A meta-analysis of the relationship between endometrial thickness and outcome of in vitro fertilization cycles

ABSTRACT

OBJECTIVE: The objective was to evaluate the relationship between endometrial thickness on the day of human chorionic gonadotropin administration and pregnancy outcome in in vitro fertilization cycles. DESIGN: This was a systematic review and meta-analysis. MATERIALS AND METHODS: We identified 484 articles using Cochrane library, PubMed, Web of Science, and Embase searches with various key words including endometrial thickness, pregnancy, assisted reproductive technology, endometrial pattern, and in vitro fertilization. A total of 14 studies with data on endometrial thickness and outcome were selected, representing 4922 cycles (2204 pregnant and 2718 nonpregnant). The meta-analysis with a random effects model was performed using comprehensive meta-analysis software. We calculated the standardized mean difference, odds ratio (OR), and 95% confidence intervals (CIs). RESULTS: There was a significant difference in the mean endometrial thickness between pregnant and nonpregnant groups (P<0.001), with a standardized mean difference of 0.4 mm (95% CI 0.22–0.58). The OR for pregnancy was 1.40 (95% CI 1.24–1.58). CONCLUSIONS: The mean endometrial thickness was significantly higher in pregnant women compared to nonpregnant. The mean difference between two groups was <1 mm which may not be clinically meaningful. Although there may be a relationship between endometrial thickness and pregnancy, implantation potential is probably more complex than a single ultrasound measurement can determine.

KEY WORDS: Assisted reproductive technology, endometrial pattern, endometrial thickness, in vitro fertilization, pregnancy

INTRODUCTION

Assisted reproductive technology (ART) has been commonly used in infertility treatment over the past two decades. The high cost, relatively low implantation, and increased multiple pregnancy rates in in vitro fertilization (IVF) cycles have led to a need to evaluate the predictors of success in these patients. One important factor is the endometrial receptivity.[1] In addition to the embryo quality, the receptivity of the endometrium also plays a role in the implantation process.

The standard method of endometrial dating is the histological evaluation of an endometrial biopsy specimen.[1] Indeed, this technique has allowed for the demonstration of a possible asynchrony in endometrial development in the course of cycles with ovarian stimulation for IVF when embryo transfer had to be cancelled.[2‑5] Obviously, the invasiveness of endometrial biopsy is not acceptable in the clinical context of ART cycles.[6] The ability to identify a receptive uterus prospectively by a noninvasive method would have an invaluable impact on treatment efficiency and success rates following ART. The need to evaluate endometrial development encouraged the use of high-resolution ultrasonography as an alternative noninvasive method of the assessment of uterine receptivity. Several sonographic parameters have been used to assess receptivity, including endometrial thickness, endometrial pattern, and endometrial and subendometrial blood flow.[6] The effect of endometrial thickness on the pregnancy rate in ART patients has been
evaluated by many authors, with controversial results.\textsuperscript{[7-16]} Using abdominal ultrasound, Glissant \textit{et al.} reported a significantly thicker endometrium in conception cycles compared with nonconception cycles;\textsuperscript{[7]} however, several reports using abdominal sonography gave contradictory findings.\textsuperscript{[18-20]} Li \textit{et al.} reported no correlation between endometrial thickness measured by abdominal ultrasound and histological dating of endometrium.\textsuperscript{[21]} Some authors demonstrated a higher pregnancy rate at a certain endometrial thickness,\textsuperscript{[8,9,15,16,22]} while others did not show a significant correlation between endometrial thickness and pregnancy rates in IVF patients.\textsuperscript{[10,12,13]} Other authors reported a threshold of <7 and/or >14 mm which was associated with a significant reduction in the implantation and pregnancy rates.\textsuperscript{[7,11]}

No conclusive cut-off value of endometrial thickness has been established in order to help clinicians in counseling the couple about the outcome. The reason for such controversy could be probably due to a relatively low number of cycles for patients with both extreme ends of endometrial thicknesses. Heterogeneity of these studies such as protocols used for controlled ovarian hyperstimulation, use of different time points and routes of ultrasonographic examination (transvaginal vs. transabdominal), and differences in the statistical evaluation of the predictive value of the endometrial thickness makes them incomparable.

