Ovulation Induction Intrauterine Insemination: Predictors of Success

Ovulation Induction Intrauterine Insemination: Predictors of Success

By

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I have reviewed this thesis. It represents work done by the author under my guidance/supervision.
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Overview

Infertility, which affects one in eight couples of reproductive age, has become a global health issue (1, 2). Intrauterine insemination (IUI), with or without ovarian stimulation (OS), is a commonly used fertility treatment, where washed sperm is directly placed into a woman's uterus to facilitate conception. As a convenient and minimally invasive procedure, IUI is thereby well-received by both physicians and patients. According to data from the European Society of Human Reproduction and Embryology (ESHRE), more than 155,000 IUI cycles are performed each year in Europe alone (3, 4).

There are many factors proposed that could affect the outcomes of IUI and pregnancy. Some of them are evidenced-based, such as female age, history of gravity/parity, OS regimens, post-washed sperm count, etc.; while the others remain a topic of debate, including body mass index (BMI), endometrial thickness (EMT), certain diagnoses of infertility, etc. (5-7).

To further investigate predictors of IUI success, for project 1, our primary focus was on EMT, in which we evaluated the differences of EMT between different OS regimens (clomiphene vs. gonadotropins) primarily by utilizing women as their own controls. Furthermore, we evaluated the potential association between EMT and cycle outcomes (namely: pregnancy) among the two regimens. For project 2, we investigated the effectiveness of IUI treatments for women with either tubal factor infertility or endometriosis in comparison to women with unexplained infertility.

Project 1: The impact of clomiphene citrate on the endometrium in comparison to gonadotropins in intrauterine-insemination treatments: Is it thinner and does it matter?

Title page

The impact of clomiphene citrate on the endometrium in comparison to gonadotropins in intrauterine-insemination treatments: Is it thinner and does it matter?

Running Title: Endometrial thickness in CC/IUI

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Capsule： Within-patient, clomiphene generally resulted in thinner EMT compared to gonadotropins. Patients who failed to conceive with clomiphene also had a thinner endometrium compared to those who eventually conceived with it. Thinner endometrium was associated with decreased chance of clinical pregnancy in clomiphene cycles, while no such association was detected in gonadotropin cycles.

Abstract

Objective: To determine whether endometrial thickness (EMT) differs between i) clomiphene citrate (CC) and gonadotropin (Gn) utilizing patients as their own controls, and ii) patients who conceived and those who did not while using CC. Furthermore, we evaluated the association between late-follicular EMT and pregnancy outcomes, in CC and Gn cycles.

Design: Retrospective study.

Setting: Academic fertility center.

Patients: To evaluate CC's impact on the endometrium, utilizing women as their own controls, we included in cohort 1 all cycles from women who initially underwent CC/IUI (CC1, n=1252), followed by Gn/IUI (Gn1, n=1307). Furthermore, to evaluate EMT differences between patients who conceived with CC and those who did not, all CC/IUI cycles from women who eventually conceived with CC during the same study period were included in cohort 2 (CC2, n=686).

Intervention(s): CC/IUI or Gn/IUI.

Outcome Measure(s):

Primary: EMT.

Secondary: Clinical pregnancy and spontaneous abortion rates (CPR, and SABR, respectively).

Statistics: CC1 cycles were compared to both Gn1, and CC2 cycles in regards to EMT. In cohort 1, CC1 and Gn1 cycles from the same patient were matched to estimate the within-patient variability of EMT. Generalized linear mixed models (GLMM) and generalized estimating equations (GEE) models were applied to account for multiple cycles from the same patient while controlling for confounders, as appropriate.

Results: When CC1 was compared to Gn1 cycles, EMT was significantly thinner [Median (IQR): 6.8 (5.5-8.0) vs. 8.3 (7.0-10.0) mm, $p<0.001$]. Within-patient, CC1 compared to Gn1 EMT was on average (mean±SD): 1.7±2.1 mm [median (IQR): 1.6 (0.5, 3.0) mm] thinner. GLMM models, adjusted for confounders, revealed similar results (coefficient: 1.69, 95% CI: 1.52-1.85, CC1 as *ref.*).

CC1 compared to CC2 EMT was also thinner both before [Median (IQR): 6.8 (5.5-8.0) vs. 7.2 (6.0-8.9), respectively, $p<0.001$] and after adjustment in GLMM models (coefficient: 0.59, 95% CI: 0.34-0.85, CC1 as *ref.*).

CPRs improved as EMT quartiles increased among CC cycles ($p<0.001$), while no such improvement was observed among Gn cycles ($p=0.94$). GEE models, adjusted for cofounders, suggested a positive association between EMT and CPR in CC cycles (adjOR: 1.12, 95% CI: 1.07-1.18, $p<0.001$) but not in Gn cycles (adjOR: 0.99, 95% CI: 0.92-1.07, $p=0.82$).

Conclusions: Within-patient, overall CC resulted in thinner EMT compared to Gn. Patients who failed to conceive with CC also had a thinner endometrium compared

to those who eventually conceived with CC. Thinner endometrium was associated with decreased CPR in CC cycles, while no such association was detected in Gn cycles.

Keywords: endometrial thickness, ovarian stimulation, clomiphene, gonadotropin, intrauterine insemination

Introduction

Infertility affects 8-15% of reproductive age couples and has become a global health issue (1, 2). Treatments such as ovarian stimulation (OS) with intrauterine insemination (IUI) are simpler, and less expensive than *in vitro* fertilization (IVF). Therefore, OS/IUI is often the recommended first-line treatment for couples with unexplained, ovulatory, and mild male factor infertility (5, 8). As a matter of fact, over 155,000 IUI cycles were performed in 2017 in Europe alone, according to data from the European Society of Human Reproduction and Embryology (ESHRE) (3).

Clomiphene citrate (CC) and gonadotropins (Gn) are frequently used for OS/IUI treatments (9). Both medications, through different mechanisms of action, promote follicular growth. However, CC also has estrogen antagonistic properties on the endometrium, eventually affecting its growth and potentially the ability of an embryo to implant in it (10). Similarly, Gn, by stimulating multi-follicular growth, increase estrogens to, on occasion, supraphysiologic levels, potentially impacting endometrial development and receptivity, as well (10, 11).

Studies evaluating the effect of the different OS regimens on endometrial thickness (EMT) and its potential association with pregnancy outcomes in IUI cycles have been limited and inconclusive. Existing data suggest that a thinner endometrium might be associated with decreased chances of pregnancy in both CC and Gn cycles (12, 13), with some studies reporting no pregnancies with EMT lower

than certain cut-offs (13, 14). A recent retrospective study of 1065 Gn cycles showed that the pregnancy rate was the highest when EMT was in the range of 10.5-13.9 mm, and lowest when EMT was less than 7 mm (15). On the contrary, data from a secondary analysis of the Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) randomized controlled trial (RCT) showed that although EMT was thinner in CC, as compared to Gn cycles, among patients with unexplained infertility, pregnancy rates were not associated with EMT in either group (6). Results from a meta-analysis including various OS regimens also found no evidence of an association between EMT and IUI outcomes (8). The existing studies, albeit compelling, have their own limitations, including either a small sample size, or a focus on a specific infertile diagnosis, such as unexplained infertility. Currently, it remains unclear whether, in the same patient, OS with CC when compared to Gn, produces a late-follicular endometrium that is thinner than that of Gn-stimulated cycles. Furthermore, it is uncertain whether such differences have a consequential effect on OS/IUI pregnancy outcomes.

The present study aimed to determine whether EMT differs between CC/IUI and Gn/IUI cycles primarily by means of utilizing patients as their own controls. Furthermore, we aspired to investigate the potential association, if any, between late-follicular EMT and pregnancy outcomes among different OS regimens in IUI cycles.

Materials and Methods

Study design

This retrospective study was approved by Partners Institutional Review Board. Data from 15980 cycles of 4783 women undergoing IUI between January 2004 and September 2021 at the Massachusetts General Hospital (MGH) Fertility Center were reviewed for eligibility. Exclusion criteria included the diagnosis of uterine factor infertility, and/or severe tubal/peritoneal factor with co-existing, untreated hydrosalpinges. Cycles with no available EMT information at the time of the last ultrasonographic evaluation were also excluded. After application of exclusion criteria two cohorts of women were included in the final analyses.

To evaluate CC's impact on the endometrium in comparison to Gn, utilizing women as their own controls, we included in cohort 1, all cycles from women who sought fertility treatments undergoing initially CC/IUI (CC1, n=1252), followed by Gn/IUI (Gn1, n=1307). Furthermore, to evaluate potential EMT differences between patients who conceived with CC and those who did not, all the CC/IUI cycles from women who eventually conceived with CC during the same study period were included in cohort 2 (CC2, n=686). CC1 cycles were compared to both Gn1, and CC2 cycles in regards to EMT.

IUI protocols

As previously reported, all couples had completed a standard infertility evaluation prior to treatment initiation (16). All women undergoing OS/IUI had at least one patent fallopian tube and partner's sperm had post processing total motile sperm count ≥ 1 million. All patients underwent at least one monitored OS/IUI cycle after receiving CC. However, women in cohort 1, after failing CC/IUI attempts, eventually underwent OS/IUI utilizing gonadotropins. Women in cohort 2 achieved pregnancy with CC/IUI treatments and did not utilize gonadotropins.

