.3%); group 3: 12/37 (32.4%); and group 4: 2/4 (50.0%). Ectopic pregnancy rate per clinical pregnancy was as following: group 1: 0/3 (0%); group 2: 1/43 (2.3%); group 3: 0/3 (0%); and group 4: 0/4 (0%).

Binary logistic analysis

Forward logistic regression stepwise binary logistic regression analyses was carried out to evaluate the effect of maternal age, body mass index, duration of infertility, baseline FSH and LH level, EMT on HCG day, oestradiol on HCG day, progesterone on the day of HCG administration, number of oocytes retrieved, and number of embryos transferred in relation to clinical pregnancy (yes/no), live birth (yes/no), spontaneous abortion (yes/no) and ectopic pregnancy (yes/no).

The logistic regression model showed that the variables predictive of clinical pregnancy were as follows: maternal age; number of embryos transferred; EMT on HCG day; progesterone on HCG day; oestradiol on HCG day; number of oocytes retrieved; and baseline FSH. Further addition of other variables did not seem to alter the prediction (Table 2). The final step of the analysis (model Cox and Snell $R^2 = 0.068$; $P < 0.001$) showed that maternal age (OR = 0.922; $P < 0.001$), progesterone on HCG day (OR = 0.612; $P < 0.001$), and baseline FSH (OR = 0.976; $P = 0.043$) were negatively correlated with clinical pregnancy, whereas EMT on HCG day (OR = 1.097; $P < 0.001$), number of oocytes retrieved (OR = 1.011; $P = 0.012$), number of embryos transferred (OR = 1.594; $P < 0.001$), and oestradiol on HCG day (OR = 1.224; $P < 0.001$) were positively correlated with improved clinical pregnancy rates (Table 2).

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

Table 2 Forward stepwise binary logistic regression analysis (clinical pregnancy served as dependent variable) for the association with clinical variables and clinical pregnancy after IVF–ICSI fresh cycle treatment.

Steps	Variables	B[h]	SE[i]	Wald[j]	Sig[k]	Rxp (B)[l]
Step 1[a]	Maternal age	−0.080	0.005	310.429	<0.001	0.923
	Constant	2.233	0.148	227.189	<0.001	9.330
Step 2[b]	Maternal age	−0.098	0.005	403.712	<0.001	0.907
	Number of embryos transferred	0.495	0.038	167.523	<0.001	1.641
	Constant	1.678	0.155	117.633	<0.001	5.353
Step 3[c]	Maternal age	−0.093	0.005	359.396	<0.001	0.911
	EMT on HCG day	0.094	0.009	113.475	<0.001	1.099
	Number of embryos transferred	0.491	0.039	161.483	<0.001	1.634
	Constant	0.488	0.191	6.556	0.010	1.629
Step 4[d]	Maternal age	−0.093	0.005	358.149	<0.001	0.911
	EMT on HCG day	0.094	0.009	112.627	<0.001	1.099
	Progesterone on HCG day	−0.317	0.062	26.152	<0.001	0.728
	Number of embryos transferred	0.506	0.039	170.408	<0.001	1.658
	Constant	0.642	0.193	11.047	0.001	1.900
Step 5[e]	Maternal age	−0.087	0.005	299.384	<0.001	0.917
	EMT on HCG day	0.093	0.009	110.425	<0.001	1.098
	Progesterone on HCG day	−0.451	0.068	44.458	<0.001	0.637
	Number of embryos transferred	0.486	0.039	155.449	<0.001	1.626
	Oestradiol on HCG day	0.278	0.046	36.876	<0.001	1.321
	Constant	0.433	0.196	4.859	0.028	1.542
Step 6[f]	Maternal age	−0.083	0.005	251.435	<0.001	0.921
	EMT on HCG day	0.092	0.009	108.171	<0.001	1.097
	Progesterone on HCG day	−0.478	0.069	48.603	<0.001	0.620
	Number of oocytes retrieved	0.013	0.004	9.139	0.003	1.013
	Number of embryos transferred	0.472	0.039	143.769	<0.001	1.603
	Oestradiol on HCG day	0.203	0.052	15.062	<0.001	1.224
	Constant	0.235	0.207	1.288	NS	1.265
Step 7[g]	Maternal age	−0.081	0.005	240.601	<0.001	0.922
	EMT on HCG day	0.092	0.009	108.438	<0.001	1.097
	Progesterone on HCG day	−0.476	0.068	48.299	<0.001	0.621
	Number of oocytes retrieved	0.011	0.004	6.271	0.012	1.011
	Number of embryos transferred	0.466	0.039	139.460	<0.001	1.594
	Oestradiol on HCG day	0.202	0.052	15.045	<0.001	1.224
	Baseline FSH	−0.024	0.012	4.096	0.043	0.976
	Constant	0.373	0.218	2.929	NS	1.452