Despite the fact that multiple studies investigated the endometrial thickness in ART cycles, it is still unknown whether the mean endometrial thickness in successful ART cycles is significantly greater than that of failed cycles. Therefore, the aim of our study was to determine if the endometrial thickness measured on the day of hCG administration had any effect on the outcome of IVF treatment with a long gonadotropin-releasing hormone analog (GnRHa) protocol, utilizing meta-analysis of previously published studies.

\textbf{MATERIALS AND METHODS}

\textbf{Study identification}

We identified 484 articles using Cochrane library, PubMed, Web of Science, and Embase searches with different combinations of various key words including endometrial thickness, pregnancy, assisted reproductive technology, endometrial pattern, and \textit{in vitro} fertilization. Initially, a total of 38 studies with data on endometrial thickness and outcome were selected. After a second review, 14 studies were selected for a systematic review representing 4922 cycles (2204 pregnant and 2718 nonpregnant). The studies were published between 1994 and 2009. Figure 1 summarizes the selection of these articles.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1}
\caption{Number of selected studies and reasons for exclusion at each step of the systematic search}
\end{figure}

\textbf{Inclusion criteria were as follows:}

1. Articles in English
2. Measurement of endometrial thickness with transvaginal ultrasound
3. Measurement of endometrial thickness on the day of hCG injection
4. Availability of the mean of endometrial thickness on the day of hCG injection in millimeters in pregnant and nonpregnant groups
5. Availability of standard deviation in each group
6. Availability of number of cycles in each group.

\textbf{Exclusion criteria were as follows:}

1. Studies that used clomiphene citrate in their stimulation protocols
2. Studies that report their data as categorical data
3. Studies that used cryopreserved embryo transfer

\textbf{Statistical analysis}

The meta-analysis with random and fixed effects models was performed using comprehensive meta-analysis software version 2 (Biostat, Englewood, NJ, USA). We calculated the standardized mean difference, and odds ratio (OR) with 95\% confidence intervals (CIs).

\textbf{RESULTS}

A total of 14 studies were selected for the systematic review representing 4922 cycles (2204 pregnant and
2718 nonpregnant). The studies were published between 1994 and 2009.

The mean age, number of oocytes retrieved, and estradiol level on the day of hCG administration for each study are presented in Table 1. Two studies did not have actual data on these parameters.

The mean endometrial thickness, standard deviation, and number of cycles in each study are demonstrated in Table 2. Four studies showed a statistically significant difference in the endometrial thickness between pregnant and nonpregnant groups.[42][44][46][49] Ten studies found no difference between two groups.

Table 3 shows the weight which was given to each study for both fixed and random effects models. Larger studies such as Al-Ghamdi and Richter were assigned 54% and 22% of the total weight in the fixed effects model, but in the random effects model these were 35% and 23%, respectively. Therefore, we chose to use the random effects model as it would allow us to avoid one or two studies skewing the results.

Table 4 and Figure 2 demonstrate the mean differences which were calculated for each study using the random effects models. In the random effects model, the standardized mean difference between pregnant and nonpregnant groups was 0.404 mm. The confidence interval did not include 0 (95% CI 0.226–0.582). Therefore, it was a significant increase in the endometrial thickness.

The odd ratios with 95% CI for each study and also for the random effects model are presented in Table 5 and Figure 3.

