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Predictive Value of Uterine Sonoelastography on the Outcome of IVF Treatment

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ABSTRACT

Objective: The knowledge of factors that influence the outcome of *in-vitro* fertilization (IVF) has increased significantly over time, yet remains insufficient. This prospective study was designed to evaluate whether uterine sonoelastography could be a tool to predict the outcome.

Materials and Methods: Patients who were to undergo the first cycle of IVF treatment were enrolled for 3 months. Sonoelastography using acoustic radiation force impulse technology was performed just before the embryo transfer in patients without a uterine abnormality. The association between the sonoelastography measurements and outcomes was evaluated.

Results: This study included 110 patients, with a pregnancy rate of 33.6%. None of the elastography measurements alone had a significant effect on the outcome, however the ratio between the measurements obtained from each half of the posterior uterine wall was significantly higher in the women who conceived compared with those who did not (controls) (1.18 vs. 0.96; p=0.01). Multivariate analysis indicated that the ratio was associated with the outcome.

Conclusion: The preliminary results of the study suggest a possible relationship between the posterior uterine wall elasticity index and the outcome of IVF treatment. Further larger studies are needed to confirm this result and to develop an examination protocol.

Keywords: Elastography, *in-vitro* fertilization, prediction, pregnancy

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INTRODUCTION

Embryo transfer is one of the most important steps of *in-vitro* fertilization (IVF) treatment. That is why it is crucial to know whether the site is suitable for this step (1). Unfortunately, measuring the endometrial thickness with ultrasonography before the transfer is currently the only way to assess whether a uterus is ready to receive an embryo. This method does not provide sufficient representation of the histological features of the whole uterus, particularly of the myometrium, which determines the fate of a pregnancy (2). There is a need for a modality that can noninvasively monitor histological changes in the uterine tissue due to ovulation induction during IVF treatment (3).

In recent decades, ultrasound-based elastography, sonoelastography, has emerged as a novel evolutionary technique. It has 3 major subtypes: transient elastography, acoustic radiation force impulse (ARFI) elastography, and strain elastography. Briefly, all of the subtypes use sound waves to assess the response to mechanical strength applied to the target organ (4, 5). Currently, it is used to assess the degree of hepatic fibrosis in chronic liver diseases and to characterize liver lesions, evaluate diffuse renal parenchymal changes and characterize renal lesions, assess thyroid nodules, screen prostate diseases, differentiate benign and malignant masses of the breast, evaluate lymph node involvement, and tendon imaging (6, 7). The promising results seen in these fields have led to interest related to obstetrical and gynecological examinations. Sonoelastography has already been studied in the prediction of preterm labor (8), evaluation of endometrial disorders (9), and adenomyosis (10). However, there are only a limited number of studies that address the relationship between elastographic features of the uterus and the success of fertility treatment.

In this study, the objective was to evaluate whether uterine sonoelastography using acoustic radiation force impulse point shear wave elastography (ARFI pSWE) focused on the endometrium as well as on the myometrium could be used as a tool to predict the outcome of IVF treatment.

MATERIALS and METHODS

Ethical Considerations

This study was conducted according to the World Medical Association Declaration of Helsinki guidelines. Informed consent was obtained from all of the patients following explanation of the purpose of the study. The study protocol was approved by the Ondokuz Mayıs University Ethical Committee (no: 2019/832).

Study Population

Patients who underwent IVF treatment at a single hospital between January 2020 and March 2020 were enrolled in this prospective study. Hysterosalpingography revealed no abnormalities in any of the patients, and all of them were to begin their first IVF cycle. Patients with known endometriosis, adenomyosis, myoma uteri, or a retroverted uterus were excluded from the study. Patients whose embryos did not develop or those whose embryos could not be transferred were also excluded. In addition, patients with a difficult embryo transfer or those who experienced hemorrhage were excluded.

