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ORIGINAL ARTICLE

Endometrial thickness as a biomarker for ongoing pregnancy in IUI for unexplained subfertility: a secondary analysis

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STUDY QUESTION: What is, in couples with unexplained subfertility undergoing IUI, the impact of gonadotrophins compared to clomiphene citrate (CC) on endometrial thickness (EMT) in relation to ongoing pregnancy?

SUMMARY ANSWER: In women with unexplained subfertility undergoing IUI with ovarian stimulation, gonadotrophins lead to a thicker endometrium compared to CC, but this does not affect ongoing pregnancy rates.

WHAT IS KNOWN ALREADY: A systematic review and meta-analysis among couples with unexplained subfertility undergoing IUI with ovarian stimulation showed that women who conceived had, on average, a thicker endometrium than women who did not conceive, but this evidence is not robust due to a high level of heterogeneity. There was insufficient data to draw any conclusions on EMT and the effect on pregnancy outcomes.

STUDY DESIGN, SIZE, DURATION: We performed a secondary analysis of a multicentre randomized controlled superiority trial in couples with unexplained subfertility undergoing IUI with adherence to strict cancellation criteria. In total, 738 couples recruited between July 2013 and March 2016 were allocated to ovarian stimulation with gonadotrophins ($n = 369$) or with CC ($n = 369$) for a maximum of four IUI cycles. According to local protocol, recombinant FSH, urinary FSH or hMG was used. Natural conceptions and cancelled cycles were removed from this secondary analysis, as they do not provide any information on pregnancy in relation to stimulation after IUI. Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We first determined the difference in EMT between women randomized to gonadotrophins (75 IU) and CC (100 mg) over all cycles using a linear mixed model. We then investigated the association between EMT and ongoing pregnancy after IUI using a logistic regression model, adjusted for the allocated drug, number of dominant follicles, female age, BMI, duration of subfertility, primary or secondary subfertility, referral status, smoking status, cycle number and total motile sperm count. To conclude, we investigated the association between EMT and ongoing pregnancy by logistic regression separately in women allocated to gonadotrophins and in women allocated to CC.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 666 couples underwent 1968 IUI cycles. Of these, 330 couples were allocated to gonadotrophins, of which 85 conceived leading to ongoing pregnancy (rate per cycle 8.9%) and 336 couples were allocated to CC, of which 71 conceived leading to ongoing pregnancy (rate per cycle 7.0%) (relative risk (RR) 1.22, 95% CI 0.92 to 1.61). The mean EMT was 8.9 mm (SD 2.1) in women treated with gonadotrophins and 7.5 mm (SD 2.1) in women treated with CC (adjusted mean difference 1.4 mm; 95% CI: 1.1–1.7). The overall mean EMT was 8.4 mm (SD 2.2) in women that conceived leading to ongoing pregnancy and 8.2 mm (SD 2.2) in women that did not conceive (adjusted odds ratio (OR): 1.03 per 1 mm increase, 95% CI 0.95–1.12). There was no association between EMT and ongoing pregnancy in women treated with gonadotrophins or CC (OR: 1.01 per 1 mm increase, 95% CI 0.90–1.13, and 1.10 per 1 mm increase, 95% CI 0.99–1.23, respectively).

LIMITATIONS, REASON FOR CAUTION: Since this is a secondary analysis, the data should be interpreted prudently as secondary analyses are prone to false-positive findings or could be underpowered to show associations that the study is not primarily set up for.

WIDER IMPLICATIONS OF THE FINDINGS: In women with unexplained subfertility and treated with IUI, gonadotrophins lead to a significantly thicker endometrium compared to CC, but there was no evidence of a consistent association between EMT in women treated with gonadotrophins or CC and the ongoing pregnancy rate. A relatively thin endometrium after CC is therefore not a valid reason to prefer gonadotrophins as the stimulation agent in IUI for unexplained subfertility.

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Key words: IUI / gonadotrophins / clomiphene citrate / endometrium / unexplained subfertility

WHAT DOES THIS MEAN FOR PATIENTS?

Intrauterine insemination (IUI) combined with stimulation of the woman's ovaries is often used for couples with unexplained subfertility. Ovarian stimulation can be done with gonadotrophin injections (e.g. LH, FSH, which normally stimulate egg development) or tablets of clomiphene citrate (a drug which causes LH/FSH release).

