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Uterine Artery and Endometrial Doppler Velocimetry before Embryo Transfer in Intra-cytoplasmic Sperm Injection: Influence on Cycle Success

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ABSTRACT

Background: Unfortunately, seeking in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) procedure is not a guarantee of success or achieving live birth. Recurrent failure of implantation remains a distressing event. There are strong efforts to assess endometrial receptivity particularly in cases subjected to assisted reproductive technology.

Objective: This study aimed to assess the role of estimating uterine artery blood flow (UABF) as well as endometrial blood flow via Doppler ultrasound prior to embryo transfer in IVF/ET cycles in prediction of pregnancy rate.

Subjects and methods: This prospective observational investigation was carried out on 80 women who were scheduled for ICSI cycles that was conducted at private ICSI centers, durıng the perıod from October 2021 tıll October 2022.

Results: The mean endometrial thickness was higher in women who got pregnant after ICSI compared to non-pregnant women after ICSI) [10.24 ± 1.6mm vs. 9.5 ± 1.89 mm respectively, P=0.05]. Additionally, the sub-endometrial indices " PI, RI, and S/D" & uterine artery RI and S/D ratio were all significantly lower in women who got pregnant than in non-pregnant women after ICSI (P<0.001). Besides, uterine artery PI was also significantly lower in pregnant cases after ICSI (P=0.001).

Conclusion: Both uterine artery RI, and uterine artery S/D ratio had significantly the best diagnostic accuracy followed by sub-endometrial RI and sub-endometrial S/D ratio. So uterine artery and sub endometrial Doppler indices may be used independently or better combined with the other factors for configuration of a predictive algorithm for ICSI implantation or pregnancy rates prediction.

Keywords: intracytoplasmic sperm injection (ICSI), Uterine artery Doppler indices, Subendometrial Doppler indices, PI, RI, S/D ratio.

INTRODUCTION

This era witnesses a breakthrough in the art of assisted reproductive technology (ART) including intracytoplasmic sperm injection (ICSI) which has awakened the hope of the infertile couples who were formerly unable to conceive or to get viable pregnancy [(1)]. Unfortunately, seeking in vitro fertilization (IVF) or ICSI procedure is not a guarantee of success or achieving live birth [(2)]. Yet, along days, ICSI are upgrading along time for the seek of achieving better acceptable pregnancy rates, less multiple births, and more healthy offspring [(3)]. Even with these achievements and the mounting experiences in this field, among the major challenges is implantation failure. Recurrent failure of implantation remains a distressing event that faces both the patients and the physician and requires a multitude of investigations with its management lines still a debate [(4)].

The crucial phase of implantation is now what prevents ICSI procedures from becoming more successful. The implantation process necessitates an intimate cross-talk bringing together a healthy embryo along with a receptive endometrium. This mechanism is orchestrated by physiological, immunological as well as molecular pathways that finally bring about and apposition, adhesion of the blastocyst to the endometrium, then trophoblast invasion into endometrial epithelial cells. All of this is mandatory for effective implantation coupled with consequent normal placentation [(5)].

It has been estimated that up to 65% of suboptimal implantation are triggered by endometrial receptivity defects. There are strong efforts to assess endometrial receptivity particularly in cases subjected to assisted reproductive technology [(6)]. Up till now, thanks to its accuracy, repeatability, real-time monitoring, in addition to higher predictability as well as non-invasiveness, transvaginal ultrasound has emerged and continued as indispensable modality in the assisted reproduction specialty, not only for monitoring follicles, but also for assessing endometrial receptivity throughout infertility treatment [(7)].

Endometrial thickness (EMT) acts as one of the most extensively researched markers for numerous years on behalf of evaluating endometrial receptivity for revealing its connotation to pregnancy success rates but results were controversial [(3)]. It has been broadly assumed that a thin endometrium is concomitant with lesser conceiving probability following IVF/ICSI and a cut-off values varying in-between 7–9 mm with triple layered endometrial pattern are the supposed endometrial thickness and pattern markers of endometrial receptivity in prior studies. Yet, a question has been addressed about the EMT threshold value above which implantation is doubtful to occur with the conflicting results [(8)].

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Uterine perfusion in terms of uterine and endometrial tissue blood flow is a crucial factor reflecting uterine receptivity. Therefore, measuring the uterine arterial impedance via uterine artery Doppler could be more objectively and reliably an efferent marker the endometrial receptivity [(9)]. Aberrant uterine artery velocimetry is proved to be coupled with unexplained sub-fertility, failed to implantation due to suboptimal uterine receptivity, plus recurrent miscarriages [(10)]. However, there are contradictory reports about their use in the IVF/ICSI therapy for pregnancy prediction [(6)]. To date, endometrial receptivity markers are still inconclusive and there is still no widely standardized procedure that has been acknowledged worldwide for the endometrial receptivity evaluation [(11)].

Therefore, the purpose of our study was to determine whether uterine artery Doppler can help to perform embryo transfers in only favorable uteri and postpone or cancel those cycles in which poor uterine score is evident, as well as to assess the role of estimating uterine artery blood flow (UABF) as well as sub-endometrial blood flow via Doppler ultrasound prior to embryo transfer in IVF/ET cycles in prediction of pregnancy rate.

PATIENTS AND METHODS

This prospective observational research was carried out on 80 women who were scheduled for ICSI cycles that was conducted at private ICSI centers, durıng the perıod of study that extended from October 2021 tıll October 2022 to assess the role of estimating UABF via Doppler ultrasound prior to embryo transfer in IVF/ET cycles in prediction of pregnancy rate.

$$n_{Case} = n_{Non-case} \geq \frac{Z^2_{1-\alpha/2} V(AUC)}{d^2}$$

$$V(AUC) = \left(0.0099 \times e^{-a^2/2}\right) \times (6a^2 + 16)$$

$$a = Z_{AUC} \times 1.414$$

Sample size justification: Based on previous study [(12)] who found that accuracy of uterine artery P1 in differentiation between control group and unexplained infertility group was 96.2% (Area under curve 0.962). Minimum sample size was calculated to be at least 72 females scheduled for ICSI cycles to be increased to 80 for assuming 10% drop-out rate according to the following formula:

Where; ncase: sample size, Alpha (α): Type 1 error, d: estimation error, AUC: Area under curve= 0.962.