IVF Procedure

For each fresh embryo transfer, recombinant follicle-stimulating hormone (FSH) (Gonal-F; Merck Serono, Geneva, Switzerland) was administered daily at a dose adjusted to the individual ovarian response beginning on the second or third day of menstruation. Once the follicles reached a diameter of ≥12 mm, a recombinant gonadotropin-releasing hormone (GnRH) antagonist (Cetrotide 0.25 mg; Merck Serono, Geneva, Switzerland) injection was administered. Upon detecting ≥2 follicles with a diameter of ≥18 mm, a recombinant human chorionic gonadotropin (hCG) (Ovitrelle 250 mcg; Merck Serono, Geneva, Switzerland) injection was given, and oocyte pick-up (OPU) was performed 36 hours later. Following OPU, the intracytoplasmic sperm injection procedure was performed. Progesterone (Progestan 50 mg; Koçak Farma, Istanbul, Türkiye) was administered beginning on the day of OPU was at a dose of 100 mg was provided to provide luteal phase support until the embryo transfer.

In all frozen embryo transfers, estrogen (Estrofem 2 mg; Novo Nordisk, Bagsværd, Denmark) was administered to the patient at a daily dose of 4 mg on days 1–4 of menstruation, 6 mg on days 5–8, and 8 mg as of day 9 to prepare the endometrium for embryo reception. Transvaginal ultrasonography was repeated on the 10^{th} day of estrogen administration. Once the endometrial thickness reached ≥7 mm, embryo transfer was scheduled and progesterone was administered at a dose of 100 mg until the embryo transfer.

The women were asked to drink water before the procedure so that their bladder was partially filled partially at the time of transfer. Using a speculum, the uterine cervix was visualized in the lithotomy position prior to the ultrasonography-guided transfer. Based on the quality, 1 or 2 embryos were transferred by a single reproductive endocrinologist in each procedure. On the 12^{th} day after the embryo transfer, chemical pregnancy was confirmed by measuring the serum level of βHCG.

Elastography Measurements

Uterine sonoelastography examinations were performed by a single operator to avoid operator-related factors, consistent with previously published studies (11, 12). Elastography measurements were obtained about half an hour before each transfer using an ACUSON S2000 ultrasound platform (Siemens Healthineers, Erlangen, Germany) that enables ARFI pSWE (Virtual Touch tissue quantification; Siemens Healthineers, Erlangen, Germany). Using a curvilinear array transducer with a bandwidth of 1.5–6 MHz, point shear wave speed measurement was performed, providing results that corresponded to the quantitative value of tissue elasticity. Both the anterior and posterior uterine walls and the endometrium were examined in the sagittal plane by setting a fixed region of excitation (ROE) at 2 locations in each target field, within a distance of 8 cm from the transducer. The first ROE for each target field was chosen within the upper half of the uterine corpus and the second within the lower half (Fig. 1). Since a distended bladder could cause miscalculation of shear wave speed, all of the participants were asked to empty their bladder before the examination.

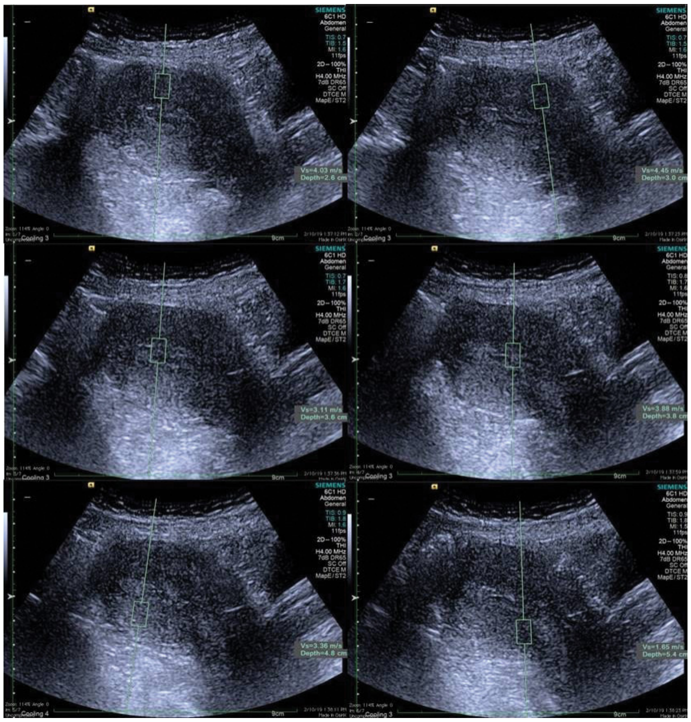

Figure 1. Uterine sonoelastography images recorded before the embryo transfer showing the elastography measurements of the anterior uterine wall (upper row), the endometrium (middle row), and the posterior uterine wall (lower row)