Studies have shown that IUI with gonadotrophin ovarian stimulation leads to a 5% increase in ongoing pregnancy rates compared to IUI with clomiphene citrate. We proposed that this difference in ongoing pregnancy rate might be explained by a difference in thickness of the endometrium: the endometrium is the inner lining of the uterus, which thickens each month in preparation for a possible pregnancy.

In this study, we performed a secondary analysis of data from patients who underwent IUI and found that injecting gonadotrophins led to a thicker endometrium compared to tablets of clomiphene citrate. However, this difference in endometrial thickness did not affect the ongoing pregnancy rate. Therefore, a relatively thin endometrium after clomiphene citrate is not a reason to prefer gonadotrophins as the stimulation agent in IUI for unexplained subfertility.

Introduction

In numerous countries, a first-line treatment for couples diagnosed with unexplained subfertility is IUI with ovarian stimulation (Calhaz-Jorge et al. 2017; The Practice Committee of the American Society for Reproductive Medicine., 2006). In Europe, 175 000 IUI cycles are performed each year (Calhaz-Jorge et al. 2017).

Ovarian stimulation in IUI can be performed with s.c. injections of gonadotrophins or with clomiphene citrate (CC) tablets taken orally. Increased serum gonadotrophin levels during the follicular phase induces growth of more than one dominant follicle, while CC occupies the estrogen receptors, blocks the negative feedback of 17β-estradiol in the hypothalamic pituitary axis and thereby indirectly increases serum gonadotrophin levels (Wu 1977; Kettel et al. 1993; Schipper et al. 1998). Although the concept of ovarian stimulation is that it is the development of multiple follicles that increases pregnancy rates, ovarian stimulation also affects endometrial thickness (EMT).

In a recent multicentre randomized controlled trial comparing gonadotrophins to CC in couples with unexplained subfertility undergoing IUI with adherence to strict cancellation criteria, the ongoing pregnancy rate was 31% in women allocated to gonadotrophins and 26% in women allocated to CC (relative risk (RR) 1.16, 95% CI 0.93 to 1.47), while there was no evidence of a statistically significant difference in the mean $\pm$ SD number of follicles $\geq$14 mm at the day of ovulation triggering (gonadotrophins 1.8 ± 1.43, CC 1.9 ± 1.11, $P=0.52$) (Danhof et al. 2018). The question then arises of whether this difference in ongoing pregnancy rates can be explained by a difference in EMT.

To investigate the association between EMT and pregnancy in women with unexplained subfertility undergoing IUI, a systematic review and meta-analysis pooled the data of two randomized controlled trials (RCTs) and five cohort studies and found a thinner endometrium after CC as compared to gonadotrophin stimulation, although the difference was not statistically significant (mean difference: 0.51 mm, 95% CI: -0.05 to 1.07; $I^2=74\%$) (Weiss et al. 2017). When comparing various drugs used for ovarian stimulation, the evidence was insufficient to draw any conclusions on the impact of the type of drug on EMT and on pregnancy outcomes (Weiss et al. 2017). Based on the existing evidence, it is unclear whether a difference in EMT following different ovarian stimulation agents in IUI can lead to a difference in pregnancy outcomes.

We therefore performed a secondary analysis of our multicentre RCT, to explore the impact of gonadotrophins compared to CC on EMT and its possible impact on ongoing pregnancy.

Materials and Methods

Study design

We conducted a secondary analysis of the SUPER study (Danhof et al. 2018). Here, we briefly discuss the trial essentials as details have been described elsewhere (Danhof et al. 2018). The Medical Ethical Committee of the Academic Medical Centre and the Dutch Central Committee on Research involving Human Subjects approved this study (CCMO NL 43131-018-13) and the board of directors of each participating site approved local execution (NTR4057). Couples diagnosed

Table I Baseline characteristics of the participating couples with unexplained subfertility undergoing IUI in the present study.