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**Table 1: Age and number of oocytes retrieved and estradiol level in both groups**

| Author name and year | Pregnant (age, years) | Nonpregnant (age, years) | P value | Number of oocytes in the pregnant group | Number of oocytes in the nonpregnant group | Estradiol on the day of hCG in the pregnant group (pg/ml) | Estradiol on the day of hCG in the nonpregnant group (pg/ml) | P value |
|----------------------|----------------------|--------------------------|---------|----------------------------------------|--------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|---------|
| Traub 2009[29]       | 32.4 ± 3.5           | 34.1 ± 4.1               | 0.019   | 15.8 ± 6.5                             | 17.3 ± 6.4                                 | 3146 ± 1255                                             | 3498 ± 1267                                              | 0.142   |
| AlGhamdi 2008[41]    | 30.2 ± 5.5           | 31.1 ± 5.3               | 0.0001  | 10.5 ± 5.4                             | 9.86 ± 5.73                                | N/a                                                     | N/a                                                     | 0.006   |
| Merce 2007[25]       | 33.3 ± 3.3           | 34.3 ± 3.4               | 0.554   | 11.2 ± 5.0                             | 8.67 ± 4.21                                | 2852 ± 1161                                             | 2449.4 ± 1050.8                                         | 0.970   |
| McWilliams 2007[28]  | 32.9 ± 3.9           | 34.0 ± 4.3               | <0.01   | 17.8 ± 11.1                            | 13.9 ± 10.9                                | 2814 ± 1436                                             | 2265 ± 1521                                             | <0.01   |
| Richter 2007[44]     | 33.5 ± 3.5           | 34.0 ± 3.7               | 0.031   | N/a                                    | N/a                                        | 2554 ± 1003                                             | 2553 ± 968                                              | 0.99    |
| Jarvela 2005[49]     | 33.5 ± 4.5           | 35.4 ± 4.2               | NS      | 13.0 ± 6.0                             | 14.0 ± 9.0                                  | N/a                                                     | N/a                                                     | 0.011   |
| Rashidi 2003[41]     | 30.9 ± 4             | 30.7 ± 5                 | 0.89    | 8.1 ± 4.0                              | 4.5 ± 3.0                                  | 1883 ± 1147                                             | 1686 ± 1057                                             | NS      |
| Yaman 2000[47]       | 32.3 ± 4.8           | 32.4 ± 5.0               | NS      | N/a                                    | N/a                                        | N/a                                                     | N/a                                                     | 0.001   |
| Lensy 1999[39]       | 30.4 ± 3.5           | 30.6 ± 3.8               | NS      | 12.5 ± 4.2                             | 10.5 ± 5.6                                  | N/a                                                     | N/a                                                     | 0.011   |
| Sharara 1999[46]     | 32.8 ± 3.4           | 33.0 ± 4.2               | NS      | 15.1 ± 5.9                             | 15.7 ± 7.0                                  | N/a                                                     | N/a                                                     | 0.003   |
| Leibovitz[45]        | 30.6 ± 4.9           | 30.7 ± 6.0               | NS      | N/a                                    | N/a                                        | N/a                                                     | N/a                                                     | 0.037   |
| Oliveira[44]         | N/a                  | N/a                      | N/a     | N/a                                    | N/a                                        | N/a                                                     | N/a                                                     | 0.000   |
| Zaidi[43]            | 32.3 ± 3.5           | 34.4 ± 4.5               | 0.004   | 10.5 ± 4.5                             | 11.0 ± 4.9                                  | N/a                                                     | N/a                                                     | 0.135   |
| Coulam[42]           | N/a                  | N/a                      | NS      | N/a                                    | N/a                                        | N/a                                                     | N/a                                                     | NS      |

All data are presented as means ± standard deviations.

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**Table 2: Author name and year, and sample size in each group**