Sample Size

The sample size calculation was performed using Student's t-test with Minitab Software, version 16 (Minitab Inc., State College, PA, USA). Based on previously published data (11), a minimum enrollment of 102 individuals was required to have 80% power with a 5% type I error to detect a minimum clinically significant difference of 0.18 units, with a standard deviation of 0.64. Assuming for the possibility of some dropouts, the study enrollment goal was set at 115 patients.

Statistical Analysis

IBM SPSS Statistics for Windows, Version 23.0 software (IMB Corp., Armonk, NY, USA) was used to perform the statistical analysis. Categorical variables were presented as numbers (%) and continuous variables as median (range, minimum–maximum). Conformity to a normal distribution was assessed using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to compare quantitative data that did not show normal distribution. A chi-squared test was used to assess categorical data. The p values obtained with single association tests were used to determine candidate variables for multivariate logistic regression. All of the independent variables that demonstrated a p value of <0.2 were included in the regression model.

Table 1. Basic characteristics of the patients according to treatment outcome

Variables	Conception n=37	No conception n=73	Total n=110	p
Age (years)	33 (20–44)	32 (22–44)	33 (20–44)	0.44[a]
BMI (kg/m²)	26 (20–45)	26 (20–40)	26 (20–45)	0.85[a]
Diagnosis of infertility				
Idiopathic	20 (39.2)	31 (60.8)	51 (45.9)	0.53[b]
Male factor	10 (29.4)	24 (70.6)	34 (30.6)	
Low ovarian reserve	2 (16.7)	10 (83.3)	12 (10.8)	
Polycystic ovary	3 (33.3)	6 (66.7)	9 (8.2)	
Tubal factor	2 (50.0)	2 (50.0)	4 (3.6)	
History of smoking				
Yes	3 (37.5)	5 (62.5)	8 (7.3)	
No	34 (33.5)	68 (66.7)	102 (92.7)	1.00[b]

Categorical variables are presented as number (%) and continuous variables are presented as median (range, min–max). a: P value calculated using the Mann-Whitney U test; b: P value calculated using a chi-squared test; BMI: Body mass index; FSH: Follicle stimulating hormone

Table 2. Comparison of variables concerning the treatment outcomes

Variables	Conception n=37	No conception n=73	Total n=110	p
Type of embryo transfer				
Fresh	29 (38.2)	47 (61.8)	76 (69.1)	0.20[a]
Frozen	8 (23.5)	26 (76.5)	34 (30.9)	
Endometrial thickness (mm)	9 (4–13)	9 (3–13)	9 (3–13)	0.40[b]
Number of oocytes collected	9 (1–30)	9 (2–34)	9 (1–34)	0.79[b]
Number of embryos transferred				
1	22 (35.5)	40 (64.5)	62 (56.4)	0.79[a]
2	15 (31.3)	33 (68.8)	48 (43.6)	
FSH level (IU/mL)	5.0 (1.1–15)	7.1 (1.0–24)	6.6 (1.0–24)	0.07[b]
Estrogen level (pg/mL)	1156 (87–3946)	422 (12–4630)	566 (12–4630)	**0.02**[b]
Progesterone level (ng/l)	0.35 (0.08–1.3)	0.30 (0.08–1.5)	0.30 (0.08–1.5)	0.63[b]

Categorical variables are presented as number (%) and continuous variables are presented as median (range, min–max). a: P value calculated using a chi-squared test. b: P value calculated using the Mann-Whitney U test. Boldface type indicates statistical significance. FSH: Follicle stimulating hormone