Characteristics	Gonadotrophins* (n = 330)	CC (n = 336)
Mean female age (years)	33.3 ± 4.0	33.7 ± 4.0
Primary subfertility	243 (74%)	245 (73%)
Median duration of subfertility (months)	24 (20–33)	24 (19–31)
Current smoking status	55 (17%)	49 (15%)
Median BMI in kg/m^2	23.3 (21–26)	23·1 (21–25)
Median total motile count ($\times 10^6$)	48 (22–98)	59 (27–113)

Data are n (%), mean (SD) or median (25th–75th percentiles)
*Recombinant FSH, urinary FSH or hMG was used
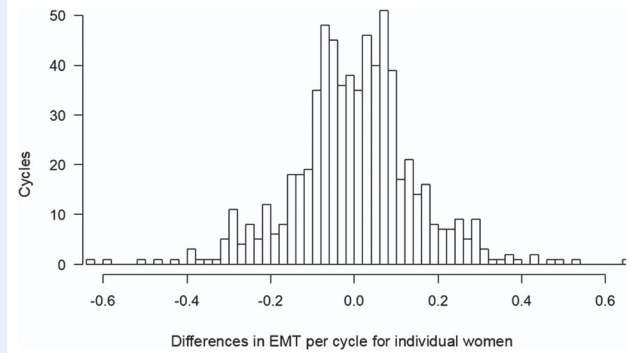CC = clomiphene citrate

Figure 1 Histogram showing the contribution of the difference in EMT over subsequent IUI cycles for all individual women. EMT = endometrial thickness.

with unexplained subfertility were scheduled for a maximum of four IUI cycles with ovarian stimulation comparing 75 IU gonadotrophins to 100 milligrams CC within a time horizon of 6 months. According to local protocol, either recombinant FSH, urinary FSH or hMG was used. Women could thus receive multiple treatment cycles. In both interventions, we monitored follicular development and EMT by transvaginal ultrasound. We cancelled insemination if more than three follicles with a diameter of ≥14 mm or five follicles with a diameter of ≥12 mm were seen at transvaginal ultrasound, regardless of the EMT. EMT was not a criterion to cancel the cycle or switch medication. Ongoing pregnancy was defined as a positive heartbeat at or beyond 12 weeks of gestation.

Statistical analysis

Data were analysed on cycle level. Natural conceptions and cancelled cycles were removed from analysis, as they are not informative on pregnancy rates after IUI in relation to EMT. First, we determined the difference in EMT between women randomized to gonadotrophins and CC over all cycles with a linear mixed model and EMT as the outcome. We handled the allocated drug as a fixed covariate and used random intercepts and random slopes for cycle number, taking into account that women could receive multiple treatment cycles. Second, we investigated the estimated trend in EMT over subsequent cycles for individual women. Third, we determined the difference in EMT between women that had an ongoing pregnancy and women that did not. Fourth, we investigated the association between EMT and ongoing pregnancy after IUI using a logistic regression model. In this model, we adjusted for the stimulation agent, female age, duration of subfertility, primary or secondary subfertility, referral status, BMI, total motile sperm count (TMSC), smoking status and the growth of two follicles versus one follicle, or three follicles versus one follicle, and for failed IUI cycles. Finally, we investigated the association between EMT and ongoing pregnancy rate by logistic regression in women allocated to gonadotrophins and in women allocated to CC.

Missing data

We decided on multiple imputations since missing data on EMT occurred in 68 out of 1968 cycles (3.5%) and multiple imputation

is generally advised in these settings. Numerical results are based on pooled estimates over 10 imputation sets using Rubin's Rules (Rubin, 2004).

We used SPSS Version 22.0 (IBM Software, USA) and R version 3.3.2 (R Core Team (2016).

Results

Study group

Between July 2013 and March 2016, we randomly allocated 369 women to ovarian stimulation with gonadotrophins and 369 women to ovarian stimulation with CC. After exclusion of natural conceptions

Table II Results for the adjusted logistic regression model.

	OR for ongoing pregnancy after IUI	95% CI
EMT, per mm	1.04	0.95–1.12
Female age, per year	0.96	0.92–1.00
Duration of subfertility, per year	1.02	0.87–1.21
Primary versus secondary subfertility	1.40	0.93–2.09
Referred by Ob/Gyn versus referred by GP	0.78	0.45–1.35
BMI, per unit	1.04	1.00–1.08
Total motile sperm count, per 10×10^6	1.00	0.98–1.02
Gonadotrophins versus CC	1.27	0.89–1.82
Smoking, yes versus no	1.01	0.64–1.60
Two follicles versus one follicle	1.59	1.10–2.30
Three follicles versus one follicle	1.99	1.24–3.21
Per failed IUI cycle	0.89	0.77–1.03

EMT = endometrial thickness, OR = odds ratio Ob/Gyn: obstetrics/gynaecology

and cancelled cycles, 666 couples remained who underwent 1968 IUI cycles in total.