The standard starting CC dose was 50 mg, with instructions to take it for 5 days starting on cycle day 2-5 after spontaneous menses or a progestin-induced withdrawal bleeding. Response to CC was monitored by serial transvaginal ultrasonography and monitoring frequency was individualized after mid-follicular phase. Ovulation was triggered with recombinant HCG (Ovidrel, Merck Serono), when at least one dominant follicle reached 16 mm in diameter. CC dose was increased to 100 mg or 150 mg in subsequent cycles either for the indication of no response to the previously administered dose or at physicians' recommendation (usually to increase follicular response). In the rare situation, where patients had an exaggerated follicular response to 50 mg, the dose was decreased to 25 mg in subsequent cycles.

Patients not conceiving with CC/IUI eventually were advanced to Gn/IUI treatments and initiated recombinant follicle stimulating hormone (rFSH) on cycle day 3. Starting dose was individualized based on age, body mass index (BMI),

ovarian reserve biomarkers, and prior response. Follicular development in Gn cycles was monitored by serial transvaginal ultrasonography and serum estradiol (E_2) levels. FSH dose was adjusted, as needed, to achieve multi-follicular response. Ovulation was triggered with Ovidrel when at least one lead follicle reached 16 mm in largest diameter.

Single IUI was performed 35-36 hours after HCG-trigger with either donor or washed partner's sperm by a trained health care professional.

A pregnancy test was performed approximately two weeks after the IUI, with a serum β-HCG level over 6 mIU/mL considered positive. Clinical pregnancy was confirmed, via transvaginal ultrasonography, with the detection of at least one gestational sac at approximately 4 weeks post IUI. Spontaneous abortion (SAB) was defined as the loss of a previous sonographically-confirmed clinical pregnancy.

Outcome Measures

The primary outcome was EMT, as measured and recorded on the last ultrasound (UTZ) before HCG-trigger (the last UTZ was performed for the most part on the day of HCG-trigger, while the remaining either one or two days prior to it). All UTZ were performed by trained health care professionals per routine clinical care. Firstly, in cohort 1, patients were used as their own controls and EMT was compared between CC1 and Gn1 cycles. Secondly, EMT was compared between CC1 and CC2 cycles (the latter including all cycles from women who eventually conceived

with CC). Finally, we evaluated pregnancy outcomes [clinical pregnancy, and spontaneous abortion rates (CPR, and SABR, respectively)] among different EMT quartiles for CC/IUI (CC1 & CC2), and Gn/IUI cycles, separately.

Statistical analysis

Normally distributed continuous variables were expressed as mean ± standard deviation (SD), while non-normally distributed continuous variables as median and interquartile range (IQR). Student's t-test or Mann-Whitney U test were used, as appropriate. Categorical variables were summarized as frequency (n) and percentage (%), and chi-square test or Fisher's exact test were used, as appropriate.

Since the last UTZ was performed on cycle days that varied between cycles, analysis was stratified according to day of last UTZ, where appropriate. Of note, almost half (47.2%) of the late-follicular EMT measurements were taken on the day of HCG-trigger, while approximately one third (36.5%), and one eighth (12.6%) were measured either one or two days prior to HCG-trigger, respectively.

In cohort 1, to estimate the within-patient variability of EMT between CC and Gn cycles, CC and Gn cycles from the same patient were matched by day of last UTZ (i.e.: EMT measured on the same day in relation to HCG-trigger), and the absolute difference of EMT was calculated among each matched cycle pair. In addition, to account for multiple cycles from the same patient, while controlling for potential confounders, generalized linear mixed models (GLMM) were utilized to

estimate potential EMT differences in cohort 1 (CC1 vs. Gn1). The same analytic approach was also utilized to estimate the EMT difference between CC1 and CC2. Results were expressed as coefficient (coeff.) and 95% confidence interval (CI). Generalized estimating equations (GEE) logistic regression models were implemented to investigate the association between EMT and pregnancy outcomes in CC (CC1 & CC2) and Gn cycles, separately. EMT was assessed either as a continuous variable or by quartile increment (Quartiles 1-4: Q1-Q4). Results were expressed as odds ratio (OR) and 95% CI. Models were adjusted for potential confounders (including age, BMI, prior gravity, and day of last UTZ).

A two-sided alpha level of 0.05 was considered statistically significant. SPSS 21.0 (SPSS Inc.) was used for all statistical analyses.

Results

Cohort 1 included a total of 2559 cycles from 556 women that initially underwent 1252 CC/IUI (CC1), subsequently followed by 1307 Gn/IUI cycles (Gn1). The CC2 cohort included a total of 686 CC/IUI cycles from 321 women that eventually conceived with CC treatments (*Fig. 1*). Characteristics of the study population are summarized in *Table 1*. As shown, the majority of women were Caucasian, and the most common diagnosis was unexplained infertility.

In cohort 1, follicular response, as assessed by total number of follicles ≥ 13

mm, did not differ clinically between CC1 and Gn1 cycles. However, HCG-trigger was on average one day later in CC1 compared to Gn1 cycles. Despite longer duration of the follicular phase in CC1 cycles, and clinically comparable follicular response, EMT in CC1 cycles was significantly thinner than that of Gn1 (6.8 vs. 8.3 mm, for CC1 vs. Gn1, $p<0.001$), a finding that was consistent and independent of the day of last UTZ (*Table 1, and Fig. 2A-C*). In addition, 46.4% of CC1 cycles had an EMT < 7 mm on the day of HCG-trigger, while in Gn1 cycles only 14.6% were below the same cut-off (*Fig. 2A*). A similar EMT distribution was noted for UTZs performed one or two days prior to HCG-trigger (*Fig. 2B-2C*).

Subsequently, CC1 cycles from the same patient were matched to Gn1 cycles based on day of last UTZ. Among the 556 patients in cohort 1, N_0=259, N_1=121, and N_2=16 CC1-Gn1 cycle matches were created based on timing of last UTZ in relation to HCG-trigger that were on the day of, one day, or two days prior to it, respectively. Mean±SD EMT difference between Gn1 and CC1 was 1.7±2.1 mm [median (IQR): 1.6 (0.5, 3.0)]. More specifically, EMT differences between Gn1 and CC1 cycles were 1.8±2.2 mm [median (IQR): 2.0 (0.5, 3.2)], 1.4±2.0 mm [median (IQR): 1.5 (0.4-2.7)], and 1.2±1.8 mm [median (IQR): 0.6 (-0.1, 1.4)] for UTZs performed on day of, one-day prior, and two-days prior to HCG-trigger, respectively.

Furthermore, a GLMM model was applied in cohort 1 to account for multiple cycles from the same patient adjusting for age, BMI, prior gravity, and day of last UTZ (*Table 2*). Overall, EMT in CC1 was significantly thinner as compared to Gn1

by 1.69 mm (coeff.: 1.69, 95% CI: 1.52-1.85, p<0.001).

CC1 women when compared to CC2, were older, with both a lower BMI and gravity/parity. Overall, EMT in CC1 cycles was significantly thinner than that of CC2 (6.8 vs. 7.2 mm, for CC1 vs. CC2, p<0.001), a finding that was independent of the day the last UTZ. Unlike CC1 cycles where 46.4% of the cycles had an EMT < 7 mm on the day of HCG-trigger, less CC2 cycles (36.2%) had an EMT below the same cut-off (*Fig. 2A*). GLMM models adjusted for age, BMI, prior gravity, and day of last UTZ suggested that CC1 EMT was 0.59 mm thinner than CC2 cycles (coeff.: 0.59, 95% CI: 0.34-0.85, p<0.001).

Pregnancy outcomes were then compared amongst EMT quartiles (Q1-Q4) in CC and Gn cycles, separately (*Fig. 2A-2B*). Among CC cycles, CPRs improved as EMT quartiles increased (15.7%, 16.3%, 21.2%, 27.5%, for Q1, Q2, Q3, and Q4, respectively, p<0.001 for all comparisons), while SABRs were similar amongst the different EMT quartiles (13.9%, 7.1%, 18.2%, 13.8%, for Q1, Q2, Q3, and Q4, respectively, p=0.22 for all comparisons). Interestingly, in Gn cycles, both CPRs and SABRs were comparable amongst different quartile groups (CPR for Q1-Q4, respectively, were: 12.0%, 14.9%, 12.8%, and 13.5%, p=0.94 for all comparisons; while SABRs were: 5.6%, 8.5%, 20.0%, and 13.7%, p=0.21 for all comparisons; for Q1-Q4, respectively).

Additionally, although most pregnancies were observed in cycles with EMT ≥ 25^{th} pct., clinical pregnancies were seen even with an EMT < 5^{th} pct. for both CC

and Gn cycles (5^{th} pct. cut-offs: 4.5, and 6 mm on the day of HCG-trigger, for CC and Gn, respectively). Among CC cycles, CPR below the 5^{th} pct. were significantly lower than those observed above the 5^{th} pct. (4.0% vs. 20.5%, for $< 5^{th}$ and $\geq 5^{th}$ pct., respectively, p=0.003). We noted no such difference in CPR among Gn cycles (9.5% vs. 13.4%, for $< 5^{th}$ and $\geq 5^{th}$ pct., respectively, p=0.59).

GEE models adjusted for age, BMI, prior gravity, and day of last UTZ suggested that in CC cycles, EMT (assessed as a continuous variable) was positively associated with CPR (adjOR: 1.12, 95%CI: 1.07-1.18, p<0.001) (*Table 3*). The odds of clinical pregnancy increased by 12% for each mm increase in EMT. Additionally, the odds of clinical pregnancy were significantly increased in EMT Q3 and Q4, compared to Q1 (adjOR: 1.46, 95% CI: 1.06-2.01, p=0.02; adjOR: 2.02, 95% CI: 1.48-2.77, p<0.001; for Q3, and Q4 vs. Q1, respectively), and cycles in EMT Q4 had 2.02 times the odds of resulting in clinical pregnancy compared to those in Q1. On the contrary, no significant associations with CPR were observed in Gn cycles, neither when EMT was analyzed as a continuous variable nor as quartiles.