All the variables entered into the model: maternal age, body mass index, duration of infertility, baseline FSH/LH, EMT/oestradol/progesterone on HCG day, number of oocytes retrieved, and number of embryos transferred.
[a]Variable entered on step 1: maternal age (model Cox and Snell $R^2 = 0.032$; $P < 0.001$).
[b]Variable entered on step 2: number of embryos transferred ($R^2 = 0.049$; $P < 0.001$).
[c]Variable entered on step 3: EMT on HCG day ($R^2 = 0.060$; $P < 0.001$).
[d]Variable entered on step 4: progesterone on HCG day ($R^2 = 0.063$; $P < 0.001$).
[e]Variable entered on step 5: oestradiol on HCG day ($R^2 = 0.067$; $P < 0.001$).
[f]Variable entered on step 6: number of oocytes retrieved ($R^2 = 0.067$; $P < 0.001$).
[g]Variable entered on step 7: baseline FSH ($R^2 = 0.068$; $P < 0.001$).
[h]B is the estimated logistic coefficient.
[i]SE is the standard error of the coefficient.
[j]Wald = [B/SE][i].
[k]Sig is the significance level of the coefficient: the coefficient on clinical pregnancy is statistically significant when less than 0.05.
[l]Exp(B) is the odds ratio of the individual coefficient.
EMT, endometrial thickness; NS, not statistically significant.

In a similar analysis, the variables predictive of live birth were as follows: maternal age and EMT on HCG day. The final step of the analysis ($R^2 = 0.038$; $P < 0.001$) showed that maternal age (OR = 0.908; $P < 0.001$) was negatively correlated with live birth, whereas EMT on HCG day (OR = 1.078; $P < 0.001$) was positively correlated with live birth rates.

Furthermore, the predictive variables of spontaneous abortion were as follows: maternal age and EMT on the day of HCG administration. The final step of forward logistic regression stepwise binary logistic regression analysis ($R^2 = 0.034$; $P < 0.001$) showed that maternal age (OR = 1.106; $P < 0.001$) was positively correlated with spontaneous abortion rate, whereas

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

EMT on HCG day (OR = 0.948; $P = 0.001$) was negatively correlated with spontaneous abortion rate.

Finally, for the logistic regression analysis on ectopic pregnancy, the predictive variables were as follows: progesterone levels on HCG day; number of embryos transferred; and EMT on HCG day. The final step of the analysis ($R^2 = 0.008$; $P < 0.001$) showed that progesterone level on HCG day (OR = 2.043; $P = 0.001$) and number of embryos transferred (OR = 1.492; $P = 0.015$) were positively correlated with ectopic pregnancy rate, whereas EMT on HCG day (OR = 0.851; $P < 0.001$) was negatively correlated with ectopic pregnancy rate.

Discussion

In this retrospective study, we have determined the cut-off values for EMT in a large cohort of women, and have examined their predictive value on clinical outcome after 10,787 fresh IVF–ICSI treatment cycles. To our knowledge, this is the largest single-centre study of its kind, and the first to show that EMT on the day of HCG administration can predict the chance of intrauterine pregnancy, ectopic pregnancy, spontaneous abortion and live birth.

Our finding concurs to some extent with a recent meta-analysis (Kasius et al., 2014) that found an EMT 7 mm or less is associated with a significantly reduced chance of conception after IVF–ICSI and embry transfer treatment. In that meta-analysis, the conception rate in women with an EMT 7 mm or less was 23.3%, significantly lower than 48.1% of those whose EMT was greater than 7 mm. Our observations are strikingly similar, with a conception rate of 23.0% (120/521) in women with an EMT less than 8 mm (lower 5th centile), and significantly increased to 41.4% (4252/10266) for women whose EMT was 8 mm or greater.