Inclusion criteria: Females aged from 20 to 40 years old with body mass index (BMI) not exceeding 35 kg/m^2, having regular cycles and with indications of ICSI due to various etiologies of infertility (male or unexplained factors), having patent tubes as checked by: (Hysterosalpingography (HSG) or Previous laparoscopy (if done) and normal uterine cavities and prepared by antagonist protocol with good quality of embryos were transferred.

Exclusion criteria: Patients aged more than 40 years old or if their body mass index was more than 35 kg/m^2. Also cases who had inappropriate endometrium for implantation, females with tubal factor infertility (after pelvic inflammatory illness or with hydrosalpinges), uterine abnormalities, and/or evident endometrial pathology (polyps, submucous myoma, or synechia). Women whose infertility is due to endocrine disorders, a history of inadequate response to ovarian stimulation, and clinically significant systemic conditions including diabetes mellitus, hypertension, malignancy of any location, uncompensated heart diseases, premature ovarian failure, advanced endometriosis (Stage III and IV) and any contraindication to pregnancy.

The chosen volunteers had rigorous history-taking, a full physical examination, an abdominal examination, blood tests, and ultrasound imaging to determine their suitability for ICSI. Semen analysis for the husband was done. According to WHO criteria, semen volume, sperm count and normal sperm morphology were all assessed.

ICSI technique:

Controlled ovarian Hyperstimulation (COH) via the antagonist protocol was used. ICSI stimulation was started with gonadotrophin injection from day 2 of the cycle. Gn RH antagonist injections to prevent premature LH surge was given either as 0.25 mg Ganirelix or citrorelix (Cetrotide®, Merck KGaA) daily from day 7 of the cycle (6 of stimulation) at the same time till and including day of triggering.

Prior to meeting the trigger criterion, ultrasound folliculometry was done every other day starting on the sixth day of Gn stimulation. Human chorionic gonadotropin (hCG) 10 000 IU (Choriomon, IBSA, Institut Biochimique, Switzerland) was used to initiate ovulation when two or more follicles reached a minimum mean diameter of 18 mm, and ovum pick up was planned for 36 hours later. Transvaginal oocyte retrieval was executed under general anesthesia 36 hours later after triggering.

In the line with that, fresh semen specimen from the husband was taken, processed after microscopic examination to achieve motile and morphologically normal sperm cells. Oocytes then were examined for integrity and graded then mature metaphase II ones were inseminated by ICSI procedures and embryos were cultured. On day 3, embryos were assessed and graded, and extra embryos were continued grown until they reached the blastocyst stage on day 5. Top-notch embryos were obtained using Gardner morphological criteria. 2 to 3 embryos from the best quality for each patient were transferred to mother uterus. 3-5 days following the ovum collection operation, embryos were transferred utilizing a Labotect semi-rigid catheter

(Labotect GmbH, Germany) under the supervision of trans-abdominal ultrasound. The vitrification procedure for the remaining embryos upon couple request was performed following standard protocols using Kitazato Freeze Kit.

Luteal-phase support consisted of daily intramuscular (IM) progesterone (Prontogest, IBSA, Institut Biochimique, Switzerland) (100 mg) coupled to rectal progesterone suppository (Prontogest, IBSA, Institut Biochimique, Switzerland) (400 mg), from the day of oocytes retrieval until serum β-HCG level was measured to confirm pregnancy.

Transvaginal ultrasonography (TVUS) of the endometrium

All ultrasound measurements were performed via SONOSCAPE (S50 Elite, China) device with transvaginal probe. On the day of the embryo transfer (ET), a preliminary transvaginal ultrasound was performed. After the patient emptied her bladder and while she was in the lithotomy position, an ultrasound examination was done. The endometrium's morphology (endometrial pattern) was evaluated, and the maximum thickness of the endometrium was determined in the midsagittal plane.

Uterine artery blood flow: Uterine artery wave form were obtained with software calculation of pulsatility (PI) and resistance indices (RI) & S/D ratio were calculated and average values were recorded (figures 1 & 2). Moreover, with a longitudinal view of the uterus, vascular signals were quantified. Sub-endometrial PI, RI & S/D ratio were calculated and average values were recorded and assessed (figures 3 & 4).

Pregnancy screening: Patients performed a serum beta HCG titer 2 weeks after embryo transfer.

Study Outcomes: Clinical pregnancy rates were the primary outcome of the study established by the presence of viable gestational sac/sacs with fetal cardiac pulsation on TVUS scan 6 weeks following embryo transfer. Secondary outcomes included chemical pregnancy rate by Serum β-hCG level at about 14 days after ET and the result wasn't deemed as being positive except for the values beyond 5 IU/ L, ongoing pregnancy rates and the usefulness of Doppler parameters to assess endometrial receptivity in patients undergoing ICSI.

Ethical approval: Faculty of Medicine, Menoufia University's Quality Education Assurance Unit granted the ethics committee approval. Before receiving their written agreement to participate in the current investigation, all participants received a thorough and understandable explanation of the study. The trial coordinator routinely checked the quality of screening, data management, and protocol adherence. All procedures involving human participants in this study were conducted in accordance with the standards indicated in the World Medical Association's Declaration of Helsinki on the conduct of research involving human participants.

Statistical analysis

Statistical analyses of data were executed via SPSS version 23. Normal distribution of variables was assessed through Shapiro–Wilks test. Numerical variables were summarized as mean ± standard deviation (SD) or median and range. Categorical variables were expressed as percentages. The significance for groups' difference was calculated via two-tailed Student’s t test for quantitative values or chi-squared χ^2test for qualitative variables. Statistical significance was indicated for probability (P) values ≤ 0.05. To determine the most sensitive and particular cutoff settings for Doppler indices using the area under the curve (AUC), the Receiver Operating Characteristic (ROC) was developed.