Discussion

Our study investigated potential differences in endometrial thickness between CC/IUI and Gn/IUI cycles, and the impact these differences might have on IUI outcomes. When patients were utilized as their own controls, our data suggested that

the endometrium was significantly thinner in CC compared to Gn cycles, despite a clinically comparable follicular response. In late-follicular phase, a remarkable percentage of CC cycles (around 40%) had an EMT < 7 mm, a cut-off considered by many to negatively affect chances of clinical pregnancy (17). As expected, in CC cycles a thinner endometrium was associated with decreased CPR in our study population. However, no such association was observed in Gn cycles. This finding implies that the two medications may be impacting the endometrium in different ways, and in the case of CC through additional mechanisms that are not directly involved to the thickness of the endometrium.

Within the same patient, our results suggested that ovarian response, as assessed by total number of follicles ≥ 13 mm, was clinically similar between CC and Gn cycles, a finding that could be translated to comparable serum estrogen levels between regimens. However, CC stimulation still resulted in a much thinner late-follicular EMT than gonadotropins, which could provide further evidence for the anti-estrogenic effect of CC on the endometrium (18). Our results indicated that within the same patient, the EMT after gonadotropin is thicker than CC stimulation by an average of 1.7 mm. Similarly, Weiss et al. in a meta-analysis reported a thicker endometrium in Gn compared to CC cycles, but the difference appeared less prominent [mean 0.33 mm (95% CI: 0.01-0.64)] (8). Studies included in the meta-analysis differed from ours in dosing of CC (only 100 mg dose regimens were utilized), diagnoses of infertility (unexplained and mild male factor only), and study

design (19, 20).

The impact of endometrial thickness on the pregnancy outcomes is still unclear and determining its clinical relevance in the fertility setting remains challenging. While a clear cut-off defining “thin” endometrium does not exist, in most studies late-follicular phase endometrium measuring less than 7 mm or 8 mm is considered to be “thin” (13). In IVF cycles, where estrogen levels are much higher and the only ovarian stimulation medications used are gonadotropins, thin endometrium, defined as less than 7 mm, is rather rare and its reported incidence varies from 1% to 2.5% (17). However, relevant data is lacking in OS/IUI cycles. Our study showed that around 40% of CC cycles had a late-follicular EMT < 7mm, while in Gn cycles only 15% were below the same cut-off. Similarly, a recent RCT reported that 45% of CC cycles had EMT ≤ 7 mm among women with a history of six failed cycles (21).

Studies evaluating the impact of a thinner endometrium on pregnancy outcomes have been inconsistent, with a few reporting that it is associated with lower pregnancy rates (22, 23), while others not (8). A retrospective study reporting on a much smaller sample of CC/IUI cycles reported that pregnancy rates did not differ substantially between EMT strata and concluded that treatment decisions regarding switching from CC to other regimens should not be influenced by the thickness of the endometrium (24). On the contrary, a recent RCT on women with a history of six failed ovulatory CC cycles reported higher live birth rates when CC was switched to Gn among subjects with EMT ≤ 7 mm in the last CC cycle. No such benefit was

reported for those who developed an “appropriately thick” endometrium with CC (EMT >7 mm) (21). In our study, pregnancies were observed even with endometria below the 5th percentile, albeit at significantly lower rates. This finding suggests a negative, but not deleterious, impact of thin endometrium on CPR among CC cycles. Our finding indicates that women developing a particularly thin endometrium following CC administration might benefit from switching to Gn.

Interestingly, in Gn cycles our data did not suggest an association between CPR and EMT. This finding is in agreement with a recent secondary analysis of the AMIGOS trial, showing no differences in EMTs between Gn/IUI cycles that led to live birth and those did not (6). Similarly, Liu et al. in a retrospective study also showed that EMT did not predict clinical pregnancy in Gn/IUI cycles (adjOR: 1.63, 95% CI: 0.71-3.77) (15).

The fact that a thinner EMT negatively impacted CPR in CC cycles but not in Gn cycles suggests that the mechanisms mediating such action are not limited to the development of a thin endometrium but might involve additional factors. Hsu et al. reported that compared to unstimulated natural cycles, CC significantly decreased uterine blood flow during the early luteal phase, potentially impairing implantation thus contributing to lower pregnancy rates (25). The significantly higher incidence of thin endometrium in CC cycles as compared to Gn and its potential effect on pregnancy rates suggests that the OS regimen should be taken into account in the definition and clinical management of thin endometrium in IUI cycles.

To the best of our knowledge, our study was the first to evaluate EMT using patients as their own controls, with obvious benefit of minimizing the impact of potential confounders and allowing for the estimate of within-patient variability. The inclusion of cycle characteristics allows us to gain a better insight in the mechanisms responsible for the observed differences (e.g. follicular response being clinically comparable between CC and gonadotropin stimulation, in part because of the mild gonadotropin stimulation protocols used in our practice). In addition, laboratory and clinical protocols were consistent in all cycles since they were all conducted within one hospital system. However, several limitations should also be taken into consideration. First, selection bias could be introduced due to the retrospective nature of the study; also there might be possibility of residual confounding as information regarding other potential confounders such as life style and relevant comorbidities was not known for the most part. Second, the CPRs appeared to be higher in CC cycles, primarily because not all the failed CC cycles during the study period were included in the analysis (patients lost to follow up, or switching to IVF were not included). However, the CPRs were only calculated to allow for an intuitive comparison amongst EMT quartiles, while the association between EMT and CPR were further evaluated by GEE logistic regression, where the results remained similar both before and after adjustment. Third, although no association between EMT and CPR was found among Gn cycles in our study population, results might not be generalizable to other populations that have not failed CC prior to

initiating gonadotropin treatments. Fourth, women included in the present study were mostly Caucasian, which could be another factor that limits the generalizability of our result. Additionally, there could be ascertainment bias in this study, as UTZs were performed by different health care professionals and blinding was lacking. Therefore, further prospective large scale cohort studies are still warranted to evaluate the impact of EMT on pregnancy and IUI outcomes.

To conclude, our study showed that CC stimulation resulted in a thinner endometrium compared to Gn; and within-patient, the EMT was thinner in CC cycles by an average of 1.7 mm. Patients who conceived with CC had a thicker endometrium compared to those who failed and had to eventually pursue gonadotropin treatments. In CC cycles, a thinner endometrium was associated with decreased CPR, while in Gn cycles, no such association was observed. However, clinical implications of these findings and whether or not this should affect patient counseling is a topic for further discussion. Future research should focus on establishing the cut-off for thin endometrium among different ovulation stimulation regimens, and its impact on IUI outcomes.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

Y.L. and I.S. were involved in study concept and design. I.S. and P.C. were involved in data collection. Y.L. and K.J. analyzed data. Y.L., K.J., P.C., and I.S. were involved in the interpretation of the data. Y.L. drafted the manuscript, and K.J., I.S., and P.C. critically revised it. C.B. and I.D. reviewed the final manuscript and provided comments. All authors reviewed and approved the final manuscript.

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Tables and figures

Table 1. Baseline characteristics (by patient); Cycle response and endometrial thickness (by cycle).

	Cohort 1		Cohort 2	p-value
	CC1	Gn1	CC2	(CC1 vs. CC2)
Baseline characteristics				
No. of patients	556		321	
Age (years)	33.5 ±4.1		32.9 ±3.5	0.01
BMI (kg/m^2)	23.1 (21.0-26.3)		23.7 (21.6-27.9)	0.02
Basal FSH (IU/L)	7.0 ±2.3		6.7 ±2.2	0.13
Ethnicity n (%)				0.09
Caucasian	430 (77.3)		232 (72.3)	
Other	126 (22.7)		89 (27.7)	
Prior gravity n (%)	186 (33.5)		135 (42.1)	0.01
Prior parity n (%)	97 (17.4)		74 (23.1)	0.05
Diagnosis n (%)				0.22
Unexplained	226 (40.6)		120 (37.3)	
Ovulatory dysfunction	124 (22.3)		88 (27.3)	
Male	69 (12.5)		40 (12.5)	
Diminished ovarian reserve	31 (5.6)		8 (2.5)	
Tubal/Peritoneal	14 (2.5)		6 (1.9)	
Combined factors	71 (12.7)		41 (12.9)	
Single Mothers/Same Sex	18 (3.3)		14 (4.4)	
Other	3 (0.5)		4 (1.3)	
Ovarian response				
No. of cycles	1252	1307	686	
No. of follicles ≥15mm [a]	1.0 (1.0-2.0)	1.0 (1.0-1.0)	1.0 (1.0-2.0)	0.25
No. of follicles ≥13mm [b]	2.0 (1.0-2.0)	2.0 (1.0-2.0)	2.0 (1.0-2.0)	0.35
Cycle trigger day [a]	12.0 (11.0-14.0)	11.0 (9.0-13.0)	12.0 (11.0-14.0)	<0.001
Day of last UTZ [a]				<0.001
Day of HCG-trigger	662 (52.9)	547 (41.9)	337 (49.1)	
One-day prior to HCG-trigger	319 (25.5)	614 (47.0)	230 (33.5)	
Two-days prior to HCG-trigger	216 (17.3)	107 (8.2)	107 (15.6)	
Endometrial thickness [a]	6.8 (5.5-8.0)	8.3 (7.0-10.0)	7.2 (6.0-8.9)	<0.001
Day of HCG-trigger [a]	7.0 (5.7-8.3)	8.9 (7.4-10.0)	7.5 (6.2-9.0)	<0.001
One-day prior to HCG-trigger [a]	6.5 (5.5-8.0)	8.0 (7.0-9.9)	7.1 (6.0-8.7)	<0.001
Two-days prior to HCG-trigger [a]	6.5 (5.5-7.9)	8.0 (7.0-9.4)	7.0 (5.7-8.2)	0.07

CC = clomiphene; Gn = gonadotropin; BMI = body mass index; FSH = follicle stimulating hormone; UTZ = ultrasound.

