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## Thicker endometrial linings are associated with better IVF outcomes: a cohort of 6331 women

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### Abstract

Our objective was to determine if a correlation exists between endometrial thickness measured on the day of ovulation trigger during an *in vitro* fertilization (IVF) cycle and pregnancy outcomes among non-cancelled cycles. We performed a retrospective cohort study looking at 6331 women undergoing their first, fresh autologous IVF cycle from 1 May 2004 to 31 December 2012 at Boston IVF (Waltham, MA). Our primary outcome was the risk ratio (RR) of live birth and positive  $\beta$ -hCG. We found that thicker endometrial linings were associated with positive  $\beta$ -hCG and live birth rates. For each additional millimetre of endometrial thickness, we found a statistically significant increased risk of positive  $\beta$ -hCG (adjusted RR: 1.14; 95% CI: 1.09–1.18) and live birth (RR: 1.08; 95% CI: 1.05–1.11). There was no association between endometrial thickness and miscarriage (RR: 0.99; 95% CI: 0.91–1.07). Similar results were seen when categorizing endometrial thickness. Compared with an endometrial thickness  $>7$  to  $<11$  mm, the likelihood of a live birth was significantly higher for an endometrial thickness  $\geq 11$  mm (adjusted RR: 1.23; 95% CI: 1.11–1.37) and significantly lower for the  $\leq 7$  mm group (adjusted RR: 0.64; 95% CI: 0.45–0.90). In conclusion, thicker endometrial linings were associated with increased pregnancy and live birth rates.

### Keywords

Endometrium; *in vitro* fertilization (IVF); pregnancy

### Introduction

Endometrial thickness, which may be a marker of uterine receptivity, is easily measured using transvaginal ultrasonography while monitoring ovarian stimulation for patients

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undergoing *in vitro* fertilization (IVF). As a result, endometrial thickness is often used clinically to decide whether to proceed with the current fresh cycle or to freeze all embryos and use them in a subsequent thaw cycle when the endometrium appears to be more receptive to implantation. These decisions may considerably impact patients' costs and experiences, and thus it is important to have robust data with which to make evidence-based treatment plans. Multiple studies have been conducted to investigate this issue and have evaluated endometrial thickness as an independent predictor of outcomes. However, the findings are inconsistent, and thus the prognostic value of endometrial thickness remains controversial.

Some investigators report that embryo implantation is impaired when the endometrium is too thin. There also are reports of no pregnancies occurring in cycles where the endometrium is as thick as 8 mm, though both studies included fewer than 200 cycles (Rashidi, Sadeghi, Jafarabadi, & Tehrani Nejad, 2005; Zenke & Chetkowski, 2004). Chen et al. (2010) published a larger study of nearly 3000 cycles that reported no pregnancies with an endometrial thickness less than 5.3 mm. Other investigators speculate that an excessively thickened endometrium is a barrier to implantation (Okohue et al., 2009; Weissman, Gotlieb, & Casper, 1999) with one small study of 150 cycles reporting that no pregnancies occurred in cycles with endometrial thickness greater than 12 mm (Rashidi et al., 2005). Yet other investigators have found no association between endometrial thickness and pregnancy outcomes, though many of these studies were conducted with sample sizes less than 750 cycles. More recently, Kasius et al. (2014) published a meta-analysis and systematic review suggesting that endometrial thickness plays a limited role in predicting pregnancy outcomes of IVF cycles. Additionally, several prior studies have been conducted using donor cycles. While the use of donor cycles provides a good opportunity to assess the independent effect of endometrial thickness on outcomes due to greater uniformity in donor age and embryo quality, these cycles may not be representative of autologous cycles, and thus may not provide the best evidence for treating autologous cycles based on varying endometrial thickness.

Given the discrepancies in the literature and the small sample sizes of many previous reports, our objective was to conduct a large, retrospective cohort study to assess the relationship between endometrial thickness measured on the day of ovulation trigger and IVF cycle outcome.

## Materials and methods

### Participants

This retrospective cohort study included all women undergoing their first, fresh autologous IVF cycle from 1 May 2004 to 31 December 2012 at Boston IVF (Waltham, MA). Only first cycles were included, and we excluded thaw cycles, cycles using donor sperm or egg, gamete intra-Fallopian transfer cycles and gestational carrier cycles. All IVF protocols were included, and oocytes were fertilized with conventional IVF methods, intracytoplasmic sperm injection or assisted hatching. Patient and cycle characteristics, as well as cycle outcomes, were collected from the medical record. The institutional review board at Beth

Israel Deaconess Medical Center in Boston, MA approved this study (Reference number: 2013P-000079).