RESULTS

This prospective observational study comprised 80 women whose overall age ranged from 23 to 39 years with a mean age of 30.84 ± 3.89 years and a mean BMI of 26.76 ± 2.53 kg/m^2. 42 (52.5%) of cases lived in rural areas while 38 (47.5%) women resides in urban areas. They were scheduled for ICSI cycles. According to the primary study outcome or the clinical pregnancy rate, our results revealed that 34 out of 80 (42.5%) women got pregnant after ICSI (Table 1).

The couples included in this study had average 7.1 ± 2.85 years of marriage (range: 2-15 years). Duration of infertility ranged between 2-12 years (Mean= 6.4 ± 2.4 year). Infertility was primary in 62 (77.5%) of the women included in this study while it was secondary in other18 women (22.5%). We evaluated the numerous etiological causes of subfertility. According to WHO 2010 criteria, it was found that 38 (47.5%) of the couples had male factor infertility. Unaccounted for infertility affected 18 (22.5%) of the women (Table 1).

In the 62 women with primary infertility, male factor was the most common cause in 29 (46.8%), unexplained infertility was found in 11 out of (17.7%) and ovulation factor or both of male and female factors in (12(19.4%), and 10 (16.1%) respectively. However, in women with secondary infertility, 9 (50%) had male factor, 7 (38.9%) had unexplained infertility, and both male factor infertility and ovarian infertility was represented in 2 (11.1%). Oligospermia, asthenozoospermia, azoospermia, teratozoopermia, oligo-athenozoospermıa and oligo-astheno-teratozoospermia were detected in 10%, 8.75%, 2.5%, 7.5, 13.75% and 20% of cases respectively. Nevertheless, 37.5% of men had good semen quality. Whereas, the remaining 37.5% of males had good semen quality according to WHO 2010 semen analysis parameters (Table 1).

In most cases in the current study, it was the first ICSI trial in 59 (73.8%) of women. 17 out of 80 had one previous ICSI attempt and 4 out of 80 women had two previous ICSI attempts. In the current ICSI trial, a single embryo was transferred to 22.5% of cases, two embryos were transferred in 32.5% of cases and three embryos were transferred to the remaining 45% of cases. In the present study the quality of transferred embryos was grade 1 in 96.2% of cases and grade 2 was only transferred to 3.8% of patients (Table 1).

Table (1): Demographic characteristics and data about the current ICSI trial of the studied cases

Variables	Mean ±SD	Frequency (n=80)	Percentage (%)
Age (years)			
20-25	30.84 ± 3.89	7	8.8%
26-30		36	45%
31-35		24	30%
>35		13	16.3%
BMI (Kg/m²)			
Underweight: ≤18.5	26.76±2.53	3	3.75%
Normal: 18.5-25		20	25%
Overweight 25-30		44	55%
obese>30		13	16.25%
Residence			
Rural	-	42	52.5%
Urban	-	38	47.5%
Years of Marriage	7.1±2.85	-	-
Years of infertility	6.4±2.4	-	-
Infertility type			
Primary		62	77.5%
Secondary		18	22.5%
Infertility cause			
Male factor	-	38	47.5%
Unexplained	-	18	22.5%
Ovarian	-	12	15%
Male + Ovarian	-	12	15%
Semen quality of the spouse			
Good qualıty	-	30	37.5%
Olıgospermıa	-	8	10%
Athenozoospermıa	-	7	8.75%
Azospermıa	-	2	2.5%
Tetrozoospermıa	-	6	7.5%
Oligo- athenospermıa	-	11	13. 75%
Oligo-astheno-teratozoospermia	-	16	20%
Prior ICSI Trials			
No (current trial is the first trial)	-	59	73.8%
One	-	17	21.2%
Two	-	4	5%
Number of embryo transfer in the current ICSI cycle			
One	-	18	22.5%
Two	-	26	32.5%
Three	-	36	45%
Quality of embryo transfer in the current ICSI cycle			
Grade 1	-	77	96.2%
Grade 2	-	3	3.8%
Clinical pregnancy rate in the current ICSI cycle			
No	-	46	57.5%
Yes	-	34	42.5%

Regarding the demographic data of pregnant and non-pregnant ICSI cases, no statistically substantial variation existed between both groups (women who get pregnant and non-pregnant women after ICSI) as regards age (P-value=0.841), BMI (P-value=0.53), marriage years (P-value =0.944), and years of infertility (P-value=0.688). Moreover on hormonal assessment that was within normal range in the scheduled cases, no statistically substantial variation existed between both groups regarding LH level (P-value =0.643), TSH level (P-value=0.579), estradiol level (P-value =0.284), and prolactin level (P-value=0.095). However, FSH was substantially higher in pregnant women (7.17 ± 2.04 mIU/ml) compared to the non-pregnant counterparts (5.7 ± 1.05 mIU/ml), (P < 0.001) (Table 2).

Table (2): Comparison between of pregnant and non-pregnant cases regarding the available data other than ultrasound

		Non pregnant women (N=46)		Pregnant women (N=34)		P
Age (years)	**Mean ± SD**	30.9	4.04	30.74	3.7	0.841
BMI(kg/m^2)	**Mean ± SD**	26.7	2.35	26.8	2.8	0.897
Years of marriage (Years)	**Mean ± SD**	7.1	2.77	7.13	2.99	0.944
	Minimum-maximum	2	15	3	15	
Years of infertility (Years)	**Mean ± SD**	6.47	2.6	6.25	2.1	0.688
	Minimum-maximum	2	12	3	11	
LH(mIU/ml)	**Mean ± SD**	5.4	1.54	5.6	1.31	0.643
FSH(mIU/ml)	**Mean ± SD**	5.7	1.05	7.17	2.04	**<0.001****
Estradiol E2(pg/ml)	**Mean ± SD**	47.9	4.2	51.11	11.7	0.284
TSH(mIU/ml)	**Mean ± SD**	2.56	1.1	2.69	0.96	0.579
Prolactin(ng/ml)	**Mean ± SD**	15.2	3.7	13	3.97	0.095

P-value >0.05 was considered insignificant.*P-value ≤0.05 was considered significant.**P-value ≤ 0.001 was considered as highly significant.