RESULTS

The study included a total of 110 patients, with a median age of 33 years (range: 20–44 years). The most common (46.4%) diagnosis for infertility in the study group was "unexplained," which means that there was no diagnosed medical reason for infertility. Of the 110 patients, 76 (69.1%) had a fresh embryo transfer and 34 (30.9%) had a frozen embryo transfer. There were no statistically significant differences between the groups (conceived and non-conceived) related to age, body mass index, the diagnosis related to infertility, or a history of smoking. Furthermore, the type of embryo transfer, endometrial thickness, the number of oocytes collected, the number of embryos transferred, and the baseline FSH level did not reveal a significant difference. It was found that the median level of estrogen on the day of embryo transfer was 1156 pg/mL in the women who conceived, whereas the level was 422 pg/mL in those who did not conceive, which represented a statistically significant difference (p=0.02). However, there was no statistically significant difference between the 2 groups regarding the median level of progesterone on the day of embryo transfer. The basic characteristics of the patients and the treatment outcomes are summarized in Tables 1 and 2.

None of the elastography measurements obtained from the described locations yielded a statistical difference between groups. Apart from the ratio of the upper half of the posterior uterine wall measurement to that of the lower half of the posterior uterine wall, there were no statistically significant differences between the ratios of the quantitative values of elastography measurements. However, the ratio was significantly higher in the women who conceived compared with those who did not (1.18 vs. 0.96; p=0.01). In addition, there was a trend toward a lower ratio in the lower half of the posterior uterine wall measurement to that of the lower half of the endometrium among those who conceived when compared with the controls (1.09 vs. 1.47; p=0.08). A comparison of elastography measurements and ratios according to treatment outcomes is

Table 3. Comparison of elastography measurements and ratios according to the treatment outcome

	Conception n=37	No conception n=73	Total n=110	p*
Measurements				
Myo-UA (m/s)	3.23 (0.62–4.49)	3.35 (0.58–4.54)	3.24 (0.58–4.54)	0.66
Myo-LA (m/s)	3.33 (0.68–4.90)	3.25 (0.67–4.88)	3.25 (0.67–4.90)	0.65
Myo-UP (m/s)	3.66 (0.84–4.86)	3.36 (0.66–4.97)	3.45 (0.66–4.97)	0.35
Myo-LP (m/s)	3.05 (0.60–4.77)	3.25 (0.67–4.88)	3.16 (0.60–4.88)	0.15
End-U (m/s)	2.12 (0.82–4.4)	2.39 (0.50–4.86)	2.33 (0.50–4.86)	0.57
End-L (m/s)	2.55 (0.63–4.53)	2.19 (0.51–4.91)	2.40 (0.51–4.91)	0.48
Ratios				
Myo-UA/Myo-LA	0.95 (0.42–3.86)	0.97 (0.19–6.78)	0.97 (0.19–6.78)	0.64
Myo-UP/Myo-LP	1.18 (0.33–3.45)	0.96 (0.20–2.18)	1.04 (0.20–3.45)	**0.01**
End-U/End-L	0.90 (0.47–3.30)	1.07 (0.28–4.36)	0.98 (0.28–4.36)	0.12
Myo-UA/End-U	1.31 (0.27–3.67)	1.31 (0.36–7.63)	1.31 (0.27–7.63)	0.70
Myo-LA/End-L	1.35 (0.22–5.49)	1.47 0.48–5.23)	1.46 (0.22–5.49)	0.60
Myo-UP/End-U	1.47 (0.36–5.47)	1.34 (0.14–6.96)	1.40 (0.14–6.96)	0.43
Myo-LP/End-L	1.09 (0.32–5.54)	1.47 (0.43–7.39)	1.22 (0.32–7.39)	0.08
Myo-UA/Myo-UP	0.89 (0.31–3.65)	0.97 (0.38–6.00)	0.94 (0.31–6.00)	0.62
Myo-LA/Myo-LP	1.09 (0.27–4.08)	1.02 (0.19–5.39)	1.06 (0.19–5.39	0.13
Myo-UA/Myo-LP	0.99 (0.24–4.40)	0.97 (0.30–2.90)	0.98 (0.24–4.40)	0.29
Myo-LA/Myo-UP	0.95 (0.19–3.38)	0.97 (0.26–3.83)	0.97 (0.19–3.83)	0.85
Myo-LA/End-U	1.45 (0.29–5.29)	1.36 (0.42–8.08)	1.38 (0.29–8.08)	0.50
Myo-UA/End-L	1.23 (0.20–5.59)	1.39 (0.42–5.00)	1.34 (0.20–5.59)	0.60
Myo-UP/End-L	1.28 (0.28–7.38)	1.31 (0.21–8.87)	1.31 (0.21–8.87)	0.77
Myo-LP/End-U	1.29 (0.36–4.20)	1.43 (0.43–6.40)	1.35 (0.36–6.40)	0.56