The published research to date seems to have provided convincing evidence to support an association between a thin endometrium (<8 mm) and a reduced conception rate. A thick endometrium is more controversial. Several previous investigators have suggested that a thick endometrium (>16, or ≥17 mm) is associated with an improved conception rate (Al-Ghamdi et al., 2008; Richter et al., 2007), whereas others have reported a detrimental effect of a thick endometrium (>14 mm) on conception rate (Weissman et al., 1999). In our study, we found a consistently positive correlation between EMT and conception rate, with patients having an EMT greater than 15 mm achieving the highest conception rate of 53.3%. Therefore, it seems that a thickened endometrium has anything but an adverse effect on the chance of clinical pregnancy. It is important to note, however, that a thickened endometrium is associated with a number of intrauterine pathologies such as polyps or fibroids, both of which has been shown to adversely affect implantation and reduce pregnancy rates (Pritts et al., 2009). Therefore, in women with an abnormally thickened endometrium, it seems prudent to carry out further investigations, such as a hysteroscopy to rule out and treat any intra-cavity lesions.

One new finding in our study is that endometrial thickness is not only related to the chance of conception, but also the likelihood of spontaneous abortion. The specific question of the relationship between EMT and spontaneous abortion was raised in the recent meta-analysis (Kasius et al., 2014), but could not be addressed, as not every reported series provided data on the spontaneous abortion rate and these data were too sparse to carry out a meaningful meta-analysis. Nevertheless, a previous report based on 37 IVF cycles (Weissman et al., 1999) has claimed that the spontaneous abortion rate was higher among those with increased endometrial thickness (>14 mm), whereas another report on 2896 IVF–ICSI cycles found that EMT did not affect the spontaneous abortion rates (Chen et al., 2010). In contrast to the above, we found that the spontaneous abortion rate was significantly reduced among those with increased endometrial thickness on the day of HCG administration, with the lowest spontaneous abortion rate of 17.5% in thickest EMT group (>15 mm), and the highest spontaneous abortion rate of 26.7% in the thinnest EMT group (<8 mm). Despite it being recognized that most spontaneous abortions are caused by embryonic factors such as aneuploidy (Hodes-Wertz et al., 2012; Macklon et al., 2002), our large cohort of over 10,000 cycles derived from a single centre with a single definition, has allowed to identify a significant relationship between EMT and spontaneous abortion rate.

A further new finding in our study relates to the association between EMT and the ectopic pregnancy rate. The notion that ectopic pregnancy is related to abnormal endometrial function is not new. Recent studies (Fang et al., 2014; Ishihara et al., 2011; Shapiro et al., 2012) suggest that the risk of ectopic pregnancy after fresh embryo transfer is significantly higher than that observed in frozen–thawed cycles especially in frozen–thawed day 5 blastocyst transfer (Fang et al., 2014), when the endometrium is not subjected to the supra-physiological levels of steroid hormones. A retrospective study (Dart et al., 1999) of patients with abdominal pain or vaginal bleeding, a positive beta-HCG value and an empty uterus by TVU, also showed that the ectopic pregnancy rate was up to 24.5% in patients with EMT less than 8 mm, compared with only 13.6% in those with EMT 8 mm or thicker, indicating that thin endometrium is a risk factor. The finding in our study adds further to the notion that abnormal endometrium is somehow related to an increased risk of ectopic pregnancy, by allowing embryo migration out of the uterine cavity.

The result of the logistic regression analyses showed that, among many factors affecting clinical outcome, EMT is consistently in the important and independent factors for intrauterine pregnancy, ectopic pregnancy, spontaneous abortion and live birth. When all the various adverse effects of a thin endometrium are considered together, it does indeed seem reasonable to offer patients with EMT less than 8 mm the option of freezing all embryos with the prospect of transferring embryos in a frozen–thawed cycle where a thicker EMT can be achieved. It remains to be confirmed, however, that such an approach would lead to an improved clinical outcome. Meanwhile, the thin endometrium (<8 mm) is a relatively uncommon phenomenon (5th centile, 521/10787), and the conception rate in this group (23.0%, 120/521) is still reasonable.