The IVF treatment protocols for ovarian stimulation, monitoring and oocyte retrieval have been described previously (Eaton, Hacker, Harris, Thornton, & Penzias, 2009). Assisted hatching and intracytoplasmic sperm injection were performed when indicated clinically. Both cleavage-stage and blastocyst-stage embryos were transferred, and that clinical decision was made independent of endometrial thickness. The number of embryos transferred was consistent with national guidelines (Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology, 2013).

## Measurements

Endometrial thickness was measured by professional sonographers for all patients on the day of ovulation trigger using transvaginal ultrasound. The lining was measured in the sagittal plane at the point of the largest anterior to posterior thickness. Due to the 8-year time frame of this study, multiple sonographers and ultrasound machines were used to measure endometrial thickness. Thin endometrium was defined as  $\leq 7$  mm (Kasius et al., 2014).

Initially, we stratified endometrial thickness into three groups based on cut-offs that seemed to possess clinical relevance:  $\leq 7$  mm,  $>7$  to  $<11$  mm and  $\geq 11$  mm. After initial analysis, we more finely stratified endometrial thickness into groups of 2-mm increments and assessed endometrial thickness as a continuous exposure.

Our primary outcome was live birth. Secondary outcomes included serum beta-human chorionic gonadotropin ( $\beta$ -hCG)  $> 5$  mIU/mL and miscarriage. Miscarriage was defined as a pregnancy with a positive  $\beta$ -hCG that did not progress to at least 20 weeks of gestation; miscarriage did not include ectopic pregnancy or therapeutic abortion. Outcomes were assessed among non-cancelled cycles.

## Statistical analysis

All analyses were conducted using SAS 9.3 (SAS Institute, Cary, NC). Data are presented as median with interquartile range, due to non-normal data distributions, or proportion. We used the Cochran–Armitage test to evaluate whether there was a trend in binomial proportions across exposure groups. We estimated the risk ratio (RR) and 95% confidence interval (CI) for each outcome using Poisson regression with robust variance estimates. Variables that might influence endometrial thickness or ovarian response, which may share a common cause with endometrial thickness, and the outcome were considered potential confounders; these included age, body mass index (BMI), peak progesterone level, peak oestradiol level and the number of oocytes retrieved. We constructed both an age-adjusted model and a model that included all potential confounders. To address concerns that markers of treatment response may vary with respect to endometrial thickness, we also constructed a model that additionally included number of embryos transferred and the number of high quality embryos that were transferred.  $p$  values  $<0.05$  were considered statistically significant.

## Results

A total of 6331 patients undergoing their first IVF cycle met the inclusion criteria for the study. There were 347 (5.5%) women with endometrial thickness  $\leq 7$  mm, 2943 (46.5%) women with endometrial thickness  $>7$  to  $<11$  mm and 3041 (48.0%) women with endometrial thickness  $\geq 11$  mm. The three groups were similar with regards to age, BMI, gravidity and the median number of embryos transferred and embryos frozen (all  $p > 0.07$ ). Peak oestradiol increased with increasing endometrial thickness ( $p < 0.001$ ); the groups also differed regarding parity and the median number of oocytes retrieved (both  $p = 0.02$ ). Overall, 151 (2.4%) cycles were cancelled on or after the day of trigger. Among women with endometrial thickness  $\leq 7$  mm, 32 cycles (9.2%) were cancelled, which was significantly more than among the  $>7$  to  $<11$  mm group (3.1%) and the  $\geq 11$  mm group (1.0%;  $p < 0.001$ ). The baseline characteristics of each group are shown in Table 1.

When stratifying into three groups of endometrial thicknesses among cycles that were not cancelled, the incidences of positive  $\beta$ -hCG and live birth increased with increasing endometrial thickness. Women in the  $\geq 11$  mm group had a significantly higher likelihood of delivering a live infant (32.2%) compared with women in the  $>7$  to  $<11$  mm group (27.1%), which yielded a statistically significant age-adjusted RR of 1.23 (95% CI: 1.11–1.37). Similar results were seen for the incidence of a positive  $\beta$ -hCG, while differences in the risk of miscarriage did not reach statistical significance (Table 2). Table 2 shows both the crude and adjusted RRs for a positive  $\beta$ -hCG, miscarriage and live birth; the RRs are adjusted all potential confounders (age, BMI, peak progesterone level, peak oestradiol level and number of oocytes retrieved) as there was no appreciable effect on the RRs for this model and the ones that was adjusted only for age. Similarly, there was no effect on the RRs when number of embryos transferred and the number of high quality embryos that were transferred were included in the model.