Regarding our aim in evaluating the ultrasound and Doppler findings among the studied cases at day of ET to forecast the outcome, the present study showed that the mean endometrial thickness was higher in women who got pregnant after ICSI compared to non-pregnant women after ICSI [10.24 ± 1.6 mm vs. 9.5 ± 1.89 mm respectively, P=0.05]. Additionally, the sub endometrial indices " PI, RI and S/D" were substantially lower in women who got pregnant [0.94 ± 0.09, 0.69 ± 0.06, and 2.22 ± 0.1 compared to 1.19 ± 0.32, 0.79 ± 0.07, and 2.53 ± 0.25 respectively in non-pregnant women after ICSI, (P<0.001). Besides, uterine artery PI was 2.28 ± 0.48 in women who got pregnant and 2.7 ± 0.55 in women who failed to be pregnant after ICSI (P=0.001). With regard to uterine artery RI and S/D ratio, substantial variation was found also when comparing women who got pregnant and those who failed to get pregnant after ICSI, as means of RI and S/D ratio were substantially lower in the former (0.81 ± 0.04 and 6.39 ± 1.19) than in the latter (1.06 ± 0.29 and 8.4 ± 1.25) group respectively (p<0.001) (Table 3) and (Figures 1, 2, 3 & 4).

Table (3): Comparison between of pregnant and non-pregnant cases regarding Ultrasound and Doppler assessment data

		All Cases N=80		Non pregnant women (N=46)		Pregnant women N=34		P
Endometrial thickness (mm) on day of ET	**Mean ± SD**	9.79	1.8	9.5	1.89	10.24	1.6	**0.05***
	Minimum-maximum	6	13	6	13	7	13	
Sub-endometrial PI	**Mean ± SD**	1.08	0.28	1.19	0.32	0.94	0.09	**<0.001****
	Minimum-maximum	0.78	2.48	0.88	2.48	0.78	1.12	
Sub-endometrial RI	**Mean ± SD**	0.75	0.08	0.79	0.07	0.69	0.06	**<0.001****
	Minimum-maximum	0.59	0.93	0.61	0.93	0.59	0.81	
Sub-endometrial S/D	**Mean ± SD**	2.4	0.26	2.53	0.25	2.22	0.1	**<0.001****
	Minimum-maximum	2.05	3.88	2.17	3.88	2.05	2.41	
Uterine artery PI	**Mean ± SD**	2.5	0.56	2.7	0.55	2.28	0.48	**0.001****
	Minimum-maximum	1.25	3.95	1.3	3.95	1.25	3.11	
Uterine artery RI	**Mean ± SD**	0.95	0.25	1.06	0.29	0.81	0.04	**<0.001****
	Minimum-maximum	0.74	2.18	0.75	2.18	0.74	0.9	
Uterine artery S/D	**Mean ± SD**	7.6	1.6	8.4	1.25	6.39	1.19	**<0.001****
	Minimum-maximum	2.06	11.8	6.21	11.75	2.06	8.35	

P-value >0.05 was considered insignificant/ *P-value ≤0.05 was considered significant/ **P-value ≤0.001 was considered as highly significant.

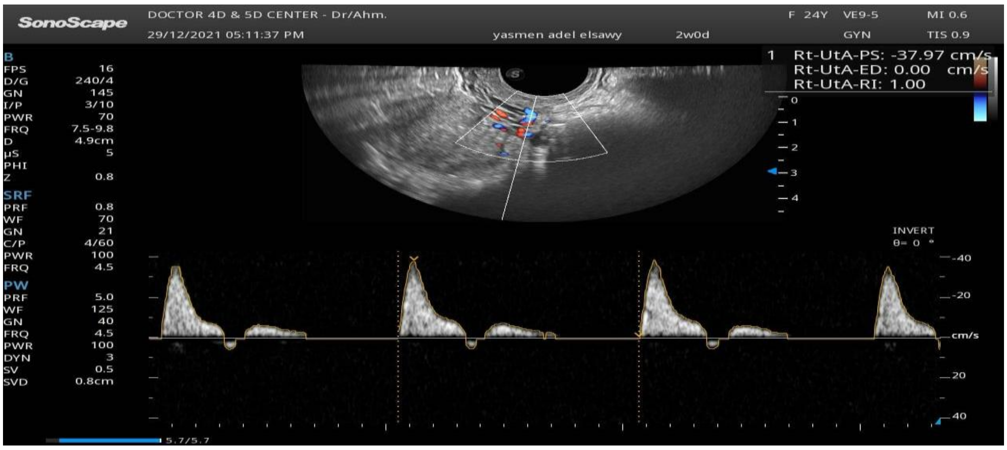

Figure (1): Uterine artery Doppler results of case who get pregnant after ICSI.