Data are shown as mean ± standard deviation (SD) if normally distributed or median and interquartile range (IQR: 25th–75th) if non-normally distributed or number (percentage).

[a] represents $p<0.001$ when CC1 vs. Gn1; [b] represents $p<0.01$ when CC1 vs. Gn1.

Table 2. Multivariate regression models for endometrial thickness

EMT[1]	**Crude**			**Adjusted**[2]		
	Coefficient	95% CI	p-value	Coefficient	95% CI	p-value
CC1 vs. Gn1						
CC1	Ref.			Ref.		
Gn1	1.67	1.51-1.83	<0.001	1.69	1.52-1.85	<0.001
CC1 vs. CC2						
CC1	Ref.			Ref.		
CC2	0.67	0.41-0.93	<0.001	0.59	0.34-0.85	<0.001

EMT = endometrial thickness; CC = clomiphene; Gn = gonadotropin; CI = confidence interval.

[1] Generalized linear mixed models were applied.

[2] Adjusted for age, BMI, prior gravity and day of last ultrasound.

Table 3. Association between endometrial thickness and chances of clinical pregnancy

Clinical pregnancy[1]	**Crude**			**Adjusted**[2]		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
CC cycles[3]						
EMT[4]	1.12	1.07-1.17	<0.001	1.12	1.07-1.18	<0.001
Q1	Ref.			Ref.		
Q2	1.05	0.74-1.49	0.79	1.04	0.77-1.48	0.82
Q3	1.45	1.05-1.99	0.02	1.46	1.06-2.01	0.02
Q4	2.04	1.50-2.78	<0.001	2.02	1.48-2.77	<0.001
Gn cycles						
EMT[4]	1.00	0.93-1.08	0.94	0.99	0.92-1.07	0.82
Q1	Ref.			Ref.		
Q2	1.26	0.81-1.96	0.30	1.39	0.88-2.19	0.16
Q3	1.05	0.65-1.72	0.84	1.14	0.68-1.91	0.63
Q4	1.13	0.72-1.77	0.60	1.11	0.69-1.78	0.67

CC = clomiphene; Gn = gonadotropin; EMT = endometrial thickness; CI = confidence interval.

[1] Generalized estimating equations logistic regression models were applied. EMT was assessed either as a continuous variable or by quartile increment (Q1-Q4).

[2] Adjusted for age, BMI, prior gravity and day of last ultrasound.

[3] CC cycles included all cycles in CC1 and CC2

[4] EMT assessed as a continuous variable.

Figure 1. Flow chart of the inclusion and exclusion criteria

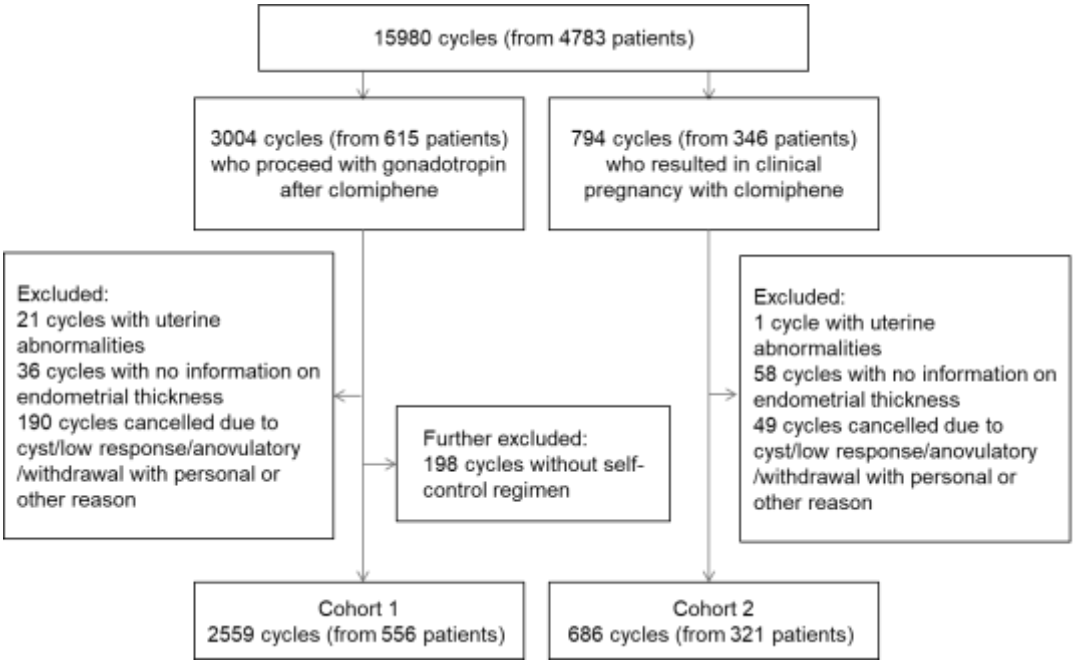
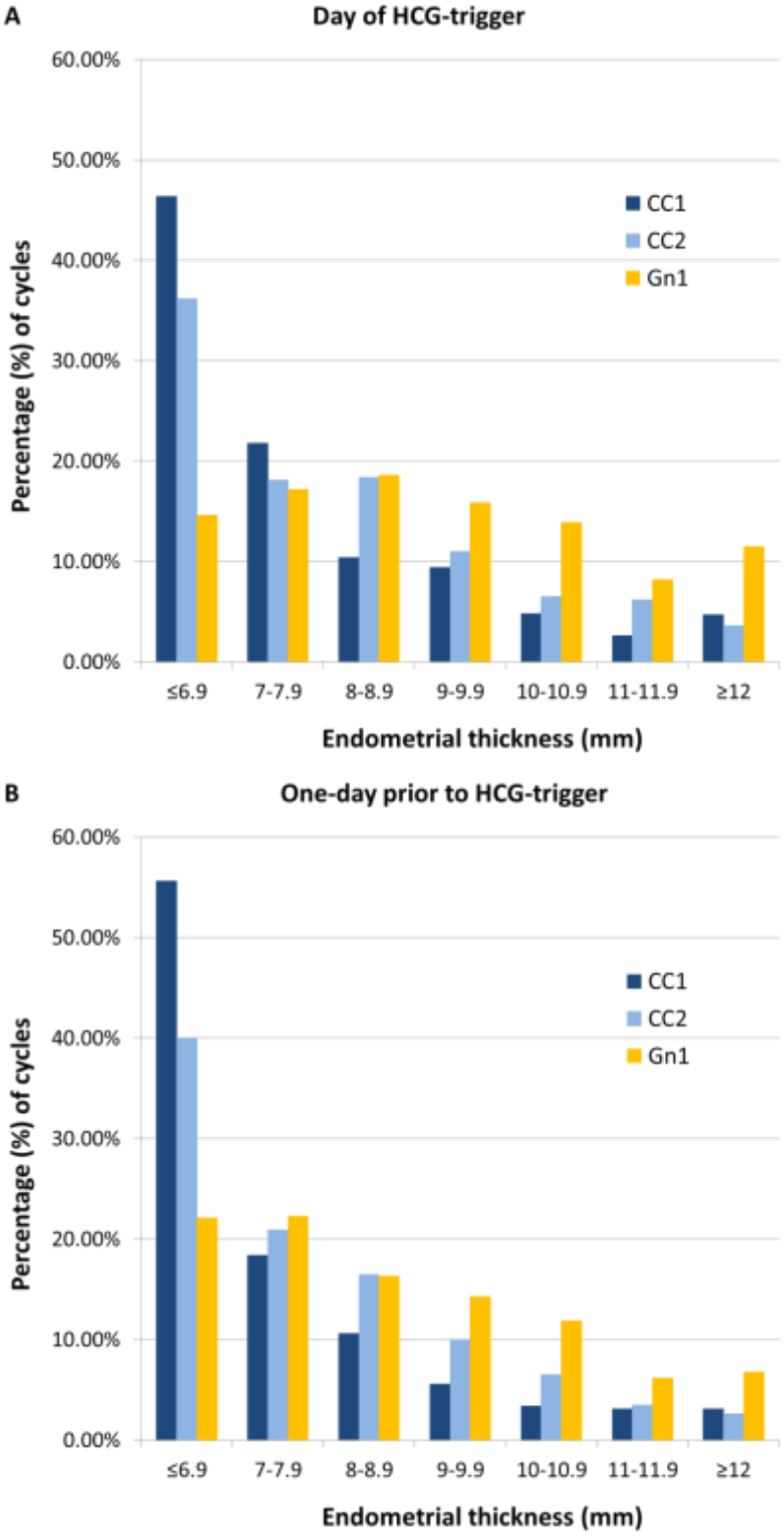
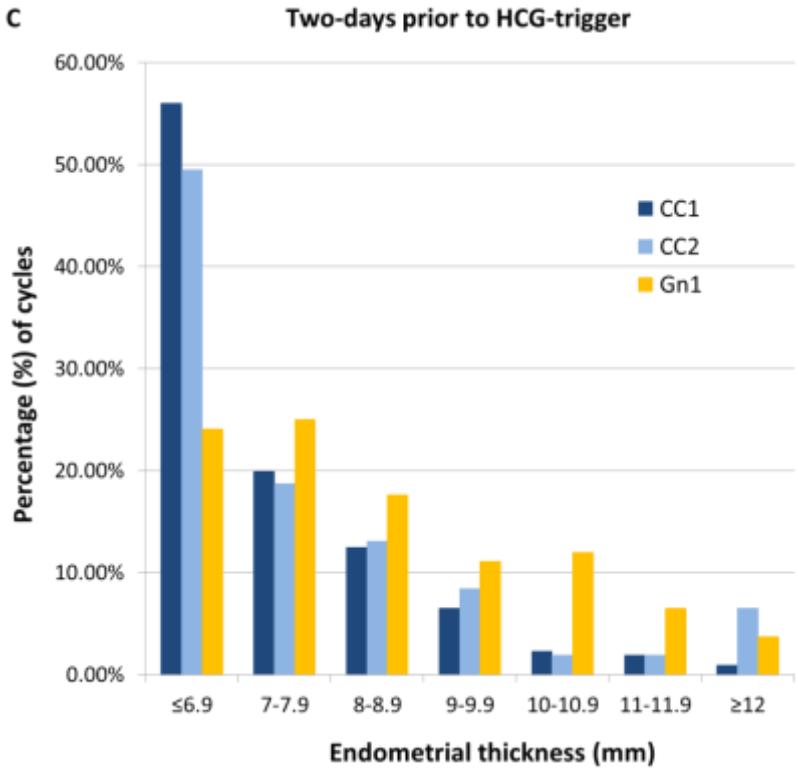