A total of 330 couples were allocated to gonadotrophins and 336 couples to CC. The baseline characteristics were well balanced between couples that were allocated to gonadotrophins or CC (Table I). In the gonadotrophin treatment arm, 85 women conceived leading to ongoing pregnancy (ongoing pregnancy rate per cycle 8.9%), while 71 women conceived leading to ongoing pregnancy (ongoing pregnancy rate per cycle 7.0%) in the CC treatment arm. In the gonadotrophin treatment arm, the median [interquartile range (IQR)] number of follicles was 1.67 (1) and in the CC treatment arm this was 1.75 (1).

EMT

The mean EMT was 8.9 mm (SD 2.1) in women treated with gonadotrophins and 7.5 mm (SD 2.1) in women treated with CC (adjusted mean difference 1.4 mm; 95% CI: 1.1–1.7). The difference in

Figure 2 The non-linear association between EMT and the estimated chance of ongoing pregnancy. (**A**) The non-linear association between EMT and the estimated chance of ongoing pregnancy after IUI with gonadotrophins. (**B**) The non-linear association between EMT and the estimated chance of ongoing pregnancy after IUI with clomiphene citrate.

EMT over subsequent cycles was on average 0.02 mm per additional cycle (Fig. 1) and ranged from −0.6 to 0.6. This suggests that there are no clear trends of EMT over multiple cycles for the same woman.

In ovarian stimulation with gonadotrophins, there was no statistically significant difference between EMT and ongoing pregnancy (odds ratio (OR): 1.01 per 1 mm increase, 95% CI 0.90–1.13). In ovarian stimulation with CC, there was also no statistically significant difference between EMT and ongoing pregnancy (OR: 1.10 per 1 mm increase, 95% CI 0.99–1.23).

Adjusting for known predictors of pregnancy, such as the stimulation agent, female age, duration of subfertility, primary or secondary subfertility, referring status, BMI, TMSC, smoking status and the growth of two follicles versus one follicle, or three follicles versus one follicle, and for failed IUI cycles, did not change results (Table II).

The non-linear association between EMT and the estimated chance of ongoing pregnancy after IUI with, respectively, gonadotrophins and CC, is shown (Fig. 2a and b). There was no statistically significant association between EMT and ongoing pregnancy in cycles with ovarian stimulation with gonadotrophins, or cycles with ovarian stimulation with CC (Fig. 2).

Discussion

This study demonstrates that in women with unexplained subfertility, undergoing IUI with ovarian stimulation EMT and ongoing pregnancy were not associated, regardless of whether they were treated with gonadotrophins or CC (OR: 1.01 per 1 mm increase, 95% CI 0.90–1.13, and OR 1.10 per 1 mm increase, 95% CI 0.99–1.23, respectively).

A strength of this study is that this analysis is based on the results of the first multicentre RCT randomizing between gonadotrophins and CC in IUI for unexplained subfertility in which EMT is measured. This is the largest study so far that investigated the association between EMT and ongoing pregnancy in women undergoing IUI for unexplained subfertility and that compared two stimulation regimens. A potential limitation of this study is that this is a secondary analysis and should thus be interpreted prudently, as secondary analyses are prone to false-positive findings or could be underpowered to show associations that the study is not primarily set up for. Also, we cannot exclude that a difference of 1.4 mm in EMT is due to interobserver variability or due to differences in equipment. Differences in EMT measurements of 1.5 mm have been reported between experienced and inexperienced transvaginal sonography examiners (Karlsson et al. 1994). The impact of this is most likely to be limited, since our study is based on a multicentre RCT with women from 24 clinics, thereby reflecting daily clinical practice.