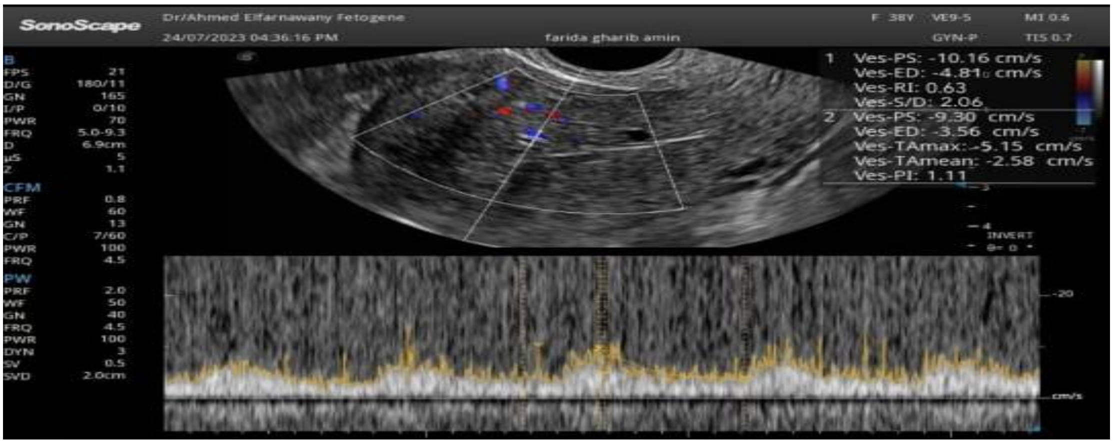

Figure (2): Uterine artery Doppler results of a case who failed to get pregnant after ICSI.

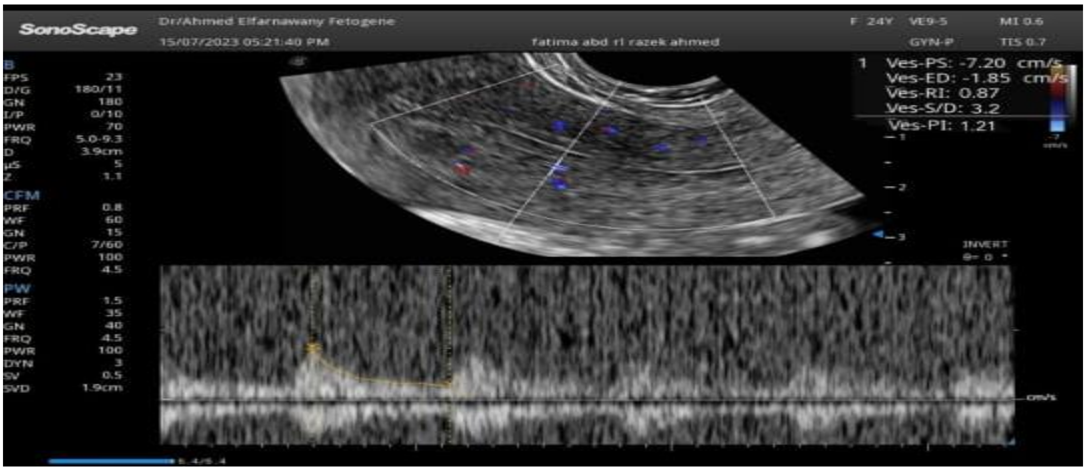

Figure (3): Subendometrial Doppler of a case who got pregnant after ICSI.

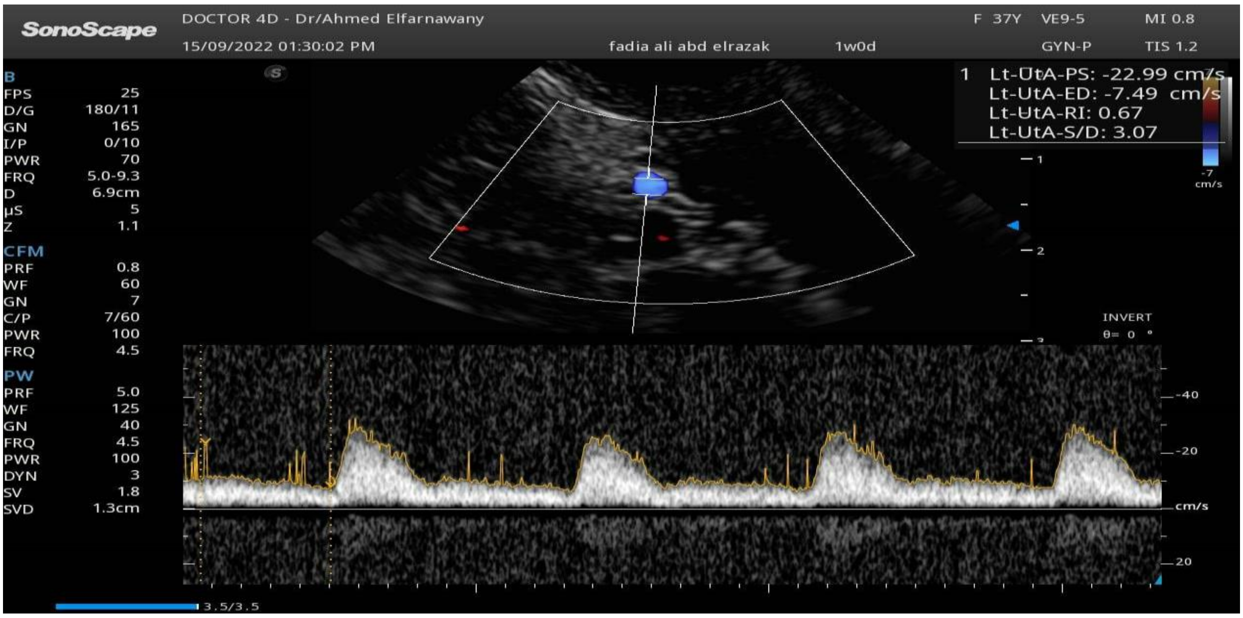

Figure (4): Subendometrial Doppler of a case who failed to get pregnant after ICSI.

Besides ROC curve analysis showed that both uterine artery RI, and uterine artery S/D ratio had significantly the best diagnostic accuracy (85% & 83.75% respectively) than other parameters in predicting occurrence of pregnancy after ICSI trial with the optimum cutoff values for uterine artery RI and S/D ratio were 0.875 and 7.325 for predicting occurrence of pregnancy after ICSI trial with specificity of 80.4% of both markers and sensitivity of 91.2% and 88.2% respectively (P<0.001). Also, sub-endometrial RI, and sub-endometrial S/D ratio were found to be good predictors for occurrence of pregnancy after ICSI trial (accuracy 80%& 81.25% respectively). While, the cut off values of endometrial thickness, sub-endometrial PI, and uterine artery PI were 8.5 mm, 1.075, and 2.58 respectively for predicting occurrence of pregnancy after ICSI trial with accuracy 55% , 70%& 73.8% respectively (Table 4 & figures 5 & 6).