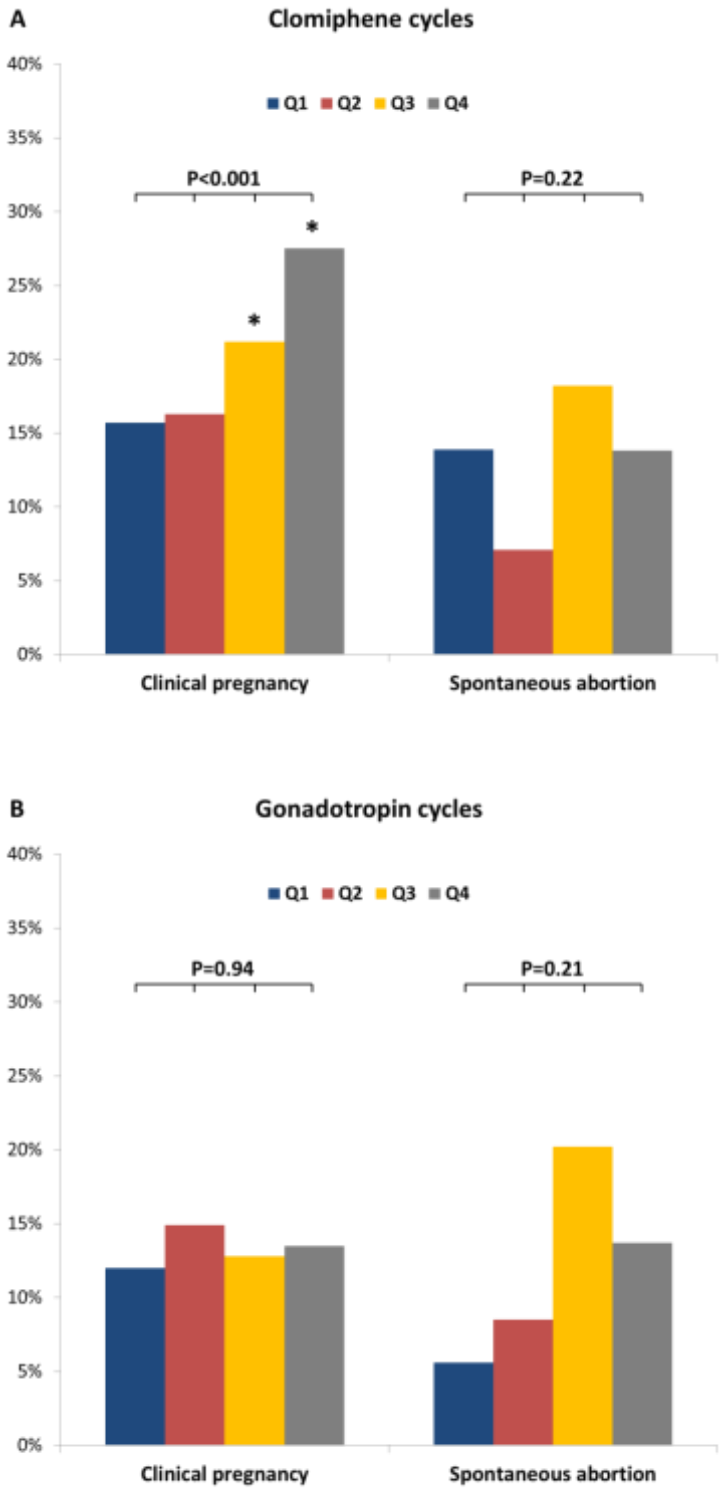
Figure 2. Distribution of endometrial thickness stratified by day of last ultrasound





CC = clomiphene; Gn = gonadotropin.

Figure 3. Pregnancy outcomes according to quartiles of endometrial thickness



Q1- Q4 represents quartiles of endometrial thickness. * represents p<0.05 compared to Q1.

Project 2: The effectiveness of intrauterine insemination treatments in women with overt or “at risk” for tubal-factor infertility

Title page

The effectiveness of intrauterine insemination treatments in women with overt or "at risk" for tubal-factor infertility

Running Title: Tubal/Peritoneal Infertility IUI outcomes

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Capsule: Overt tubal factor infertility was associated with impaired IUI outcomes, namely increased ectopic and decreased ongoing pregnancy rate as compared to unexplained infertility, whereas our results do not suggest such associations for women “at-risk” for tubal factor such as those with endometriosis.

Abstract

Objective: To investigate the effectiveness of intrauterine insemination (IUI) for women with “overt” tubal factor (TF) infertility or “subtle” TF, such as those with endometriosis, in comparison to those with unexplained infertility.

Design: Retrospective cohort study.

Setting: Academic fertility center.

Patients: Women who underwent IUI cycles due to tubal factor infertility (TF, 269 cycles from 105 women), endometriosis (ENDO, 242 cycles from 87 women), or unexplained infertility (UE, 4102 cycles from 1433 women) between January 2004 and October 2021 were included.

Intervention(s): IUI with or without ovarian stimulation (OS).

Main Outcome Measure(s): The primary outcome was ongoing pregnancy rate (OPR). Secondary outcomes included positive HCG rate, clinical, multiple, and ectopic pregnancy rates, as well as rate of spontaneous abortion (CPR, MPR, EcPR, and SABR, respectively).

Results: While CPRs were similar among the three groups (TF: 10.0%, ENDO: 10.3%, and UE: 12.6%, p=0.30 for all comparisons), TF had 8.17 times the risk for EcPR compared to UE group (TF: 11.1% vs. UE: 1.4%, p=0.01; RR: 8.17, 95% CI: 2.24-29.87, UE: *ref.*). While OPRs per initiated cycle were comparable (p=0.12), OPRs per identified clinical pregnancy were lowest among patients with TF (TF:

63.0%, ENDO: 92.0%, UE: 80.8%, p=0.03 for all comparisons). After adjusting for age, BMI, basal FSH, prior parity, OS regimen, and total progressive motile sperm count, results showed that cycles in TF group had a 47% lower chance for ongoing pregnancy compared to those with UE (adjOR: 0.53, 95% CI: 0.31-0.91, p=0.02), while no such association was observed in ENDO compared to UE. Interestingly, although cumulative OPRs after 3 or 4 IUI cycles were lowest in TF group, the differences among groups did not reach statistical significance (p=0.18 and 0.08, for 3 and 4 cycles, respectively).

Conclusions:

Overt tubal factor infertility seemed to be associated with impaired IUI outcomes with regard to increased EcPR and decreased OPR as compared to unexplained infertility, whereas our results do not suggest such associations for women “at-risk” for TF such as those with endometriosis.

Keywords: intrauterine insemination, tubal factor, endometriosis, unexplained infertility, pregnancy outcome

Introduction

Intrauterine insemination (IUI) is a widely-used procedure for many women seeking fertility treatment all around the world, as it is simpler, more convenient, and less costly than *in vitro* fertilization (IVF) (26, 27). Despite the fact that there are many indications for IUI, efficacy varies depending on the cause of infertility (7). While IUI has been proved to be effective and recommended as the first-line treatment for infertile couples with unexplained infertility and mild male factor, it still remains a topic of debate whether it should be considered as an effective treatment for women with tubal factor infertility or mild endometriosis (2, 5, 9).

