

Association between Myometrial Thickness and Assisted Reproductive Technologies Outcomes: A Prospective Cohort Study

Tahereh Madani, M.D.¹, Nadia Jahangiri, M.Sc.¹, Seyedeh Masoumeh Moosavisadat, M.Sc.^{1,2}, Elaheh Mirzaagha, B.Sc.¹, Saman Maroufizadeh, Ph.D.³, Shohreh Irani, Ph.D.^{4*} , Firoozeh Ahmadi, M.D.^{4*} 

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

2. Asali Charity Hospital, Lorestan University of Medical Science, Lorestan, Iran

3. Department of Biostatistics and Epidemiology, School of Health, Guilan University of Medical Sciences, Rasht, Iran

4. Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Background: Myometrial thickness has been expected to be a prognosticator for lower uterine segment function. An abnormal function of the uterine muscle layer can cause common and important reproductive problems. This study aimed to evaluate the relationship between baseline myometrial thickness and assisted reproductive technologies (ART) outcomes.

Materials and Methods: In this prospective cohort study, 453 infertile women undergoing ART cycles without any obvious uterine pathology, participated in this prospective cohort study from February 2013 to May 2015. In order to measure the myometrial thickness in the anterior and posterior of the uterine, trans-vaginal ultrasounds were conducted on days 2-4 of the cycle (menstrual phase) preceding ovarian stimulation and the day of human chorionic gonadotropin (hCG) injection. We defined three groups based on the baseline myometrial thickness in the anterior and posterior, including (A) <25 mm, (B) 25-29.9 mm and (C) ≥30 mm. Ovarian stimulation, oocyte retrieval and luteal phase support were performed in accordance with the standard long protocol. Two weeks after embryo transfer, the patients underwent a pregnancy test by checking their serum β-hCG levels. The primary outcome measure was clinical pregnancy rate. Secondary outcome measures were, implantation rate, abortion rate and live birth rate.

Results: The clinical pregnancy (P=0.013) and implantation (P=0.003) rates were significantly lower in group A than in two other groups. Although the live birth rate was lower in group A than two other groups, this decrease was not statistically significant (P=0.058).

Conclusion: The findings may be a way for clinicians to draw focus on providing therapeutic strategies and a specific supportive care for women with a baseline myometrial thickness <25 mm in order to improve the reproductive outcome of *in vitro* fertilization/intracytoplasmic sperm injection (IVF-ICSI).

Keywords: Embryo Implantations, Myometrium, Pregnancy Rate, Ultrasonography

Citation: Madani T, Jahangiri N, Moosavisadat SM, Mirzaagha E, Maroufizadeh S, Irani Sh, Ahmadi F. Association between myometrial thickness and assisted reproductive technologies outcomes: a prospective cohort study. *Int J Fertil Steril.* 2024; 18(2): 123-127. doi: 10.22074/IJFS.2023.555447.1314

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Introduction

Uterine contractions are effective in varied reproductive processes of menstruation, gamete and embryo transport, implantation, pregnancy and to parturition (1, 2). An abnormal function of the uterine muscle layer can cause disorders including dysmenorrhea, infertility, implantation failure, spontaneous abortion or preterm delivery, that are common and important challenges of couples in fertility age (3-6). Based on the mathematical modeling, it has been proven that an uterine wall stress is an inversely

proportional factor of the myometrium thickness (7). Some studies suggested that interactions between the innermost layer of the myometrium (junctional zone) and the endometrium, it seems it plays a significant role in the implantation process (8, 9).