Table (4): ROC analysis of Ultrasound and Doppler assessments in predicting outcome of ICSI trial

Parameters	Cutoff	AUROC	Sensitivity	Specificity	PPV	NPV	Overall Accuracy
Endometrial thickness (mm)	**8.5**	**0.618**	**82.4%**	**34.8%**	**48.3%**	**72.7%**	**55%**
Sub-endometrial PI	**1.075**	**0.825**	**91.2%**	**60.9%**	**63.3%**	**90.3%**	**73.8%**
Sub-endometrial RI	**0.74**	**0.835**	**82.4%**	**78.3%**	**73.7%**	**85.7%**	**80%**
Sub-endometrial S/D ratio	**2.315**	**0.886**	**79.4%**	**82.6%**	**77.1%**	**84.4%**	**81.25%**
Uterine artery PI	**2.58**	**0.718**	**79.4%**	**63%**	**61.4%**	**80.6%**	**70%**
Uterine artery RI	**0.875**	**0.916**	**91.2%**	**80.4%**	**77.5%**	**92.5%**	**85%**
Uterine artery S/D	**7.325**	**0.902**	**88.2%**	**80.4%**	**76.9%**	**90.2%**	**83.75%**

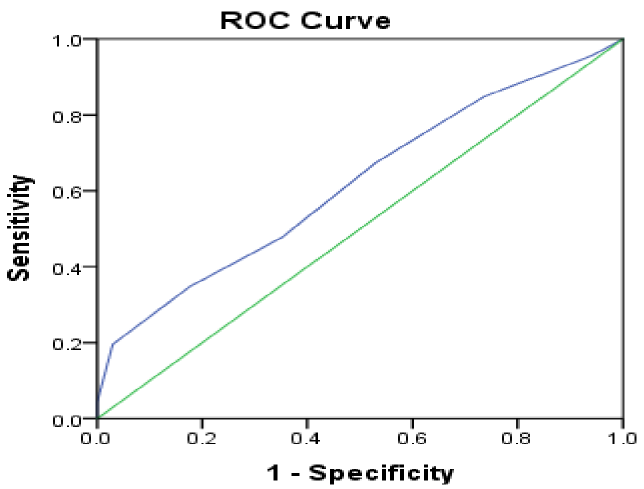

Figure (5): ROC curve analysis of endometrial thickness for predicting occurrence of pregnancy after ICSI trial.

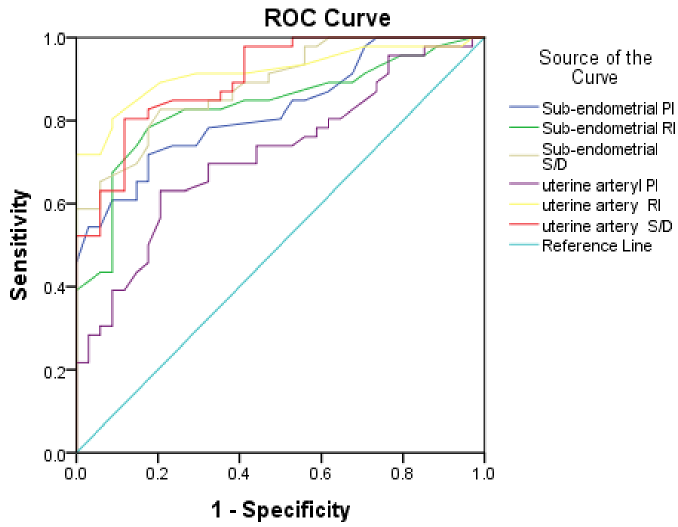

Figure (6): ROC curve analysis of Doppler parameters (Sub-endometrial PI, RI, S/D ratio & uterine artery PI, RI, and S/D ratio) for predicting occurrence of pregnancy after ICSI trial.

DISCUSSION

The protocol used in our work for the purpose of controlled ovarian hyperstimulation (COH) was the antagonist protocol for the entire cases. **Arora *et al.*** [13] also chose the antagonist protocol for their study. While, in **Hussain *et al.*,** [14] study 33.3% were stimulated via the antagonist protocol, 60% by the short agonist and the remaining 6.7% by the long agonist one. Yet, **Prasad *et al.*,** [10] reported that the ovarian stimulation in their study was achieved by long agonist protocol or antagonist protocol.

Although the long GnRH agonist (GnRH-a) protocol is a conventional protocol, possibly the most widely consumed throughout the world. The introduction of GnRH antagonists (GnRH-ant) in ART to prevent LH surge opened up the gate to what is called friendly IVF/ICSI [15]. Thus, our choice to the GnRH antagonist protocol is reinforced by other studies that mentioned it as the favored protocol for ICSI patients especially in polycystic ovary syndrome (PCOS) cases bearing in mind the less cost-effectiveness, the reduction of ovarian hyperstimulation syndrome (OHSS) risk, the brief stimulation time and the better probability of pregnancy [16, 17].