Tubal factor is one of the most common causes for infertility, which accounts for 11-67% of the cases depending on the population studied (2, 28, 29). The prevalence of tubal factor infertility is still increasing, primarily due to the rising cases of pelvic inflammatory disease and sexually transmitted infections (30). Women diagnosed with bilateral tubal occlusion are advised to proceed with either IVF where resources are available, or surgical intervention if younger and wishing to avoid IVF. Although there is no consensus on treatment recommendations for women with unilateral tubal occlusion (UTO), IUI is considered to be a reasonable initial approach, when at least one fallopian tube is patent, to avoid more invasive and costly treatments such as IVF and surgery (31). Several studies in the IUI setting showed that women with UTO had similar pregnancy rates compared to unexplained

infertility (31-33); whereas a recent retrospective cohort reported decreased clinical pregnancy rates following IUI cycles in UTO patients when compared to those with unexplained infertility (34). However, all of these studies were limited either by sample size or lack of information on ectopic pregnancy, which is regarded as a particular concern in the setting of tubal factor infertility, due to its associated morbidity and mortality.

Endometriosis, characterized by growth of functional endometrial-like tissue outside of the uterus, is another common gynecological condition often associated with infertility (35). It is estimated that approximately 30-50% of women with endometriosis have infertility, while endometriosis accounts for 8-35% of female infertility (36-38). Endometriosis could have an impact on female fertility through mechanisms acting on different levels. Small endometrial-like tissue implants on the surface of fallopian tubes, and endometriosis-related chronic inflammation induces a toxic pelvic environment, both of which could in turn affect the fallopian tube (35). As such, women with endometriosis are often considered to be “at risk” for tubal factor, despite sometimes subtle tubal damage associated with endometriosis may not be visible at hysterosalpingography (HSG) (39, 40). Additionally, ovarian endometriomas could result in decreased ovarian function or even diminished response to ovarian stimulation (OS) (37). Nevertheless, IUI is considered to be an effective treatment for women with minimal to mild endometriosis according to the 2014 guidelines of European Society of Human Reproduction and Embryology

(ESHRE) that were based on evidence showing that pregnancy rates following IUI in endometriosis patients were similar to those with unexplained infertility (41, 42). On the contrary, the 2019 *Endometriosis Treatment Italian Club (ETIC) position statement* recommended against the use of IUI in endometriosis patients independent of disease stage, arguing that existing studies recommending its use were of low quality and with conflicting results (43).

Therefore, the goal of the present study was to investigate the effectiveness of IUI treatments for women with either tubal factor infertility or endometriosis in comparison to women with unexplained infertility.

Materials and methods

Study design

The study was approved by Massachusetts General Hospital (MGH) Partners Healthcare Institutional Review Board. Data from all IUI cycles performed at the MGH Fertility Center between January 2004 and October 2021 were retrospectively reviewed. In this time period, 1641 women with either unexplained, tubal or endometriosis related infertility underwent 5155 IUI cycles and data were reviewed to determine eligibility. Cycles lacking information on pregnancy outcomes and those cancelled after cycle initiation were excluded.

Patients were classified into three groups. Patients were classified as having

tubal factor (TF) infertility when either UTO was diagnosed by HSG or history was significant for either a prior ectopic pregnancy and/or unilateral salpingectomy. The endometriosis (ENDO) group consisted of patients who had been diagnosed with endometriosis, either by laparoscopy, or by imaging modalities, such as transvaginal sonography (identifying an ovarian endometrioma) and/or magnetic resonance imaging (MRI). The control group consisted of women with unexplained infertility (UE). In the latter group couples had normal ovarian reserve and semen analysis parameters, and bilateral tubal patency documented by HSG.

IUI protocols

As previously reported, all couples had completed a standard infertility evaluation prior to the initiation of IUI treatments (16). Decisions regarding OS regimen, if any used, were made following a comprehensive patient consultation and were based on patient's preference and clinician's recommendation. IUI cycles were either natural or following administration of oral ovulation inducing agents (clomiphene citrate or letrozole) or injectable ovarian stimulation regimens (i.e.: gonadotropins). During natural cycles, patients were most often monitored by transvaginal ultrasonography and ovulation was triggered with recombinant HCG (r-HCG, Ovidrel, Merck Serono), or less frequently monitored by urinary luteinizing hormone (LH) kits. Regarding clomiphene (CC) and letrozole (LTZ) cycles, the usual starting dose was 50 mg and 2.5 mg, respectively, with instructions to take the medications for 5 days

starting from cycle day 2-5 post spontaneous menstruation or progesterone-induced withdrawal bleeding. Gonadotropin stimulation was initiated on cycle day 3 and initial dose was determined based on patient's age, body mass index (BMI), ovarian reserve biomarkers, and prior response, when available. Ovarian response was monitored via transvaginal ultrasonography and serum estradiol (E_2) measurements. Dose and monitoring frequency were then adjusted according to it. When at least one dominant follicle reached 16 mm diameter, ovulation was triggered with Ovidrel. Single IUI was performed with either washed partner's sperm or donor sperm within 24 hours from the LH surge or 35-36 hours after trigger.

Serum β-HCG level was used to evaluate the outcome approximately two weeks after the insemination, with a level higher than 6 mIU/mL considered positive. A pregnancy was considered clinical once a gestational sac was visualized via transvaginal ultrasonography at approximately 4 weeks after the IUI procedure, including both intrauterine and extrauterine pregnancies. An ectopic pregnancy was diagnosed by transvaginal ultrasonography, with a gestational sac detected at any site other than the endometrial lining of the uterine cavity. A pregnancy was considered ongoing if continuing beyond 12 weeks of gestation.

Measured Outcomes

The primary outcome was ongoing pregnancy rate (OPR). Secondary outcomes included positive HCG rate, clinical (CPR), multiple (MPR), and ectopic pregnancy

rate (EcPR), as well as spontaneous abortion rate (SABR).

Statistical analysis

Normally distributed continuous variables were expressed as mean ± standard deviation (SD), while non-normally distributed continuous variables as median and interquartile range (IQR). Analysis of variance (ANOVA) or Kruskal-Wallis test were implemented for statistical analysis accordingly. Categorical variables were summarized as frequency (n) and percentage (%) and the distribution were compared by chi-square or Fisher's exact test. Generalized estimating equations (GEE) with an exchangeable working correlation structure were utilized to account for multiple cycles, while multivariate logistic regression models were used to control for potential confounders including age, BMI, basal FSH, prior parity, OS regimen, and total progressive motile sperm count. Results were expressed as odds ratio (OR) and 95% confidence interval (CI). The risk ratio (RR) and 95% CI for the incidence of ectopic pregnancy was also calculated. Kaplan-Meier curves were calculated for the cumulative ongoing pregnancy events and were compared using the log-rank test. A two-sided alpha level of 0.05 was considered statistically significant. SPSS 21.0 (SPSS Inc.) was used for statistical analyses.

Results

Overall, 269 cycles from 105 patients in the TF, 242 cycles from 87 patients in the ENDO, and 4102 cycles from 1433 patients in the UE group were included in our analysis (*Fig. 1*). Patient demographics and cycle characteristics are summarized in *Table 1*. There were no differences in age, BMI, basal FSH, ethnicity, prior gravity and parity among groups.

As expected, more cycles in TF and ENDO groups utilized gonadotropins for OS as compared to the UE group ($p<0.001$). While the number of follicles ≥13mm, number of follicles ≥15mm, and day of trigger were similar among groups, endometrial thickness was thicker in the ENDO group compared to UE (9.0 vs. 8.2 mm, $p<0.001$).

Positive HCG rate, CPR, SABR, and MPR were all similar among groups (*Table 2*). Incidence of ectopic pregnancy was significantly higher in the TF group as compared to UE group, both per identified clinical pregnancy (11.1% vs. 1.4%, $p=0.01$), and per patient (2.9% vs. 0.5%, $p=0.03$). After confirmation of clinical pregnancy, a cycle in the TF group had 8.17 times the risk of resulting in ectopic pregnancy as compared to the UE group (RR: 8.17, 95% CI: 2.24-29.87); while overall, women in the TF group had 5.85 times the risk of having an ectopic pregnancy compared to those in the UE group (RR: 5.85, 95%CI: 1.54-22.29).

While conception rates between cycles were comparable, OPRs per identified

clinical pregnancy were lowest among patients with TF (63.0% vs. 92.0% vs. 80.8%, for TF vs. ENDO vs. UE, respectively, p=0.03) (*Fig. 2*). After adjusting for age, BMI, basal FSH, prior parity, OS regimen, and total progressive motile sperm count *(Table 3)*, results showed that cycles in TF had a 47% lower chance for ongoing pregnancy compared to those with UE (adjOR: 0.53, 95% CI: 0.31-0.91, p=0.02), while no such association was observed for those with ENDO.

Interestingly, the cumulative probability for achieving an ongoing pregnancy after 3 or 4 cycles were also lowest in the TF group, but the differences among groups did not reach statistical significance (p=0.18 and 0.08, for 3 and 4 cycles, respectively). *Fig. 3* illustrates the results of Kaplan-Meier analysis for cumulative OPRs among groups. Log-rank test also showed no significant differences in cumulative OPRs among groups (p=0.30 and 0.17, for 3 and 4 cycles, respectively).

Discussion

Our study investigated the potential differences in IUI outcomes among women with tubal factor infertility, endometriosis, and unexplained infertility. Our results suggested that women undergoing IUI cycles due to tubal factor infertility were at a significantly increased risk of ectopic pregnancy and had lower chances to achieve an ongoing pregnancy, whereas no such associations were observed for women with endometriosis. These results are important to provide evidence-based guidance for

patient counseling and fertility treatments in women with these diagnoses.