The pathophysiology of thin endometrium remains elusive. One speculation relates to oxygen tension. Constriction of spiral arteries after ovulation significantly diminishes blood flow to the surface endometrium (Rossman and Bartelmez, 1957), thus reducing oxygen tension in the functional epithelium around the time of implantation. It is widely recognized that embryos cannot develop well in high oxygen tensions due to the production of reactive oxygen species (Catt and

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

Henman, 2000; Yang et al., 1998). When the endometrium is thin, it is the functional layer that is particularly affected, with the result that the transferred embryo(s) is closer to enriched vascular network of the basal endometrium with higher oxygen concentrations or hyperoxia (Casper, 2011). Regardless of the pathophysiological mechanism, various modalities have been proposed to increase endometrial thickness in patients with thin endometrium, including low-dose aspirin (Weckstein et al., 1997), pentoxifylline and tocopherol (Ledee-Bataille et al., 2002), sildenafil citrate (Sher and Fisch, 2002) and extended estrogen administration (Chen et al., 2006), intra-uterine perfusion with granulocyte colony-stimulating factor (G-CSF) (Gleicher et al., 2011, 2013) or platelet-rich plasma (Chang et al., 2015). Firm evidence confirming treatment benefit relating to these treatment modalities, however, is lacking.

The observations in our study were based on fresh embry transfer in IVF stimulated cycles; the same conclusion may not apply to embryo transfer in unstimulated cycles, which may apply to frozen embryo transfer in natural cycles or fresh embryo transfer in HRT cycles following oocyte or embryo donation. A recent study with a total of 737 donor oocyte cycles, however, found no statistically significant difference in clinical pregnancy rates and live birth rates in cycles with EMT less than 6 mm compared with those with EMT greater than 6 mm (Dain et al., 2013). In our study, we have not included data from donor oocytes cycles because they are rarely carried out in China. Adequately powered studies are needed to compare the effect of EMT on pregnancy outcomes in donor oocyte cycles and cryopreserved embryo transfer cycles to determine if the observations on EMT in stimulated IVF cycles are applicable in FET or fresh donor oocyte cycles.

In analysing the results of a subgroup of patients aged less than 40 years, FSH less than 10 IU/L, who underwent long agonist protocol and had single embryo transfer, there was no longer any significant difference between the groups, although a similar trend to that of the whole group was observed. The striking feature in this subgroup was the rather low pregnancy and live birth rates compared with the entire group. The most likely explanation is that most patients in our centre during the period of the study wished to have the transfer of more than one embryo if at all possible; elective single embryo transfer was therefore rather uncommon. Consequently, most women who had single embryo transfer in this study represented a group with poor prognosis producing only a single viable embryo suitable for transfer. The outcomes would have been rather different in a group of women with good prognostic features who elected to have single embryo transfer, and it would be of interest for future research to report on such a group.

In conclusion, this study indicated that EMT is a significant and independent predictor of intrauterine pregnancy, ectopic pregnancy, spontaneous abortion and live birth after IVF–ICSI treatment. The frequently reported cut-off of 7 mm is not only related to a lower chance of pregnancy, but also related to a higher probability of spontaneous abortion and ectopic pregnancy. Women with thin endometrium should be properly counselled about the lower chance of conception, and, should conception occur, an increased risk of spontaneous abortion and ectopic pregnancy. The option of offering cryopreservation and deferring embryo transfer should not be discounted. Future research should be directed towards understanding the pathophysiology of the thin endometrium and how it can be successfully managed.

References

Al-Ghamdi, A., Coskun, S., Al-Hassan, S., Al-Rejjal, R., Awartani, K., 2008. The correlation between endometrial thickness and outcome of in vitro fertilization and embryo transfer (IVF-ET) outcome. Reprod. Biol. Endocrinol. 6, 37–41.

Alcázar, J.L., 2006. Three-dimensional ultrasound assessment of endometrial receptivity: a review. Reprod. Biol. Endocrinol. 4, 56.

Casper, R.F., 2011. It's time to pay attention to the endometrium. Fertil. Steril. 96, 519–521.