When further stratifying endometrial thickness into 2-mm increments, among non-cancelled cycles we found a significant trend of increasing likelihood of positive  $\beta$ -hCG with increasing endometrial thickness ( $p$  trend  $< 0.001$ ). We found similar results for the incidence of live birth ( $p$  trend  $< 0.001$ ). In contrast, endometrial thickness was not associated with miscarriage ( $p$  trend = 0.58). Similar results were seen when adjusting for all potential confounders. These results are shown in Table 3. Again, there was no appreciable effect on the RRs in the models adjusting only for age or for those adjusting for all potential confounders plus the number of embryos transferred and the number of high quality embryos transferred.

After controlling for all potential confounders, among non-cancelled cycles we found a statistically significant increased likelihood of a positive  $\beta$ -hCG (RR: 1.14; 95% CI: 1.09–1.18) and live birth (RR: 1.08; 95% CI: 1.05–1.11) with each additional millimetre of endometrial thickness. There was no association between endometrial thickness and the risk of miscarriage (RR: 0.99; 95% CI: 0.91–1.07). Among women who delivered, 15% of those with the thinnest endometrial linings had a multiple pregnancy, compared to 24% and 25% of those with linings  $>7$  to  $<11$  mm and  $\geq 11$  mm, respectively ( $p = 0.67$ ). The thinnest endometrial thickness at which pregnancy occurred was 3.7 mm, and this pregnancy resulted

in a live birth. The thickest endometrial thickness at which pregnancy occurred was 27 mm, and this pregnancy also resulted in a live birth.

We performed a post hoc analysis of the association between endometrial thickness and live birth (reported as the risk for each additional mm of thickness) stratified by the number of oocytes retrieved. We observed no significant differences across strata of <4 oocytes (RR: 1.09; 95% CI: 1.02–1.16), 4 to <7 oocytes (RR: 1.05; 95% CI: 1.01–1.09) and ≥7 oocytes (RR: 1.04; 95% CI: 1.02–1.06).

## Discussion

Our data suggest that there is no minimum endometrial thickness below which pregnancy and live birth cannot be achieved. However, and not surprisingly, pregnancy and live birth rates were lower with thinner endometrial linings. Likewise, we did not find an upper limit of endometrial thickness beyond which implantation markedly diminished; rather, thicker endometrial linings were associated with an increased likelihood of a positive pregnancy test and live birth. This association was observed both when stratifying endometrial thickness into 2-mm increments and when treating endometrial thickness as a continuous exposure. However, despite this association, one cannot imply that thicker endometrial linings necessarily cause higher pregnancy and live birth rates. There does not appear to be an upper limit at which pregnancy is guaranteed or a lower limit of endometrial thickness at which pregnancy cannot be achieved. This suggests that there are likely to be other uterine and endometrial factors that influence the likelihood of live birth. Furthermore, although we did not observe an effect of oestradiol and ovarian response on endometrial thickness in our study, as evidenced by the similarity between the crude and adjusted RRs, a potential association still exists given the physiology of endometrial proliferation.

Some investigators have proposed freezing all fresh embryos and performing thaw embryo transfer in a subsequent cycle, postulating that ovarian stimulation may create an unfavourable uterine environment and impair implantation. However, our results indicate that there are many women, especially with thicker endometrial linings, for whom that is not necessary. Few studies have stratified endometrial thickness at the thicker end of the spectrum. For example, Weissman et al. (1999) and Okohue et al. (2009) grouped values of endometrial thickness >14 mm together. Our study included women with endometrial thickness of >15 to 17 mm and >17 mm, and demonstrated that women with endometrial thickness of >15 to 17 mm had the highest incidence of positive β-hCG, while the incidence of live birth was highest among women with endometrial thickness of >17 mm. Given that the highest pregnancy rates occurred with thicker endometrial linings, our results suggest patients with thicker endometrial linings may benefit from continuing with a fresh cycle given the high probability of pregnancy and live birth among those patients rather than freezing the embryos for a future cycle.