Continuous variables are presented as median (range, min–max). *: P values calculated using the Mann-Whitney U test. Boldface type indicates statistical significance. End-L: Endometrium, lower half of the uterine corpus; End-U: Endometrium, upper half of the uterine corpus; Myo-LA: Myometrium, lower half of the uterine corpus, anterior; Myo-LP: Myometrium, lower half of the uterine corpus, posterior; Myo-UA: Myometrium, upper half of the uterine corpus, anterior; Myo-UP: Myometrium, upper half of the uterine corpus, posterior

summarized in Table 3. Univariate analysis revealed that the ratio of the upper half of the posterior uterine wall measurement to that of the lower half of the posterior uterine wall was associated with the outcome (p=0.03); however, multivariate analysis did not disclose any significance. Table 4 provides a summary of the significance of the relationships tested in the model.

DISCUSSION

Unlike earlier research related to uterine sonoelastography, our study focused on the impact of endometrial elasticity as well as myometrial elasticity on the outcome of IVF treatment. We found that none of the elastography measurements obtained, as defined in the Materials and Methods section, produced a significant difference between those who conceived and those who did not. But perhaps most notably, we observed that the ratio of the value measured in the upper half of the posterior uterine wall to that of the lower half of the same wall was significantly higher in the women who conceived. Thus, our results suggest a possible association between the posterior uterine wall elasticity index and achieving a successful pregnancy in patients undergoing IVF treatment.

The available literature proposes that the elastographic features of any tissue are a histological reflection of the content thereof (13). The capability for noninvasive detection of the changes in the histological infrastructure of soft tissues has enabled sonoelastography to be used in many clinical areas, including obstetrics and gynecology. In recent years, many studies have been conducted in this field, investigating the possible clinical usages of this modern modality. The research, particularly on fertility treatment, has demonstrated some exciting results. For instance, pregnancy rates are higher in patients with a high pre-insemination intrauterine elasticity index (the ratio of subendometrial elasticity to endometrial elasticity). In other words, it has been proposed that pregnancy rates increase as myometrial stiffness increases (14). A recent study of patients with adenomyosis has revealed greater stiffness of the myometrial tissue (15). This suggested that there might be a relation between muscular stiffness and problems with fertility (15–17). In another study examining adenomyosis, it has been reported that the stiffness of adenomyotic lesions was significantly greater when compared with that of uterine fibroids, which, in turn, was significantly greater when compared with that of myometrial controls. The researchers observed a positive correlation between lesional stiffness and the extent of lesional fibrosis, and a negative correlation regarding

Table 4. Univariate and multivariate logistic regression showing the impact of single factors on the success of IVF treatment

Variables	Univariate analysis			Multivariate analysis		
	SE	OR (95% CI)	p	SE	OR (95% CI)	p
FSH level	0.04	1.01 (0.92–1.11)	0.75	0.50	1.01 (0.92–1.12)	0.73
Estrogen level	0.00	1.00 (1.00–1.00)	0.92	0.00	1.00 (1.00–1.00)	0.87
Myo-LP measurement	0.18	0.76 (0.53–1.10)	0.15	0.28	0.92 (0.53–1.60)	0.79
Myo-UP/Myo-LP ratio	0.45	2.66 (1.09–6.49)	**0.03**	0.56	2.66 (0.87–8.12)	0.08
End-U/End-L ratio	0.34	0.67 (0.34–1.32)	0.25	0.42	0.64 (0.27–1.50)	0.31
Myo-LP/End-L ratio	0.19	0.78 (0.52–1.15)	0.21	0.25	0.97 (0.59–1.60)	0.91
Myo-LA/Myo-LP ratio	0.27	1.39 (0.81–2.38)	0.22	0.39	0.93 (0.42–2.03)	0.86