Catt, J.W., Henman, M., 2000. Toxic effects of oxygen on human embryo development. Hum. Reprod. 15, 199–206.

Chang, Y., Li, J., Chen, Y., Wei, L., Yang, X., Shi, Y., Liang, X., 2015. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. Int. J. Clin. Exp. Med. 8, 1286.

Chen, M.-J., Yang, J.-H., Peng, F.-H., Chen, S.-U., Ho, H.-N., Yang, Y.-S., 2006. Extended estrogen administration for women with thin endometrium in frozen-thawed in-vitro fertilization programs. J. Assist. Reprod. Genet. 23, 337–342.

Chen, S.-L., Wu, F.-R., Luo, C., Chen, X., Shi, X.-Y., Zheng, H.-Y., Ni, Y.-P., 2010. Combined analysis of endometrial thickness and pattern in predicting outcome of in vitro fertilization and embryo transfer: a retrospective cohort study. Reprod. Biol. Endocrinol. 8, 30–36.

Dain, L., Bider, D., Levron, J., Zinchenko, V., Westler, S., Dirnfeld, M., 2013. Thin endometrium in donor oocyte recipients: enigma or obstacle for implantation? Fertil. Steril. 100, 1289–1295.

Dart, R.G., Dart, L., Mitchell, P., Berty, C., 1999. The predictive value of endometrial stripe thickness in patients with suspected ectopic pregnancy who have an empty uterus at ultrasonography. Acad. Emerg. Med. 6, 602–608.

De Geyter, C., Schmitter, M., De Geyter, M., Nieschlag, E., Holzgreve, W., Schneider, H.P., 2000. Prospective evaluation of the ultrasound appearance of the endometrium in a cohort of 1,186 infertile women. Fertil. Steril. 73, 106–113.

Ding, C., Li, T., Zeng, Y., Hong, P., Xu, Y., Zhou, C., 2014. Effects of in-vitro or in-vivo matured ooplasm and spindle-chromosome complex on the development of spindle-transferred oocytes. Reprod. Biomed. Online 29, 708–716.

Fang, C., Huang, R., Wei, L.-N., Jia, L., 2014. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. Fertil. Steril. 103, 655–61.e3.

Gleicher, N., Vidali, A., Barad, D.H., 2011. Successful treatment of unresponsive thin endometrium. Fertil. Steril. 95, 2123, e2113-2123. e2117.

Gleicher, N., Kim, A., Michaeli, T., Lee, H., Shohat-Tal, A., Lazzaroni, E., Barad, D., 2013. A pilot cohort study of granulocyte colony-stimulating factor in the treatment of unresponsive thin endometrium resistant to standard therapies. Hum. Reprod. 28, 172–177.

Gonen, Y., Casper, R.F., Jacobson, W., Blankier, J., 1989. Endometrial thickness and growth during ovanan stimulation a possible predictor of implantation in in vitro fertilization. Fertil. Steril. 52, 446–450.

Hodes-Wertz, B., Grifo, J., Ghadir, S., Kaplan, B., Laskin, C.A., Glassner, M., Munné, S., 2012. Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos. Fertil. Steril. 98, 675–680.

Ishihara, O., Kuwahara, A., Saitoh, H., 2011. Frozen-thawed blastocyst transfer reduces ectopic pregnancy risk: an analysis of single embryo transfer cycles in Japan. Fertil. Steril. 95, 1966–1969.

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002

Järvelä, I., Sladkevicius, P., Kelly, S., Ojha, K., Campbell, S., Nargund, G., 2005. Evaluation of endometrial receptivity during in-vitro fertilization using three-dimensional power Doppler ultrasound. Ultrasound Obstet. Gynecol. 26, 765–769.

Kasius, A., Smit, J.G., Torrance, H.L., Eijkemans, M.J., Mol, B.W., Opmeer, B.C., Broekmans, F.J., 2014. Endometrial thickness and pregnancy rates after IVF: a systematic review and meta-analysis. Hum. Reprod. Update 20, 530–541.

Khoudja, R.Y., Xu, Y., Li, T., Zhou, C., 2013. Better IVF outcomes following improvements in laboratory air quality. J. Assist. Reprod. Genet. 30, 69–76.