Limitations of the study include its retrospective design and the higher incidence of cancellation among cycles with endometrial thickness ≥7 mm (9%) as compared with endometrial thickness <7 mm (1%). Assuming that women with cancelled cycles had lower fertility potential and thus a lower probability of a successful outcome compared to women

with non-cancelled cycles, by excluding the cancelled cycles we potentially overestimated the pregnancy and live birth rates. This overestimate would have been most substantial in the lowest range of endometrial thickness and thus would have led to a less pronounced difference in outcomes. Despite this, our results show increasing positive  $\beta$ -hCG and live birth rates with increasing endometrial thickness. Due to the 8-year time frame of this study, multiple sonographers and several different ultrasound machines were used to measure endometrial thickness. However, inter-observer variability and measurement error were minimized by the fact that these professional sonographers used a standard technique for measuring endometrial thickness. Furthermore, investigators Delisle, Velleneuve, and Voulvain (1998) reported an excellent inter-observer agreement of 94% between sonographers when measuring transvaginal endometrial thickness, with a kappa value of 0.74. Lastly, there is no evidence that the accuracy of endometrial thickness measurements has changed over time with the introduction of newer ultrasound machines. Any temporal variability likely would lead to random measurement error and thus non-differential misclassification of exposure, which would serve only to bias results towards the null. Thus, our findings would be an underestimate of the true association between endometrial thickness and IVF success. Finally, our study does not address the relationship of endometrial thickness in donor cycles or subsequent thaw cycles, though there are some data to suggest that endometrial thickness may play less of a role in pregnancy outcomes of donor cycles (Barker, Boehnlein, Kovacs, & Lindheim, 2009; Dain et al., 2013).

Strengths of this study include the large sample size, which allowed us to both stratify by small increments of endometrial thickness and to assess endometrial thickness as a continuous exposure. Our sample size is substantially larger than other studies published on this topic and is the largest to our knowledge. We were able to maintain a large sample size while using only first cycles, which eliminated the need to account for repeated measures. Many prior reports used multiple cycles per woman, and in several it appears that the investigators did not account for the correlation among these cycles (Barker et al., 2009; Chen et al., 2010; Dain et al., 2013; Kovacs, Matyas, Boda, & Kaali, 2003; Weissman et al., 1999). An additional strength of this study is that our database has undergone multiple iterations of verification for data accuracy and completeness, leading to a robust dataset with high fidelity.

Future research should examine the relationship of endometrial thickness with pregnancy and live birth rates among donor cycles and subsequent thaw cycles, particularly those from which all embryos were frozen in a prior fresh cycle, as well as the outcomes of repeated cycles among patients. It also would be useful to examine repeated endometrial thickness measurements within a single cycle, as there are data from McWilliams and Frattarelli (2007) that suggest that the rate of change of endometrial thickness over the cycle is more predictive of outcomes than a single measurement. Correlation with endometrial function and a search for biomarkers predictive of implantation beyond endometrial thickness alone should continue.

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Table 1

Participant and cycle characteristics for the first cycle.

Characteristic	All women (n = 6331)	Endometrial thickness			p
		7 mm (n = 347)	7 to <11 mm (n = 2943)	11 mm (n = 3041)	
Age (years)	35.6 (32.2–39.2)	36.0 (32.4–39.5)	35.8 (32.2–39.4)	35.4 (32.3–38.9)	0.07
Body mass index	24.0 (21.7–28.1)	24.0 (21.6–29.1)	24.0 (21.6–28.0)	24.0 (21.8–28.1)	0.92
Gravidity					0.93
0	3363 (53.1)	184 (53.0)	1575 (53.5)	1604 (52.8)	
1	1492 (23.6)	82 (23.6)	678 (23.0)	732 (24.1)	
2 or more	1476 (23.3)	81 (23.3)	690 (23.5)	705 (23.2)	
Parity					<0.001
0	4789 (75.6)	277 (79.8)	2312 (78.6)	2200 (72.3)	
1	1172 (18.5)	54 (15.6)	489 (16.6)	629 (20.7)	
2 or more	370 (5.8)	16 (4.6)	142 (4.8)	212 (7.0)	
Oocytes retrieved	10.0 (6.0–15.0)	9.0 (6.0–14.0)	9.0 (6.0–14.0)	10.0 (6.0–15.0)	0.02
Embryos transferred <sup>a</sup>					0.71
0	356 (9.1)	24 (11.4)	154 (9.1)	178 (8.8)	
1	612 (15.6)	28 (13.3)	257 (15.1)	327 (16.2)	
2	2115 (53.8)	118 (55.9)	914 (53.7)	1083 (53.7)	
3	847 (21.6)	41 (19.4)	377 (22.2)	429 (21.3)	
Embryos frozen <sup>a</sup>					0.69
0	3614 (60.5)	185 (61.9)	1686 (61.0)	1743 (59.9)	
1–2	1089 (18.2)	48 (16.1)	512 (18.5)	529 (18.2)	
3–4	621 (10.4)	31 (10.4)	285 (10.3)	305 (10.5)	
5	647 (10.8)	35 (11.7)	279 (10.1)	333 (11.4)	
Cycles cancelled <sup>b</sup>	151 (2.4)	32 (9.2)	90 (3.1)	29 (1.0)	<0.001
Peak oestradiol (pg/mL)	1711 (1012–2691)	1331 (804–2447)	1676 (984–2677)	1767 (1052–2719)	<0.001
Progesterone (ng/mL)	0.8 (0.5–1.3)	0.8 (0.5–1.3)	0.8 (0.5–1.2)	0.8 (0.5–1.3)	0.76

Data are presented as median (interquartile range) or n (%).