An ultrasound imaging is a noninvasive method to evaluate an infertility and its treatment progress. Ultrasonography is an effective tool in improving the quality of services provided by the assisted reproductive technologies (ART) with facilitating timely diagnosis

Received: 08/June/2022, Revised: 19/March/2023, Accepted: 07/May/2023

*Corresponding Address: P.O.Box: 16635-148, Department of Reproductive Imaging, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Emails: sh.irani@royaninstitute.org, f_ahmadi@royaninstitute.org



Royan Institute
International Journal of Fertility & Sterility

and appropriate management (10, 11). Transabdominal and transvaginal ultrasonography have been used as reliable methods to measure the myometrial thickness in predicting pregnancy outcome (12). In a first study by Lesny et al. (13), they observed that the myometrium thickness was significantly higher in the pregnant group in comparison with the non-pregnant group on the down regulation day, day 8 of ovulation induction and human chorionic gonadotropin (hCG) injection day during *in vitro* fertilization-embryo transfer (IVF-ET) cycles. However, Youm et al. (14) argues that a myometrial thickness more than 2.50 cm on trans-vaginal ultrasonography (TVUS) may predict adverse outcomes of IVF-ET for women with adenomyosis.

Apart from Lesny et al. (13), the effect of myometrial thickness on reproductive outcome has not been paying attention as much as it needed. To our knowledge, the present study is the first prospective cohort analysis was designed to assess the relationship between the thickness of the myometrium and outcomes of IVF/intracytoplasmic sperm injection (ICSI) cycles.

Materials and Methods

Patients

This prospective cohort study was carried out in 453 infertile women undergoing IVF/ICSI cycles from February 2013 to May 2015 at the Infertility Center of Royan Institute, Tehran, Iran. The Institutional Review Board approved this study and the ethical clearance was issued by the Royan Institute Ethics Committee (EC/1020/91), Tehran, Iran, in compliance with the Helsinki Declaration. Also, a written informed consent was obtained upon their arrival at the clinic.

The inclusion criteria were as follows: no obvious pathology of uterine, being at their first IVF/ICSI cycle, having 2-3 excellent or good quality embryos, fertilization rate above 50%, and endometrial thickness of at least 7 mm on the day of the hCG injection. Patients were excluded from participation if either have obvious anomalies of the uterus, uterine myoma, uterine septum, or their husbands underwent testicular sperm extraction (TESE) or testicular sperm aspiration (TESA) or percutaneous epididymal sperm aspiration (PESA).

Sonographic procedure

All patients underwent TVUS to measure the myometrial thickness of anterior and posterior uterine on the days 2-4 of the menstrual phase preceding an ovarian stimulation. Measurements were performed in the mid-sagittal plane by one sonographer using with an Aloka- α 10 ultrasound system (Alok, Japan) equipped with a 5-8 MHz transvaginal probe, from one endometrial-myometrial interface to the uterine serosa as end point where the area appears to be at its thickest in the fundus.

The summation of myometrial thickness in anterior and posterior was divided into three following groups: <25 mm

(group A), 25-29.9 mm (group B) and \geq 30 mm (group C).

Ovarian stimulation cycle

All patients were treated according to the standard long gonadotropin-releasing hormone (GnRH) agonist protocol (Buserelin acetate, Aventis Pharma Deutschland, Germany). Ovarian stimulation started with follicle stimulating hormone (FSH, Gonal F 75 IU, Merck Serono, Italy) with or without human menopausal gonadotrophin (hMG, Menogan 75 IU, Ferring, Germany or Menopur 75 I, Ferring, Germany) according to the ovarian response. The cycle monitoring was performed with sequential TVUS and measurement of the serum estradiol level. When at least one follicle was detected with a diameter of \geq 18 mm and serum estradiol level reached to the 500-2000 pg/ml level, a dose of 10000 IU hCG (DarouPakhsh Co., Iran) was injected. The luteal phase was supported by a daily dose of the progesterone ampoule 50 mg (Aburaihan Co., Tehran, Iran) until 2 weeks after an ET. And 34-36 hours later, oocytes retrieval was performed under transvaginal ultrasound guidance to collect follicles. An embryo transfer was done after 48-72 hours. Pregnancy was detected by measuring the serum hCG level, two weeks after the embryo transfer.

Outcome definitions

The implantation rate was defined as the ratio of gestational sacs number per the ET number that were observed 4-6 weeks after ET. Clinical pregnancy was defined as the presence of at least one intrauterine gestational sac with the detectable fetal heart activity by TVUS 6-8 weeks after the embryo transfer. The abortion rate was defined as the total number of abortions obtained before 20 completed weeks gestation to the ET cycles.