Ledee-Bataille, N., Olivennes, F., Lefaix, J., Chaouat, G., Frydman, R., Delanian, S., 2002. Combined treatment by pentoxifylline and tocopherol for recipient women with a thin endometrium enrolled in an oocyte donation programme. Hum. Reprod. 17, 1249–1253.

Macklon, N.S., Geraedts, J.P., Fauser, B.C., 2002. Conception to ongoing pregnancy: the 'black box'of early pregnancy loss. Hum. Reprod. Update 8, 333–343.

Ng, E.H.Y., Chan, C.C.W., Tang, O.S., Yeung, W.S.B., Ho, P.C., 2006. The role of endometrial and subendometrial blood flows measured by three-dimensional power Doppler ultrasound in the prediction of pregnancy during IVF treatment. Hum. Reprod. 21, 164–170.

Pritts, E.A., Parker, W.H., Olive, D.L., 2009. Fibroids and infertility: an updated systematic review of the evidence. Fertil. Steril. 91, 1215–1223.

Rabinowitz, R., Laufer, N., Lewin, A., Navot, D., Bar, I., Margalioth, E.J., Schenker, J.J., 1986. The value of ultrasonographic endometnal measurement in the prediction of pregnancy following in vitro fertilization. Fertil. Steril. 45, 824–828.

Richter, K.S., Bugge, K.R., Bromer, J.G., Levy, M.J., 2007. Relationship between endometrial thickness and embryo implantation, based on 1,294 cycles of in vitro fertilization with transfer of two blastocyst-stage embryos. Fertil. Steril. 87, 53–59.

Rossman, I., Bartelmez, G., 1957. The injection of the blood vascular system of the uterus. Anat. Rec. 128, 223–231.

Schild, R.L., Knobloch, C., Dorn, C., Fimmers, R., Van Der Ven, H., Hansmann, M., 2001. Endometrial receptivity in an in vitro fertilization program as assessed by spiral artery blood flow, endometrial thickness, endometrial volume, and uterine artery blood flow. Fertil. Steril. 75, 361–366.

Senturk, L.M., Erel, C.T., 2008. Thin endometrium in assisted reproductive technology. Curr. Opin. Obstet. Gynecol. 20, 221–228.

Shapiro, B.S., Daneshmand, S.T., De Leon, L., Garner, F.C., Aguirre, M., Hudson, C., 2012. Frozen-thawed embryo transfer is associated with a significantly reduced incidence of ectopic pregnancy. Fertil. Steril. 98, 1490–1494.

Shen, X., Xu, Y., Zhong, Y., Zhou, C., Zeng, Y., Zhuang, G., Ding, C., Li, T., 2011. Preimplantation genetic diagnosis for α-and β-double thalassemia. J. Assist. Reprod. Genet. 28, 957–964.

Sher, G., Fisch, J.D., 2002. Effect of vaginal sildenafil on the outcome of in vitro fertilization (IVF) after multiple IVF failures attributed to poor endometrial development. Fertil. Steril. 78, 1073–1076.

Wang, L., Qiao, J., Li, R., Zhen, X., Liu, Z., 2010. Role of endometrial blood flow assessment with color Doppler energy in predicting pregnancy outcome of IVF-ET cycles. Reprod. Biol. Endocrinol. 8, 122.

Weckstein, L.N., Jacobson, A., Galen, D., Hampton, K., Hammel, J., 1997. Low-dose aspirin for oocyte donation recipients with a thin endometrium: prospective, randomized study. Fertil. Steril. 68, 927–930.

Weissman, A., Gotlieb, L., Casper, R.F., 1999. The detrimental effect of increased endometrial thickness on implantation and pregnancy rates and outcome in an in vitro fertilization program. Fertil. Steril. 71, 147–149.

Yang, H.W., Hwang, K.J., Kwon, H.C., Kim, H.S., Choi, K.W., Oh, K.S., 1998. Detection of reactive oxygen species (ROS) and apoptosis in human fragmented embryos. Hum. Reprod. 13, 998–1002.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 17 November 2015; refereed 25 April 2016; accepted 10 May 2016.

Please cite this article in press as: Xi Yuan, et al., Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVF–ICSI cycles, Reproductive BioMedicine Online (2016), doi: 10.1016/j.rbmo.2016.05.002