<sup>a</sup>Calculated among cycles with an oocyte retrieval.



$\eta$  Calculated only among cycles cancelled at or after the time of trigger.

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Table 2

Outcome of non-cancelled first cycle by endometrial thickness measured on day of ovulation trigger.

Endometrial thickness	N	Positive $\beta$ -hCG			Miscarriage <sup>a</sup>			Live birth		
		Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)	Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)	Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)
7 mm	315	103 (32.7)	0.80 (0.68–0.94)	0.74 (0.59–0.94)	38 (12.1)	0.98 (0.72–1.34)	0.96 (0.64–1.45)	62 (19.7)	0.73 (0.58–0.92)	0.64 (0.45–0.90)
>7 to <11 mm	2853	1169 (40.8)	REF	REF	351 (12.3)	REF	REF	772 (27.1)	REF	REF
11 mm	3012	1340 (44.5)	1.09 (1.02–1.15)	1.12 (1.04–1.21)	329 (10.9)	0.89 (0.77–1.02)	0.97 (0.81–1.15)	970 (32.2)	1.19 (1.10–1.29)	1.23 (1.11–1.37)

RR: risk ratio; CI: confidence interval.

<sup>a</sup>Excludes ectopic pregnancy and therapeutic abortion.

<sup>b</sup>Adjusted for age, body mass index, peak progesterone, peak oestradiol, and number of oocytes retrieved.

**Table 3**  
Outcomes of non-cancelled first cycles by endometrial thickness on day of ovulation trigger.

Endometrial thickness (mm)	N	Positive $\beta$ -hCG				Miscarriage <sup>a</sup>				Live birth			
		Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)	Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)	Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)	Incidence N (%)	Crude RR (95% CI)	Adjusted <sup>b</sup> RR (95% CI)
5	50	13 (26.0)	0.60 (0.38–0.97)	0.70 (0.39–1.27)	4 (8.0)	0.71 (0.26–1.90)	0.97 (0.31–3.03)	9 (18.0)	0.60 (0.33–1.08)	0.61 (0.26–1.44)			
>5 to 7	265	90 (34.0)	0.79 (0.66–0.94)	0.72 (0.56–0.92)	34 (12.8)	1.14 (0.80–1.62)	1.09 (0.68–1.74)	53 (20.0)	0.66 (0.52–0.85)	0.57 (0.39–0.82)			
>7 to 9	1090	420 (38.5)	0.90 (0.82–0.98)	0.92 (0.83–1.03)	147 (13.5)	1.20 (0.98–1.46)	1.30 (1.03–1.66)	257 (23.6)	0.78 (0.69–0.88)	0.77 (0.65–0.89)			
>9 to 11	2679	1152 (43.0)	REF	REF	302 (11.3)	REF	REF	808 (30.2)	REF	REF			
>11 to 13	1287	563 (43.8)	1.02 (0.94–1.10)	1.07 (0.97–1.18)	124 (9.6)	0.85 (0.69–1.05)	1.00 (0.77–1.30)	418 (32.5)	1.07 (0.97–1.19)	1.10 (0.97–1.25)			
>13 to 15	527	241 (45.7)	1.06 (0.96–1.18)	1.13 (1.0–1.29)	72 (13.7)	1.21 (0.94–1.57)	1.29 (0.92–1.80)	161 (30.6)	1.01 (0.88–1.17)	1.13 (0.95–1.35)			
>15 to 17	192	95 (49.5)	1.15 (0.99–1.34)	1.27 (1.05–1.53)	23 (12.0)	1.06 (0.70–1.62)	1.13 (0.65–1.99)	72 (37.5)	1.24 (1.03–1.51)	1.41 (1.10–1.80)			
>17	90	38 (42.2)	0.98 (0.77–1.26)	1.0 (0.67–1.47)	12 (13.3)	1.18 (0.66–2.11)	1.12 (0.46–2.73)	26 (28.9)	0.96 (0.69–1.33)	1.03 (0.61–1.75)			

RR: risk ratio; CI: confidence interval.

<sup>a</sup>Excludes ectopic pregnancy and induced abortion.

<sup>b</sup>Adjusted for age, body mass index, peak progesterone, peak oestradiol, and number of oocytes retrieved.