| Study name            | Pregnant group mean | Pregnant group std. dev. | Pregnant group sample size | Nonpregnant group mean | Nonpregnant group std. dev. | Nonpregnant group sample size |
|----------------------|---------------------|--------------------------|---------------------------|------------------------|------------------------------|-------------------------------|
| Traub 2009[29]       | 11.2                | 3.1                      | 57                        | 10.1                   | 2.6                          | 57                            |
| AlGhamdi 2008[41]    | 11.64               | 2.13                     | 882                       | 11.26                  | 2.17                         | 1582                          |
| Merce 2007[25]       | 12.29               | 2.71                     | 38                        | 12.15                  | 2.31                         | 39                            |
| McWilliams 2007[28]  | 10                  | 1.9                      | 70                        | 9.1                    | 2.3                          | 62                            |
| Richter 2007[44]     | 11.9                | 2.4                      | 864                       | 11.3                   | 2.4                          | 430                           |
| Jarvela 2005[49]     | 12.5                | 3.2                      | 13                        | 11.5                   | 2.5                          | 22                            |
| Rashidi 2003[41]     | 10.1                | 1                        | 30                        | 10.2                   | 2                            | 120                           |
| Yaman 2000[47]       | 11                  | 2                        | 21                        | 11                     | 2                            | 44                            |
| Lensy 1999[39]       | 12.9                | 2.7                      | 30                        | 12.4                   | 2.7                          | 30                            |
| Sharara 1999[46]     | 10                  | 2.1                      | 47                        | 9.6                    | 1.7                          | 56                            |
| Leibovitz 1998[45]   | 11                  | 2.7                      | 29                        | 11.7                   | 2.5                          | 46                            |
| Oliveira 1997[44]    | 10.8                | 2.1                      | 45                        | 10.2                   | 2.2                          | 105                           |
| Zaidi 1995[43]       | 10.9                | 1.8                      | 31                        | 11.3                   | 2.2                          | 65                            |
| Coulam 1994[42]      | 11.5                | 2.7                      | 47                        | 11.2                   | 2.4                          | 60                            |
The OR for pregnancy in the random effects model was 1.402 (95% CI 1.240–1.585) which was statistically significant.

**DISCUSSION**

To our best knowledge, this study is the first meta-analysis that addresses the effect of endometrial thickness on the pregnancy rate in IVF cycles with the long GnRHa protocol. Multiple studies in the literature showed that the endometrial thickness was significantly higher in pregnant women compared to nonpregnant women.\[^{[6,8,9,13-17,20,22-24,26-38]}\]

However, there are just as many studies that failed to find a significant difference.\[^{[10,12,13,18,19,25,39-70]}\] The publication year of all these papers ranged from 1984 to 2009.

In reviewing the IVF cycles stimulated by human menopausal gonadotrophin/human chorionic gonadotrophin (HMG/hCG), Rabinowitz et al. described a daily growth of 0.5 mm starting from 3 days prior to the hCG administration up to the day of oocyte retrieval.\[^{[39]}\] The growth continued through
the luteal phase at a slower rate of 0.1 mm/day. Conception cycles were characterized by an accelerated growth compared with nonconception cycles starting 17 days after the hCG administration.\textsuperscript{19} Imoedemhe \textit{et al.} have also found a positive correlation between the endometrial thickness in the luteal phase and conception rates in IVF cycles.\textsuperscript{23} On the other hand, Lesny \textit{et al.} have reported that the maximal endometrial thickness is reached at the time of hCG injection followed by a small decrease or no increase at the time of oocyte retrieval and embryo transfer.\textsuperscript{39}

Weisman \textit{et al.} investigated the association between the endometrial thickness and the pregnancy rate by questioning whether there was a maximal value for endometrial thickness above which pregnancy was unlikely to occur.\textsuperscript{11} They found that pregnancy rates were significantly lower above a maximum thickness of 14 mm in their patient population. Similarly, Dickey \textit{et al.} reported increased biochemical pregnancy rates with an endometrial thickness >14 mm.\textsuperscript{17} Rashidi \textit{et al.} also showed no pregnancies with an endometrial thickness >12 mm.\textsuperscript{71} However, there are case series which reported successful pregnancies in women with an endometrial thickness ≥20 mm.\textsuperscript{72,74}