There have been several studies evaluating the efficacy of IUI in women with tubal factor infertility. A meta-analysis conducted in 2018 by Tan et al. included 10 cohort studies and showed that no significant difference was observed in CPR (OR: 0.88, 95%CI 0.69-1.12) between patients with UTO and unexplained infertility, which is consistent with our finding (44). However, in our study in spite of similar CPRs, patients with tubal factor infertility had 5.85 times the risk of ectopic pregnancy, which translated to decreased OPR compared to patients with unexplained infertility. A recent retrospective cohort study published in 2020 including a total of 148 patients also demonstrated that women with UTO seemed to have lower live birth rate (LBR) compared to those with unexplained infertility (10.3% vs. 20.0%, p=0.096) (34).

As for patients with endometriosis, we included them because they are often considered to be “at risk” for, or have “functional” tubal factor infertility. As tubal damage in the pelvis may not always be visible at HSG (39, 40), it is a possible subtle “tubal” factor infertility group that often gets overlooked. However, results of our study did not reveal a negative effect of endometriosis on pregnancy outcomes. This finding is in line with results from a recent analysis using propensity score matching, where no significant differences were detected in both per-cycle and cumulative CPRs between women with endometrioma-associated infertility and those with unexplained infertility (47). In addition, endometrial thickness was

obviously thicker in ENDO cycles, which could be explained by the fact that endometriosis has been proved to produce excessive estrogen through different mechanisms, including increased cell survival, inflammation, and deficient differentiation (48, 49).

To the best of our knowledge, this is so far the largest study evaluating the effectiveness of IUI for patients with tubal factor and endometriosis in comparison to those with unexplained infertility. As detected by our results, the significantly increased incidence and risk of ectopic pregnancy among women with tubal factor infertility in IUI cycles were particularly relevant to both physicians and patients in clinical practice, indicating that these women might benefit more from earlier transition to IVF. Ectopic pregnancies, on top of being life-threatening, have a significant negative impact on patient's already fragile psychology and overall well-being, which will also delay transition to IVF and potentially delay live birth by at least 4-6 months. The diagnosis and treatment of an ectopic pregnancy whether with methotrexate or with a surgical intervention might often exceed the cost of an IVF cycle. An additional strength of our study is the inclusion of the endometriosis group, which in a sense ensures that cases of "subtle/undiagnosed" tubal/peritoneal factor were not missed.

Yet, our study might have several limitations to be noted. Firstly, selection bias cannot be ignored due to the retrospective nature of the study. However, our results remained the same after adjusting for potential confounders in the GEE model,

indicating that it's unlikely that the differences in IUI outcomes were related to the selection bias alone. Yet, there might still be possibility of residual confounding in our results, as data regarding other potential confounders such as life style was lacking in the study. Secondly, the sample sizes in the TF and ENDO group were smaller than the control group, which in part reflects the routine practice, but may also have impacted our ability to test the between-group differences for certain pregnancy outcomes, including ectopic pregnancy in the ENDO group. Therefore, our results should be interpreted with caution. Third, since the majority of patients included in the study were Caucasian, our results may not be generalizable to other population. Additionally, tubal factor infertility was diagnosed by HSG in our study; and some of the patients in the ENDO group didn't have pathologically-confirmed diagnosis for endometriosis, because they preferred to seek fertility treatment before surgery. Moreover, information regarding the type of tubal occlusion and stage of endometriosis was not known for the most part. Although IUI was routinely recommended for patients with "mild to moderate" endometriosis without combined overt tubal factor, it is difficult to investigate whether different subgroups of tubal occlusion or endometriosis would have varied effects on IUI outcomes. Consequently, well-designed prospective cohort studies are necessary to confirm our results.

In conclusion, even though some women have good outcomes with IUI, it might not be the best approach for all infertility diagnoses. Our study provides

evidence of significantly higher risk of ectopic pregnancy and lower probability of ongoing pregnancy for women with confirmed tubal factor infertility. In these cases, there might be additional advantages from moving to IVF earlier. On the other hand, IUI seemed to be an appropriate approach for patients with potential "subtle" tubal/peritoneal factor infertility such as those with endometriosis who had similar pregnancy outcomes as patients with unexplained infertility. Our results should be interpreted with caution, and well-designed prospective studies are still warranted to further verify these findings and provide tools to efficiently counsel patients with overt and "subtle" tubal factor infertility considering OS/IUI treatments.

Disclosure of interests

None declared. Completed disclosure of interests form available to view online as supporting information.

Contribution to authorship

Y.L. and I.S. were involved in study concept and design. Y.L. and K.J. analyzed data. Y.L., V.J., P.C. and I.S. drafted the manuscript, and I.S. had a primary responsibility for final content. All authors were involved in acquisition of the data collection, interpreted the data, provided critical input to the manuscript and approved the final manuscript.

Funding

None.

Tables and figures

Table 1. Patient demographics and cycle characteristics

	Tubal factor	Endometriosis	Unexplained	p-value		
				Overall	TF vs. UE	ENDO vs. UE
No. of patients	105	87	1433			
Age (years)	34.5 (4.0)	33.9 (3.7)	34.6 (3.5)	0.17		
BMI (kg/m^2)	24.0 (21.6-27.0)	22.8 (21.0-25.3)	23.4 (21.1-26.3)	0.41		
Basal FSH (IU/L)	7.0 (1.9)	7.4 (2.3)	7.0 (1.9)	0.18		
Ethnicity n (%)				0.15		
Caucasian	82 (78.1)	57 (65.5)	1023 (71.4)			
Other	23 (21.9)	30 (34.5)	410 (28.6)			
Prior gravity n (%)	46 (43.8)	32 (36.8)	580 (40.5)	0.61		
Prior parity n (%)	30 (28.6)	16 (18.4)	288 (20.1)	0.10		
No. of cycles	269	242	4102			
Stimulation regimen n (%)				<0.001	<0.001	0.02
Natural	4 (1.5)	15 (6.2)	160 (3.9)			
Oral medication	54 (20.1)	74 (30.6)	1551 (37.8)			
Gonadotropins	211 (78.4)	153 (63.2)	2391 (58.3)			
No. of follicles ≥13mm	2.0 (1.0-2.0)	2.0 (1.0-3.0)	2.0 (1.0-2.0)	0.21		
No. of follicles ≥15mm	1.0 (1.0-2.0)	1.0 (1.0-2.0)	1.0 (1.0-2.0)	0.33		
Day of trigger (day)	11.2 (2.3)	11.0 (2.0)	11.2 (2.2)	0.74		
Endometrial thickness (mm)	8.3 (2.2)	9.0 (2.5)	8.2 (2.3)	<0.001	0.33	<0.001
TPMSC (million)	42.9 (20.8-86.5)	33.2 (15.8-63.2)	41.9 (18.7-81.8)	0.06		
Cancelled cycles n (%)	20 (7.4)	20 (8.3)	263 (6.4)	0.45		

TF =tubal factor; ENDO = endometriosis; UE = Unexplained; TPMSC = Total progressive motile sperm count.

Data are shown as mean and standard deviation (SD) if normally distributed, or as median and interquartile range (25th–75th) if non-normally distributed, or as n (%).

Table 2. Pregnancy outcomes

	Tubal factor	Endometriosis	Unexplained	p-value		
				Overall	TF vs. UE	ENDO vs. UE
No. of cycles	269	242	4102			
Positive HCG	28/269 (10.4)	26/242 (10.7)	575/4102 (14.0)	0.10		
Biochemical pregnancy loss	1/28 (3.6)	1/26 (3.8)	60/575 (10.4)	0.40		
Clinical pregnancy	27/269 (10.0)	25/242 (10.3)	515/4102 (12.6)	0.30		
Spontaneous abortion	7/27 (25.9)	2/25 (8.0)	92/515 (17.9)	0.22		
Multiple pregnancy	2/27 (7.4)	4/25 (16.0)	44/515 (8.5)	0.41		
Ectopic pregnancy						
Per clinical pregnancy	3/27 (11.1)	0	7/515 (1.4)		0.01	
Per patient	3/105 (2.9)	0	7/1433 (0.5)		0.03	
Ongoing pregnancy						
Per initiated cycle	17/269 (6.3)	23/242 (9.5)	416/4102 (10.1)	0.12		
Per clinical pregnancy	17/27 (63.0)	23/25 (92.0)	416/515 (80.8)	0.03	0.02	0.20
Cumulative ongoing pregnancy						
After 3 cycles per patient	17/105 (16.2)	15/85 (17.2)	323/1433 (22.5)	0.18		
After 4 cycles per patient	17/105 (16.2)	16/85 (18.8)	354/1433 (24.7)	0.08		

TF = tubal factor; ENDO = endometriosis; UE = Unexplained.

Data are shown as n (%).

Table 3. Associations between causes of infertility with ongoing pregnancy

	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Ongoing pregnancy				
Unexplained infertility	Ref.		Ref.	
Tubal factor infertility	0.60 (0.36-1.00)	0.05	0.53 (0.31-0.91)	0.02
Endometriosis	0.93 (0.57-1.52)	0.77	0.81 (0.46-1.42)	0.46

OR= odds ratio; CI = confidence interval.

Logistic regression analyses were conducted with generalized estimating equations to account for multiple cycles, adjusting for age, BMI, basal FSH, prior parity, ovarian stimulation regimen, and total progressive motile sperm count.