The live birth rate was defined as the ratio of deliveries number that resulted in at least one live born baby to the ET cycles (15).

Statistical analysis and sample size calculation

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, SPSS Inc., Chicago, IL, USA) version 20. Continuous variables are presented as mean \pm standard deviation (SD) and categorical variables are shown as number (percentage). Demographic and clinical characteristics were compared between groups using chi-square test and one-way analysis of variance (ANOVA). Logistic regression was used to examine the relationship between myometrial thickness and cycle outcome (clinical pregnancy). A $P < 0.05$ was considered statistically significant.

Results

Totally, 453 couples participated in our study that divided in to three groups, including A (n=52), B (n=199) and C (n=202). Demographic and clinical characteristics of the three groups (A, B and C) are detailed in Table 1.

Table 1: Demographic and clinical characteristic of our participants

Parameters	Group			P value*
	A [†] (n=52)	B [‡] (n=199)	C [§] (n=202)	
Age (Y)	30.69 ± 5.06	30.86 ± 4.46	32.93 ± 4.67	<0.001
Body mass index (kg/m ²)	26.30 ± 3.86	25.93 ± 3.60	26.23 ± 4.37	0.698
Duration of infertility (Y)	6.50 ± 4.24	6.36 ± 4.16	6.47 ± 4.08	0.958
Cause of infertility				
Male factor	22 (42.3)	116 (58.3)	106 (52.5)	0.284
Tubal factor	3 (5.8)	7 (3.5)	14 (6.9)	
Anovulatory	8 (15.4)	28 (14.1)	32 (15.8)	
Recurrent abortion	1 (1.9)	2 (1.0)	4 (2.0)	
Unexplained factor	6 (11.5)	23 (11.6)	27 (13.4)	
Multi-factor	12 (23.1)	23 (11.6)	19 (9.4)	
Ovulation induction information				
No. of ampoules used	25.86 ± 8.07	26.70 ± 10.61	26.81 ± 9.73	0.896
Length of ovarian stimulation (days)	13.03 ± 3.34	13.02 ± 3.22	12.98 ± 3.23	0.990
Endometrial thickness on the day of hCG administration (mm)	9.18 ± 1.69	9.66 ± 1.85	10.31 ± 2.12	<0.001
No. of oocytes retrieved	7.93 ± 3.51	8.88 ± 3.98	8.10 ± 3.97	0.250
No. of injected oocytes	6.83 ± 3.45	7.09 ± 3.28	6.76 ± 3.64	0.761
Embryo's information				
No. of 2PN embryos	3.86 ± 2.51	4.50 ± 2.82	4.83 ± 2.91	0.244
No. of embryos transferred	2.57 ± 0.66	2.42 ± 0.75	2.52 ± 0.74	0.226
No. of excellent and good quality embryos	2.09 ± 0.91	2.02 ± 0.96	2.08 ± 0.91	0.729

Values are given as mean ± SD or n (%).[†]; Anterior-posterior myometrial diameter <25 mm, [‡]; Anterior-posterior myometrial diameter between 25-29.9 mm, [§]; Anterior-posterior myometrial diameter ≥30 mm, hCG; Human chorionic gonadotropin, 2PN; two-pronuclear, and *; Chi-square test for categorical variables and one-way analysis of variance for continuous variables.

The data showed that 94.7% patients had primary infertility and 5.3% had secondary infertility. Causes of infertility were as follows: male factor 53.9%, tubal factor 5.3%, anovulatory factor 15.0%, recurrent abortion 1.5%, mixed 11.9% and unexplained factor 12.4%.

Clinical characteristics of the three groups (A, B and C) are detailed in Table 1. Clinical characteristics including body mass index, duration of infertility, causes of infertility, number of ampoules used, length of ovarian stimulation, number of oocytes retrieved, number of injected oocytes, number of two-pronuclear embryos (2PN), number of embryos transferred, and excellent and good quality embryos number showed no significant differences among the three groups (Table 1).

Age and endometrial thickness on the day of hCG administration were significantly higher in group C as compared to groups A and B. The mean values of

myometrial thickness in the anterior and posterior of the uterus were as follows: 14.30 ± 2.41 mm (range, 9.10-24.60 mm) and 15.47 ± 2.44 mm (range, 10.0-23.80 mm), respectively.