Regarding comparison between pregnant and non-pregnant cases, there was no statistically substantial variation between the two groups (women who get pregnant after IVF and non-pregnant women after IVF) regarding age (P-value=0.841), BMI (P-value=0.53), years of marriage (P-value =0.944), and years of infertility (P-value=0.688), or the LH level (P-value =0.643), TSH level (P-value=0.579), Estradiol level (P-value =0.284), and Prolactin level (P-value=0.095). However, FSH hormone was significantly higher in women who got pregnant (7.17 ± 2.04 mIU/ml) compared to those failed to be pregnant (5.7 ± 1.05 mIU/ml), (P<0.001). In line with our results, **Ragheb and his colleagues** [6] found that in terms of age (p = 0.068), BMI (p = 0.53), LH (p = 0.156), E2 (p = 0.75) and TSH levels (p = 0.52), there was no statistical substantial variation between the two groups. Also, the mean FSH hormone was significantly different among both groups (P= 0.048). Yet, unlike our findings, statistically significant difference was existing regarding serum prolactin (P<0.001), duration of marriage (P =0.04) and duration of infertility (P =0.0399), between their pregnant and non-pregnant groups. Our finding are in agreement with **Maged *et al.*** [18] who showed that age, BMI, duration, type, and cause of infertility, basal hormonal levels (including basal FSH, LH, E2, TSH, and prolactin), and E2 levels did not differ statistically (P>0.05). **Moustafa** [3] in a recent work also reported no substantial variation between both groups concerning basal characteristics, hormonal data, duration of infertility, and infertility causes (P > 0.05). This also is in the case of **Abuelghar *et al.*** [19] study. Any difference between the results may be attributed to the discrepancy in the inclusion and exclusion criteria of the included female cases and their varying hormonal status between our study and others.

Our results revealed that infertility was primary in 62 (77.5%) of the women included in this study while it was secondary in other18 women (22.5%). **Maged *et al.*** [18] reported that the percentage of primary infertility in their work was 67%, while the remaining 33% complained of secondary infertility. Also, in **Sini *et al.*** [20] study 24 (82.8%) had primary infertility compared to 5 (17.2%) cases with secondary infertility.

It was observed in our study that 38(47.5%) of couples had male factor, 12 (15%) women had ovarian causes, 18 (22.5%) had unexplained infertility and both male factor infertility and ovarian infertility were detected in 12 (15%) of women. Moreover, male factor was the most common cause (46.8%) in couples with both primary as well as secondary infertility 9 (50%). Our results are in agreement with **Sini *et al.*** [20] who revealed that 41.4% of their couple had infertility due to male factor and 37.9% complained of unexplained infertility. **Moustafa** [3] also agrees with us in that most ICSI cases were due to male factor infertility (35%). And this is similar to **Ragheb *et al.*** [6] study as 45% had male factor followed by 23.5% unexplained infertility in their findings. Unlike our findings, **Maged *et al.*** [18] didn't exclude tubal factors of infertility that accounted for the most common causes in their study (40.5%) followed by unexplained infertility (22.5%).

Regarding prior ICSI trials, our results revealed that in most cases it was the first ICSI trial in 59 (73.8%) of women. 21.2% had previous one ICSI attempt and 5% women had two previous ICSI attempts. Our results are nearly similar to that of **Maged and his colleagues** [18] whose most cases also were in their first trial (82.2%) and the remaining 17.8% had ≥ 2 attempts. While, **Ragheb *et al.*** [6] only 51.75% has not formerly exposed to prior ICSI cycles and the remaining nearly half of cases had unsuccessful trials before. Another study agrees with us in their ICSI patient data was **Abuelghar *et al.*** [19] that reported that the average age of the women examined was 27.9 ± 4.2 years, their average BMI was 28.3±5.3 kg/m2, and the average duration of infertility was 4.9±3.1 years. 76% of their studied women were for the reason that of primary infertility, and 24% were due to secondary infertility and 24% of their studied women had prior ICSI trials, while the remaining 76% of the studied cases had no prior ICSI trials. In addition in our study, a single embryo was transferred to 22.5% of cases, two embryos were transferred in 32.5% of cases, while three embryos were transferred to the remaining 45% of cases**.** The transferred embryos were grade I in 96.2% of cases and grade 2 was only transferred to 3.8% of patients. In concordance to our findings, **Ragheb *et al.*** [6] reported that single embryo was transferred to 35% of cases, while two embryos were transferred to 30% of and the remaining 35% of cases had three embryos transferred with 96.75% of embryos were grade I too. **Sini *et al.*** [20] also reported that the median number of embryo transferred was 1 (ranged from 1–2). **Ibrahim *et al.*** [20] in 2020 also reported that the median number of transferred embryos were 2.16 ± 0.73 and the entire of them were grade 1 (100%).

Regarding the main outcome of our study, clinical pregnancy was achieved in 34 out of 80 (42.5%) of the included cases. In line to our findings, the clinical pregnancy rates were 41.8% in **Wang *et al.*** [21], 46.25% in **Ragheb *et al.*** [6], 40.2% in **Arora *et al.*** [13], 37.9% in **Sini *et al.*** [2)] and 39% in **Moustafa** [3]. While lower success rates were reported in **Sardana *et al.*** (35.43%) [22]**, Abuelghar *et al.*** (36%) [19] & **Maged *et al.*** (25.5%) [18] that may be owed to different patient criteria or different protocol used.

Regarding our aim in measuring the ultrasound and Doppler assessment among studied cases at day of ET to forecast the outcome, the present research showed that the median endometrial thickness was greater in women who got pregnant after ICSI compared to non-pregnant women after ICSI [10.24± 1.6mm vs. 9.5±1.89 mm; respectively; P=0.05]. Additionally, the sub endometrial indices " PI, RI, and S/D" & uterine artery RI and S/D ratio were all significantly lower in women who got pregnant than in non-pregnant women after ICSI, (P<0.001). Besides, uterine artery PI was also significantly lower in pregnant cases after ICSI (P=0.001).