We found a thinner endometrium in women who had ovarian stimulation with CC, which can be explained by the anti-estrogenic effect of CC, since endometrium proliferates under the influence of estrogen. In histological studies on the effect of CC on the development of the endometrium, CC had a deleterious effect on the maturity of the endometrium (Yeko et al. 1992; Massai et al. 1993; Unfer et al. 2001). The relation between these histological changes, and the EMT measured by ultrasound has not been clarified yet (Zaidi et al. 1995; Unfer et al 2000). Nevertheless, we feel histology has a very limited impact, since the average EMT was very similar for women who conceived and women who did not conceive and there was no
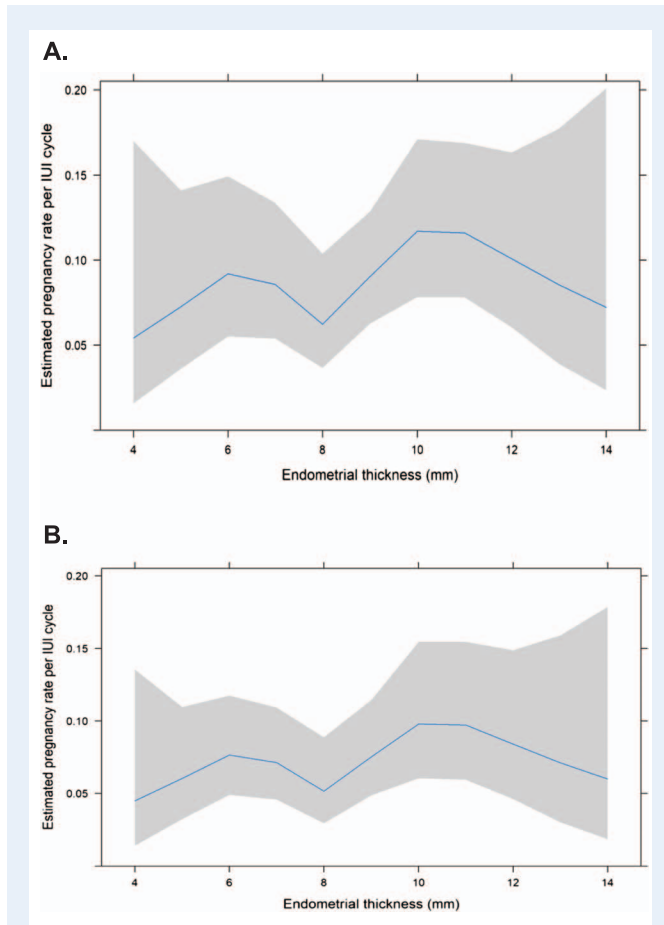
association between EMT and ongoing pregnancy when looking at women allocated to either gonadotrophins or CC. Since the sample size was too small to draw any firm conclusions on EMTs of less than 4 mm or more than 12 mm, uncertainty remains in these cases.

Our data regarding the difference in EMT as a result of ovarian stimulation with either gonadotrophins or CC and the lack of any association with ongoing pregnancy are in agreement with a recently performed systematic review and meta-analysis (CC versus gonadotrophins; two studies, mean difference: −0.33, 95% CI: −0.64 to −0.01) (Weiss et al. 2017). Our study also confirms the results of another systematic review on EMT, and pregnancy rates performed in women with World Health Organization group II anovulation, with respect to a thinner endometrium after CC compared to gonadotrophins (one study, weighted mean differences −0.08, 95% CI −0.89 to 0.73) and no evidence of a significant difference in the association between EMT and pregnancy (one study, RR 0.71, 95% CI 0.23 to 2.15) or live birth (one study, RR 0.85, 95% CI 0.26 to 2.73) (Gadalla et al. 2018). Our findings are in contrast with a systematic review and meta-analysis on EMT and pregnancy rates after IVF, which found that the chance of a clinical pregnancy with an EMT ≤ 7 mm was lower compared to women with an EMT >7 mm (60/258 versus 4981/10354, OR 0.42, 95% CI 0.27 to 0.67) (Kasius et al. 2014). The difference between the association of EMT and pregnancy rates in IUI versus IVF might be explained by the difference in hormone treatment. In IVF, a GnRH agonist or GnRH antagonist is used for downregulation of the menstrual cycle, which might influence the development of endometrial lining. In IUI, it is not common to use downregulation.

In conclusion, CC leads to a significantly thinner endometrium compared to gonadotrophins, but since there was no evidence of a consistent association between EMT and the ongoing pregnancy rate, a relatively thin endometrium after CC is not a valid reason to prefer gonadotrophins as the stimulation agent in IUI for unexplained subfertility.

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Authors' roles

N.D. is responsible for the overall logistical aspects of the study and drafted the paper. M.M., F.v.d.V. and M.W. designed the study. R.v.E. is responsible for the statistical analysis. All authors contributed and approved the final version of the paper.

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Conflict of interest

Prof. Dr B.W.J.M. is supported by a NHMRC Practitioner Fellowship (GNT1082548) and reports consultancy for Merck, ObsEva and Guerbet. The other authors declare no conflicts of interest.

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