Boldface type indicates statistical significance. CI: Confidence interval; End-L: Endometrium, lower half of the uterine corpus; End-U: Endometrium, upper half of the uterine corpus; FSH: Follicle stimulating hormone; IVF: *In-vitro* fertilization; Myo-LA: Myometrium, lower half of the uterine corpus, anterior; Myo-LP: Myometrium, lower half of the uterine corpus, posterior; Myo-UP: Myometrium, upper half of the uterine corpus, posterior; OR: Odds ratio; SE: Standard error

E-cadherin and progesterone receptor expression levels (13). It may be that myometrial tissue with increased stiffness consists of fibrotic tissues that have a higher response to estrogen but a lower response to progesterone (18). Another study that evaluated 101 secondary infertile patients with endometrial elastography demonstrated an increase in endometrial stiffness in patients with chronic endometritis (19). Given the knowledge that endometritis adversely affects implantation (20), one could conclude that there may be a relationship between increased endometrial stiffness and infertility. Finally, it has been reported that cervical elasticity measurement may predict difficulties in the embryo transfer (21–23).

The promising results seen thus far led us to design a study designed to evaluate the relationship between recorded sonoelastography features of the endometrium and the myometrium and the outcome of IVF treatment. Since there is no standardized elastography measurement protocol for this kind of examination, the existing system of rules was observed (11, 12). Taken in sum, our results did not reveal any significant difference in the elastography measurements of each target field between the women who conceived and the women who did not conceive. This was somewhat unexpected, since a possible effect of endometrial/myometrial elasticity on fertility has been implied in several studies. Interestingly, however, among all of the elasticity indices we studied, the posterior uterine wall elasticity index did demonstrate a statistically significant association with the outcome of IVF treatment. The ratio of the upper half of the posterior uterine wall measurement to that of the lower half was higher in women who conceived compared with those who did not.

Univariate analysis of the individual indices also revealed a significant association. This is notable, given that the implantation site in the human uterus is usually in the upper and posterior wall in the midsagittal plane (24). In addition, in the women who successfully achieved conception, there was a trend toward a lower ratio of the lower half of the posterior uterine wall measurement to that of the lower half of the endometrium, confirming data that the stiffness of the lower half of the posterior uterine wall measured in those who conceived was lower than that of those who did not, despite being statistically nonsignificant. Given that successful implantation requires a receptive endometrium as well as a muscle layer without contraction (25, 26), our findings may reflect a negative correlation between myometrial stiffness and uterine contraction. In this respect, we recommend that the impact of elastographic features of the posterior uterine wall on the fate of pregnancy needs to be further investigated with a larger series.

There are some limitations of this study that need to be addressed. Perhaps most importantly, we performed only a single measurement in each target field, as in the study used as a basic guide for this research. Multiple values (i.e., at least 3 measurements) at each point and using the averages in the analysis would be preferable. Another limitation was the design of the study itself: the impact of independent variables was based on the elevation of βHCG level following the transfer, but a study design that includes greater detail would perhaps yield important new results. Nevertheless, measurements performed by a single radiologist constitute a valuable and important aspect of the present study.

CONCLUSION

Sonoelastography could aid IVF clinicians in predicting the success of pregnancy due to its capability to reflect histological properties of the uterus, just as in all soft tissues. The creation and use of a standardized examination protocol is necessary to provide maximal benefit from the potential of this valuable tool.

Ethics Committee Approval: The Ondokuz Mayıs University Clinical Research Ethics Committee granted approval for this study (date: 13.12.2019, number: 2019/832).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – FU, AZÖ, BA, AİS, DG, ARAD; Design – FU, AZÖ, BA, AİS, DG, ARAD; Supervision – FU, AZÖ, BA, AİS, DG, ARAD; Resource – FU, AZÖ, BA, AİS; Materials – FU, AZÖ, BA, AİS, DG, ARAD; Data Collection and/or Processing – FU, AZÖ, BA; Analysis and/or Interpretation – FU, AZÖ, BA; Literature Search – FU, AZÖ, ARAD; Writing – FU, AZÖ; Critical Reviews – FU, AZÖ, BA, DG.

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