There is still a paucity of data regarding the use of Doppler parameters (Sub-endometrial & uterine artery PI, RI, and S/D ratio) at the time of ET for predicting occurrence of pregnancy after ICSI trial. **Moustafa** [3] in agreement with our results found that subendometrial PI, RI were substantially decreased in the pregnant group (1.35 ± 0.13 and 0.82 ± 0.07 respectively) compared to those who did not achieve pregnancy (1.58 ± 0.21 and 0.85 ± 0.06 respectively) (p<0.001 &P= 0.003) in order. They also reported that ET was significantly increased (11.38 ± 0.41mm) in the pregnant cases vs 10.83 ± 0.39 mm in the non-pregnant counterparts (P<0.001). In concordance to our results also, **Adibi and his colleagues** [23] declared that their study included 65 IVF women; 32 (49.2%) and 33 (50.8%) of these women had successful and failed IVF outcomes, respectively. Women who experienced successful IVF treatment outcomes had substantially lower mean (SD) PI and RI values (P <0.01) than those who did not. **Wahab and colleagues** [24] stated that good quality uterine and endometrial blood flow is a crucial prerequisite for effective implantation and pregnancy maintenance and lower endometrial blood flow presented as higher uterine artery flow resistance is concomitant with recurrent pregnancy loss. Unlike our results, **Ragheb and his colleagues** [6] in comparison between pregnant and non-pregnant ICSI groups as regards ultrasound and Doppler parameters reported that there were no statistically substantial variation between both groups regarding the mean endometrial thickness, Sub-endometrial RI & S/D ratio as well as uterine artery PI, RI, and S/D ratio. The only marker in their work that yielded statistically significant difference was the Sub-endometrial PI (P= 0. 01). **Kader *et al.*** [25] **in a** prospective observational investigation that was conducted on 100 IVF/ICSI women, and the findings showed that there was no statistically important distinctions in the Doppler indices of the sub-endometrial blood vessels between pregnant and non-pregnant women when EMT, morphology, and subendometrial blood flow were assessed using TVUS on the day of hCG. **Ng *et al.*** [26] concluded in older work that the 3D-power measurement of the vascularity of the endometrium and subendometrial layers when examined at only one time point, Doppler ultrasonography was not a reliable indicator of pregnancy during stimulated IVF and fresh embryo transfer cycles. However, the fact that endometrial and subendometrial vascularity was much greater in expectant women who delivered a live child than in those who had a miscarriage supports us in their study. Varying research designs, varying numbers of patients included, various endometrial preparation protocols, endometrial thickness at the moment of transfer, and the Doppler technology used to measure endometrial blood flow may all contribute to the discrepancy from our results. **Prasad *et al.*** [10] suggested that Doppler parameters may alter amongst women with various causes of infertility. Those with male and tubal factors have been reported to have lower PSV and uterine perfusions than those with unexplained infertility.

Even though the topic of endometrial thickness (EMT) and pregnancy outcomes has been the subject of countless research, there is still disagreement. The inconsistent outcomes may be attributed to a number of possible confounders, including female age, the stimulation strategy used, the numbers of harvested eggs and transplanted embryos. According to other scientists, considerably higher pregnancy rates are associated with greater EMT, which is unrelated to the quantity and caliber of the embryos as in **Khan *et al.*** [27], the pregnancy rate diminished as the EMT reduced like our findings. In the study of **Liu *et al.*** [28] revealed that with each millimeter fall in endometrial thickness below 8 mm in 24,363 fresh cycles, clinical pregnancy and live birth rates declined (P <0.0001), while pregnancy loss rates rose (P = 0.01). Our findings are also comparable to published research by **Yuan *et al.*** [29] that produced notable evidence of a linkage between a thin endometrium (less than 8 mm) and a worse conception rate or in other words that a thick endometrium is allied to an elevated pregnancy probability. However, other research showed that EMT on the day of ET is just a marginally reliable indicator of IVF success [18, 30, 31].

ROC curve analysis showed that both uterine artery RI, and uterine artery S/D ratio had significantly the best diagnostic accuracy (85% & 83.75% respectively) than other parameters in predicting occurrence of pregnancy after ICSI trial with the optimum cutoff values for uterine artery RI, and S/D ratio were 0.875 and 7.325 for predicting occurrence of pregnancy after ICSI trial with specificity 80.4% of both markers and sensitivity 91.2% and 88.2% respectively (P < 0.001). Also, subendometrial RI, and sub-endometrial S/D ratio were found good predictors for occurrence of pregnancy after ICSI trial (accuracy 80% & 81.25% respectively). While, the cut off values of endometrial thickness, sub-endometrial PI, and uterine artery PI were 8.5 mm,1.075, and 2.58 respectively for predicting occurrence of pregnancy after ICSI trial with accuracy 55%, 70% & 73.8% respectively.

Moustafa [3] reported that concerning uterine artery PI it had cut off value of 1.33 with sensitivity 66.7% and specificity 98.4% (p < 0.0001) and RI had cut off value of 0.775, 79.5% sensitivity and 98.4% specificity (p= 0.001). Endometrial thickness had cut off value 10.37 mm 97.4% sensitivity and 98.4% specificity (p < 0.0001). In **Adibi and his colleagues** [23] are in agreement with us, the uterine artery RI had more predictive value for predicting ICSI success with sensitivity 72.7.4% and specificity was 90.6%, while the sensitivity of PI for uterine artery was 63.6% and specificity was 84.4% with an accuracy of 81.5% & 73.8% for uterine artery RI & PI respectively. In **Ragheb *et al.*** [6] research the ROC curve of sub endometrial PI that only was significantly different among pregnant and non-pregnant cases showed that the AUC was 0.750 with cut off < 1.3 , sensitivity of 91.5 % and specificity of 50.2%. While **Maged *et al.*** [18] reported that endometrial thickness at Day of ET at cut off value 10.95 mm had lower sensitivity 57.1%, lower specificity 57.4%, and lower overall accuracy 59.2%.

CONCLUSION

Uterine artery and sub endometrial Doppler indices (PI, RI, and S/D ratio) were significantly lower in cases who developed clinical pregnancy in ICSI. It was found that for predicting incidence of pregnancy after ICSI trial, both uterine artery RI, and uterine artery S/D ratio had significantly the best diagnostic accuracy followed by sub-endometrial RI, and sub-endometrial S/D ratio. Thus, from our work, uterine artery and sub endometrial Doppler indices may be used independently or better combined with the other factors for configuration of a predictive algorithm for ICSI implantation or pregnancy rates.

DECLARATIONS

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