The overall rate of pregnancy based on the positive βhCG was 42.8% (194/453). Among the 194 pregnancies (based on the positive βhCG), 160 clinical pregnancies, 16 blighted ovums, 7 missed abortions and 11 biochemical pregnancies occurred. The overall rates of clinical pregnancy, implantation and live birth were 35.3, 18.7 and 32.3%, respectively. There were significant differences regarding the clinical pregnancy and implantation rates among three groups (P=0.013 and P=0.003, respectively). Loss to follow up after pregnancy was 5 (3.1%) in groups of B (n=3) and C (n=2) (Table 2). Although, the live birth rate was lower in group A than two other groups, this difference was not statistically significant (P=0.058). It is likely this lack of statistical significance is due to the small sample size.

Table 2: Cycle outcomes in our participants

Outcomes	Group			P value [‡]
	A [†] (n=52)	B [‡] (n=199)	C [§] (n=202)	
Clinical pregnancy rate	9/52 (17.3)	78/199 (39.2)	73/202 (36.1)	0.013
Implantation rate	12/134 (9.0)	105/482 (21.8)	94/511 (18.4)	0.003
Abortion rate	0/9 (0)	5/78 (6.4)	3/73 (4.1)	0.631
Live birth rate	9/52 (17.3)	67/196 (34.2)	66/200 (33.0)	0.058

Values are given as number/n (%).[†]; Anterior-posterior myometrial diameter <25 mm, [‡]; Anterior-posterior myometrial diameter between 25-29.9 mm, [§]; Anterior-posterior myometrial diameter ≥30 mm, and *; Chi-square analysis.

In unadjusted analysis, the clinical pregnancy rate was significantly higher in the group B than group A (39.2 vs. 17.3%) with an odds ratio of 3.08 [95% confidence interval (CI): 1.42-6.67], while it changed only slightly after being adjusted for age and endometrial thickness on the hCG day to 3.10 (95% CI: 1.42-6.73). The group C had approximately the same results as the group B (Table 3).

Table 3: Crude and adjusted odds ratios for clinical pregnancy in our participants

Group	Clinical pregnancy rate, n (%)	Crud (unadjusted) OR		Adjusted OR ^a	
		OR (95% CI)	P value	OR (95% CI)	P value [*]
A (n=52) ^b	9 (17.3)	1	-	1	-
B (n=199)	78 (39.2)	3.08 (1.42-6.67)	0.004	3.10 (1.42-6.73)	0.004
C (n=202)	73 (36.1)	2.70 (1.25-5.86)	0.012	2.85 (1.29-6.31)	0.010

^a; Adjusted for age and endometrial thickness on hCG day, ^b; Reference group, OR; Odds ratio, CI; Confidence interval, *; Logistic regression, and hCG; Human chorionic gonadotropin.

Discussion

According to the International Society of Ultrasound in Obstetrics and Gynecology (ISUOG), anterior-

posterior diameter is 2.00-5.00 cm in reproductive age women (16) that is compatible with our results. In our study, the findings showed that in ART cycles, the baseline myometrial thickness (anterior-posterior diameter) <25 mm is associated with a lower implantation and pregnancy rates in comparison with a myometrial thickness ≥ 25 mm.

Our results showed there was a significant reduction in the rates of clinical pregnancy and implantation and an insignificant reduction in the live birth rate of group A. Although, the reduction observed in the live birth rate was insignificant, this difference was clinically important; therefore, IVF-ET/ ICSI outcomes in the group A were lower than those of group B and group C. Youm et al. (14) findings showed the implantation and clinical pregnancy rates were considerably lower in patients with a myometrial thickness ≥ 2.50 cm in comparison with the patients with a myometrial thickness <2.00 cm and 2.00-2.49 cm. As it is clear, the study population in our study is different to those of Youm et al. (14). The patients in that study had adenomyosis, while in our study all had a normal uterine. So far as is known, the smooth muscle cells from normal myometrium differs ultrastructurally from adenomyosis cells (17). This is supported by the hypothesis that the adenomyosis tissue results from the invasion of endometrial tissue through the endomyometrial junctional zone (JZ) into the myometrium. Adenomyosis causes a variable degree of cellular hyperplasia and hypertrophy surrounding the heterotopic endometrial tissue (18), and myocytes in adenomyosis show differences in cytoplasmic organelles, nuclear structures, and intercellular junctions (17). A growing literature has proposed that this thickness and distortion of the myometrium can alter the coordinated peristaltic activity of the inner myometrium (19) which interfere with the sperm transportation and the embryo implantation, and can adversely affect the fertility potential (18).