A triple-layer endometrial pattern and an endometrial thickness greater than 7 mm have also been proposed as markers of endometrial receptivity but have yielded a high percentage of false-positive results.\textsuperscript{6} However, some authors think that endometrial thickness is a distinct parameter, unrelated to the endometrial pattern on the day of hCG administration.\textsuperscript{18,21,22,27} Several studies have evaluated the endometrial lining at different time points during the stimulation cycles. The day of hCG administration,\textsuperscript{11,12,13,40,71} the day before hCG administration,\textsuperscript{9,11,13,22,24,25,28,29,39,41-49,73} the day of oocyte retrieval,\textsuperscript{13,20,26,46} and the day of embryo transfer\textsuperscript{13,15,50,74} were used in various studies. Another factor which is also different among studies is that different treatment and stimulation protocols were applied including natural cycles with cryopreserved embryo transfer,\textsuperscript{42,75} natural cycles with fresh embryo transfer,\textsuperscript{40} ovarian stimulation cycles for IVF with different stimulation protocols such as long GnRHα down-regulation,\textsuperscript{16,30,31,40,42,51-53} clomiphene citrate with HMG, short GnRHα down-regulation,\textsuperscript{22,54} HMG only,\textsuperscript{76} and hormone replacement therapy with oocyte donation.\textsuperscript{19,32,42,55,77,78}

These studies used various fertility treatment regimens, endometrial thickness evaluation methods, and time points. Therefore, the study populations are extremely heterogeneous making it hard to duplicate the results. In a review by Friedler \textit{et al.} published in 1996, patients also suffered from the same issues as natural cycles, fresh IVF cycles, and oocyte donation cycles with hormone replacement therapy were included.\textsuperscript{6} Therefore, we decided to study a more homogenous study population that underwent the same type of stimulation protocol and endometrial thickness evaluation. We chose the day of hCG administration as an inclusion criteria for our systematic review, for two main reasons. First, most of the authors used that day as the preferred day for endometrial evaluation.\textsuperscript{1,9,11-13,22,25,28,29,39,41-49,72,73} Second, that day is the best day to formulate the plan for the ongoing cycle. Among various ovarian stimulation protocols for fresh IVF cycles, the long GnRHα down-regulation protocol is internationally accepted and used by most centers as the standard of care. Therefore, we chose to analyze studies where patients underwent fresh IVF cycles with the long GnRHα protocol.

Using more homogenous study population enabled us to detect a significant difference in endometrial thicknesses between pregnant and non-pregnant groups. On the other hand, this limits the generalization of our findings. Also, we could not identify a cut-off value for endometrial thickness in our study, as studies analyzed did not report any linear data of endometrial thickness.

Calculating endometrial volume could be an option to find differences which could be meaningful clinically. Some authors actually used endometrial volume instead of endometrial thickness for their evaluation.\textsuperscript{47,56,79} However, more studies on endometrial volume are needed before reaching any conclusions.

In summary, a continuing use of transvaginal ultrasound to evaluate endometrial thickness and the changes occurring during ovarian stimulation can aid providers in counselling patients and predicting IVF success. It is unclear if the improved IVF success is the result of a more responsive endometrial lining or the responsiveness of the endometrial lining is only a marker of a better hormonal stimulation of the ovary with downstream effects on the endometrium. It is important to note that the correlation between endometrial thickness and pregnancy outcomes described here does not necessarily imply a causal relationship; also it is our limitation that in these studies, we cannot indentify if the endometrial thickness was taken into consideration before making the decision for hCG administration or not. The relationship may merely result from a correlation with some other confounding factors that are directly responsible for differences in receptivity such as blood flow or some other underlying machinery responsible for cyclic endometrial development. Therefore, even if the treatment protocols resulting in significant improvements in endometrial thickness are identified, such therapies may not necessarily have any clinical benefits in terms of pregnancy rates.\textsuperscript{24}
Finally, in our systematic review, the mean endometrial thickness is significantly higher in pregnant women compared to non-pregnant. The difference between two groups is <1 mm which may not be clinically meaningful. Although there may be a relationship between endometrial thickness and pregnancy, the implantation potential is probably more complex than a single ultrasound measurement can determine.

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