Figure 1. Flow chart of the inclusion and exclusion criteria

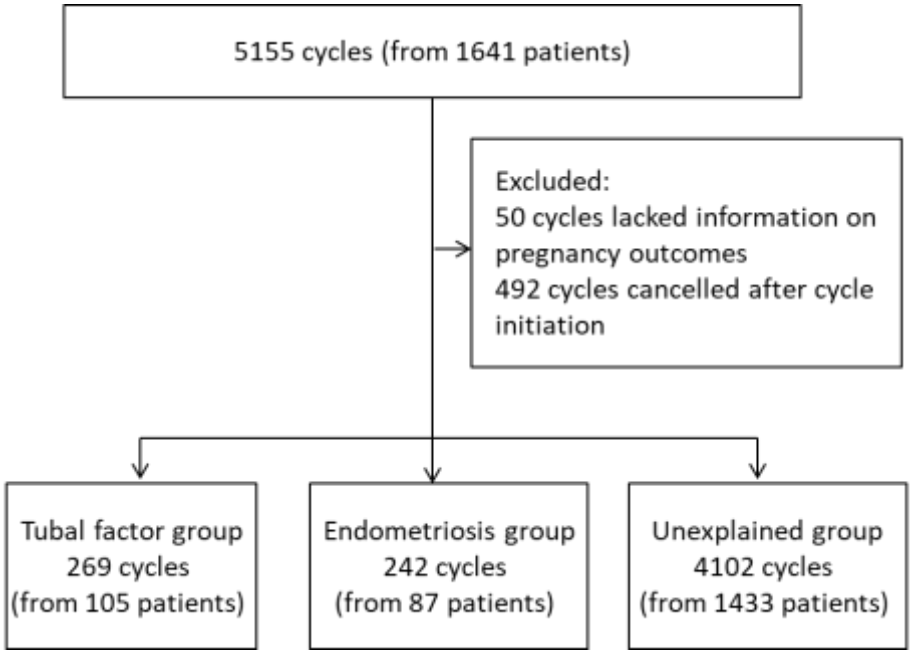
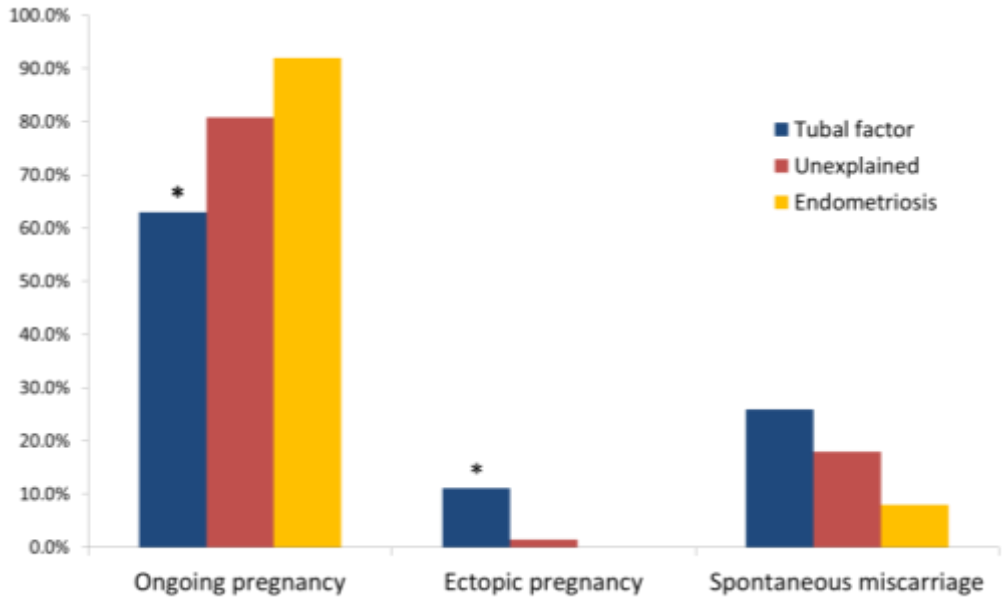
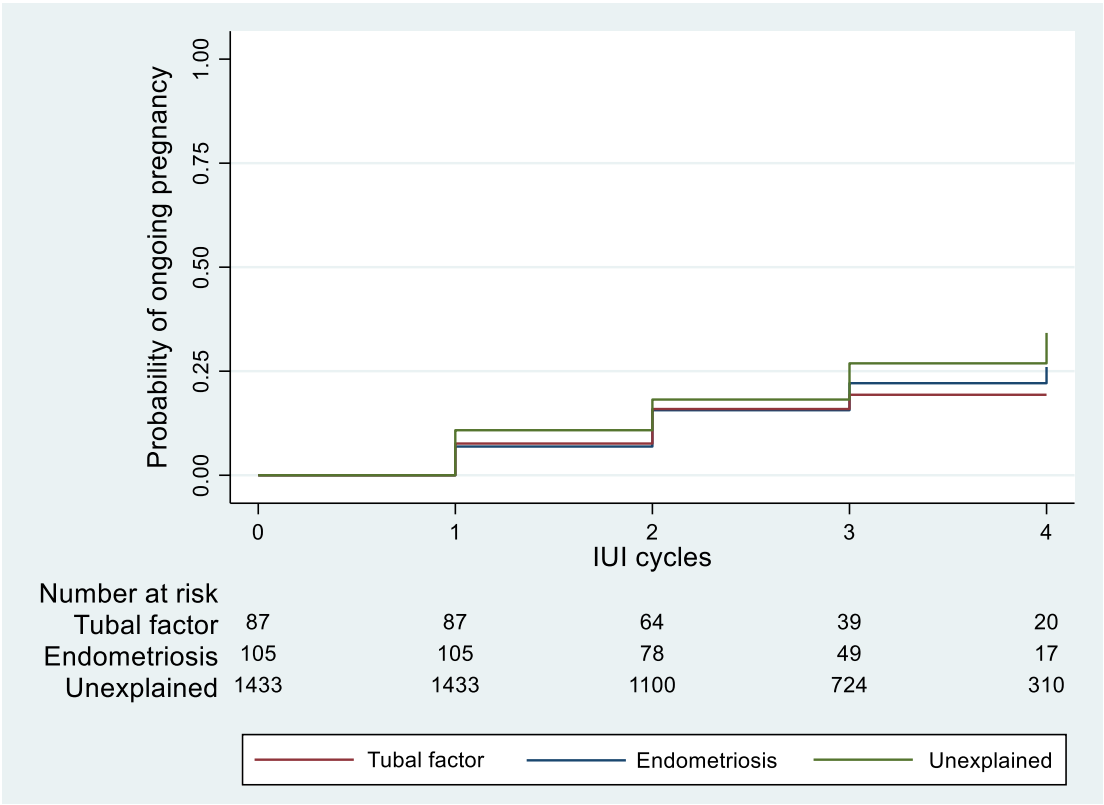


Figure 2. Prognosis among patients with different causes of infertility after clinical pregnancy



* represents $p<0.05$ compared to the unexplained group.

Figure 3. Kaplan-Meier analyses for cumulative ongoing pregnancy among different causes of infertility



Summary of conclusion

This body of work focused on two debatable predictive factors and their potential effects on IUI success and pregnancy outcomes.

In project 1, we investigated the differences in EMT between CC/IUI and Gn/IUI cycles, and the impact of these differences on IUI outcomes. Our study showed that CC stimulation resulted in a thinner endometrium compared to Gn; and within-patient, the EMT was thinner in CC cycles by an average of 1.7 mm. Patients who conceived with CC had a thicker endometrium compared to those who failed and had to switch to Gn treatments. Additionally, we found that in CC cycles, a thinner endometrium was associated with decreased CPR, while in Gn cycles no such association was observed, indicating that women developing a particularly thin endometrium with CC stimulation might benefit from switching to Gn.

In project 2, we investigated the potential differences in IUI outcomes among women with tubal factor infertility, endometriosis, and unexplained infertility. Our results suggested that women with tubal factor infertility were at a significantly increased risk of ectopic pregnancy and had lower chances to achieve an ongoing pregnancy in IUI cycles, which suggested that for this group of women there might be additional advantages from moving to IVF earlier. On the other hand, similar pregnancy outcomes were observed in women with endometriosis compared with those with unexplained infertility, indicating that IUI seemed to be an appropriate approach for patients with endometriosis that might also have a component of undiagnosed tubal factor infertility.

Discussion and perspectives

In project 1, our study was the first to evaluate EMT using patients as their own controls, with obvious benefit of minimizing the impact of potential confounders, and allowing for the estimate of within-patient variability. Our study provided firm evidence for the anti-estrogenic effect of CC on the endometrium. Additionally, our findings that thinner endometrium was associated with decreased CPR in CC cycles, but not in Gn cycles, implies that the two medications may be impacting the endometrium in different ways, and OS regimen should be taken into account in the definition and clinical management of thin endometrium in IUI cycles. However, clinical implication of these findings is a topic for further discussion. Future research should focus on establishing appropriate cut-offs for thin endometrium among different OS regimens, the impact of thin endometrium on IUI outcomes and the underlying mechanisms responsible for the associations observed.

In project 2, our study was the largest to date that evaluated the effectiveness of IUI for women with tubal factor infertility or endometriosis. Our results revealed significantly increased risk of ectopic pregnancy among women with tubal factor infertility in IUI cycles, which was particularly relevant to both physicians and patients in clinical practice, indicating that earlier transition to IVF could provide an additional advantage for these women. However, due to the retrospective design and the small number of ectopic cases in the study, well-designed prospective studies are still warranted to verify our results.

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