Our finding is in agreement with Lesny et al. (13) study, in which they observed the thicker myometrium in the pregnant group in comparison with the non-pregnant group on the day of down regulation during IVF-ET cycles. However, there are the limited published and/or available evidences. Based on our results, we propose that a thin myometrium may be involved in the failure of implantation and pregnancy, but how to directly determine this relation is a question. It seems possible that these results are due to the association between myometrial thickness and uterine contraction. However, very little was found in the literature on this topic, too. Previous studies have demonstrated that each uterine contraction originates from a local contraction, which transiently increases the intrauterine pressure. Subsequently, a high intrauterine pressure increases the stress level throughout the uterine wall which causes contractions of more regions (20). Accordingly, Deyer et al. (7) showed that uterine wall stress (defined as the applied force per unit cross-sectional area of material) is inversely related to the myometrium thickness. Therefore, the thicker the myometrium, the

lower the uterine wall stress or uterine contraction. Data suggest that there are no contractile fibers in the endometrium, so these contractions were produced in the myometrium (21). Compared to the outer myometrial layer, the JZ as the innermost layer of the myometrium consists of higher density of compacted myocytes (22). Lesny et al. (13) demonstrated significantly thicker JZ and lower JZ contractions related to the higher pregnancy rate in ART cycles. This was supported by significant temporal and dynamic variations at the time of the oocyte's retrieval and embryos transfer. It shows that the thinner myometrium provides further contraction (23).

In line with these observations, major roles of the uterine contractions in the process of implantation and pregnancy were reported (1, 24, 25). In menstrual cycles, the frequency and direction of uterine contractions differ during the menstruation. The frequency attains a peak immediately prior to the ovulation time (26) with a mainly cervico-fundal wave form (27) to enable an effective ascending migration of sperm to fallopian tubes (28). Subsequently, the contractile activity decreases, thus creating an ideal environment for implantation (29). Zhu et al. (26) confirmed that in both fresh and frozen embryo cycles, uterine contraction frequencies were significantly lower in women who conceived than non-conceived women. The combination of these findings can provide some support for the correlation between myometrial thickness and ART Outcomes. However, more research on this topic needs to be undertaken.

This present study has two limitations that should be noted when interpreting the results, including (1) it was a single center study, and (2) the complete report of live birth rate was not achieved. The information on live birth was not stated in 3.1% (5/160) of clinical pregnancies of this study, which might impact the statistical power of the test for the live birth parameter.

Conclusion

In conclusion, it seems that the myometrial thickness (anterior-posterior diameter) <25 mm measured on menstrual phase may have an adverse effect on IVF-ET/ ICSI outcomes. The findings may be a way for clinicians to draw focus on providing therapeutic strategies and specific supportive care in order to improve reproductive outcome of IVF/ICSI in these women.

Acknowledgments

The authors wish to express their gratitude to the Royan Institute and their staff. The authors sincerely thank the patients who kindly participated in this study. This work was supported by the Royan Infertility Research Centre. The authors declare that they have no conflicts of interest in this study.

Authors' Contributions

T.M.; Conceptualization, Supervision, and Revised manuscript critically for important intellectual content.

Sh.I., E.M.; Data acquisition and Investigation. N.J., S.M.M.; Methodology, Validation, and Writing- review and editing. S.M.; Statistical analysis. F.A.; Supervision, Project administration, and Revised it critically for important intellectual content. All authors have critically reviewed and approved the final manuscript.

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