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Enhancing IVF Success: The Role of Endometrial Receptivity and Uterine Scoring in Predicting Pregnancy Outcomes

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Abstract: ***Introduction: Infertility is defined as the failure to conceive after 12 months or more of regular unprotected intercourse. Infertility can create divisive among the affected couples, as well as among their relatives and friends. The achievement of a successful*** **pregnancy** ***hinges on a multifaceted process, requiring both a high - quality embryo and a receptive endometrium. Endometrial receptivity specifically refers to the endometrium's capability to effectively engage with the embryo, providing nourishment and sustaining its viability. It has become absolute necessity to evaluate uterus and endometrium prior to embryo transfer, so that optimum results are obtained in favourable uteri. Materials and Methods: In this prospective pilot study, 65 women underwent frozen - thawed embryo transfer (FET) cycles at our reproductive medicine centre from March to October 2023. Uterine receptivity was assessed by analysing ultrasonographic and Doppler parameters on the day of initiating progesterone. The assessment utilized the Applebaum Uterine Scoring System, encompassing various parameters such as endometrial thickness, morphology, endometrial blood flow within zone 3, myometrial echogenicity, uterine artery pulsatility index (PI), end - diastolic blood flow, presence of endometrial motion, and myometrial blood flow. To add on, we also analysed the endometrial volume using the 3D scan and the direction of endometrial motion as ultrasonic markers. Results: Out of total 65 subfertility patients for embryo transfer 20 conceived, which gives 30.8% pregnancy rate. No patients had a perfect score of 20. Patients with score of 17 - 19 had pregnancy rate of 82.4 %. Endometrial thickness of 10 - 14mm gave optimum result of 55.6 %. Pulsatility index with 2.2 - 2.49 was associated with pregnancy rate of 40.7%. No significant association was found between Endometrial Volume and pregnancy rate. Conclusion: Applebaum's USSR scoring is a simple and noninvasive method for prediction of pregnancy rate in ICSI and embryo transfer cycle. Endometrial receptivity including thickness and vascularity are predominant in predicting outcome of pregnancy.***

Keywords: Endometrial receptivity, applebaum uterine scoring system, ICSI, embryo transfer

1. Introduction

Endometrial receptivity, which refers to the ability of the endometrium to accept embryo adhesion, implantation, and subsequent development, is a critical factor influencing the success of in vitro fertilization (IVF). This aspect is essential in establishing an optimal environment for embryo development and placenta formation, ultimately determining the likelihood of IVF success. Research on endometrial receptivity and the characteristics of the implantation window has been ongoing for over 80 years, with Rock and Bartlett (1937) being pioneers in describing histological changes in the endometrium during implantation[1]. Implantation failure can result from impaired embryo development potential or compromised endometrial selectivity/receptivity, negatively impacting embryo–endometrial cross - talk[2]. Studies indicate that approximately one - third of implantation failures are due to factors related to the embryo, while the remaining two - thirds are associated with suboptimal endometrial receptivity and disruptions in the dialogue between the embryo and the endometrium[3]. Achieving synchrony between these functions is crucial, defining a specific timeframe known as the nidation/implantation window.

Extensive studies have been aimed to develop a specific marker for uterine receptivity and are based on:

1) Biochemical evaluation
2) Ultrastructure study of pinopods
3) Ultrasound scoring systems
4) Ultrasound assessment of endometrium and its blood flow.

Understanding the intricate molecular and cellular mechanisms governing endometrial receptivity is paramount for comprehending the complexities of successful embryo implantation, minimizing the risk of implantation failure or early pregnancy loss[4]. Despite the profound understanding of the processes involved in embryo - endometrial cross - talk, there has been limited headway in clinically integrating this knowledge into diagnostic tests and treatments for suboptimal endometrial receptivity[5]. A single marker may be difficult to accurately reflect endometrial receptivity.

Transvaginal sonography is a simple, reliable and inexpensive method for evaluation of endometrial receptivity[6]. Endometrial thickness is one of the most widely used evaluation markers of endometrial receptivity[7]. The morphological pattern of the endometrium with a five line or a trilaminar pattern can be used as one of the predictors of clinical pregnancy[8]. The inner part of the myometrium has been revealed to generate contractions controlled by oestrogen and progesterone changes, causing the endometrium to generate wave - like or peristaltic movements which contributes to implantation[9]. The relationship between the frequency, direction, and amplitude of endometrial movements and pregnancy outcome needs to be further studied in the field of assisted reproduction[10]. Endometrial blood flow can be measured by 2D or 3D ultrasound. The assessment of uterine artery resistance is a clue to implantation potential of endometrium. The often - overlooked ultrasound indicator, endometrial volume, emerges as a viable marker in the evaluation of endometrial receptivity compared to focusing solely on the thickness of a specific section[11].

These sonographic findings are weighed according to uterine scoring system for reproduction (USSR) or uterine biophysical profile (UBP) by Applebaum[12]. It has become absolute necessity to evaluate uterus and endometrium prior to embryo transfer, so that optimum results are obtained in favourable uteri[13]. The purpose of the study was to association between Uterine Scoring System for Reproduction (USSR) and endometrial receptivity in assessing the pregnancy rates in women undergoing frozen embryo transfer

2. Methodology

Patient Recruitment and Counselling:
This Prospective single - centre pilot study was conducted over a period of 8 months, in the department of Institute of Reproductive Medicine at Madras Medical Mission. A total of 65 women who have undergone frozen embryo transfer cycles from March 2023 to October 2023 were enrolled in the study. Informed consent was taken from them to ascertain their willingness. Due anonymity of all responders was maintained at each step. Our study included subfertile women who underwent frozen embryo transfer after following the exclusion criteria. Exclusion criteria included the presence of uterine anomaly, thin endometrium, Progesterone >1.5 ng/ml on the day prior to starting progesterone.

ICSI Treatment Protocol:
The subfertile patients underwent controlled ovarian stimulation with antagonist protocol, when atleast two follicle reaches >=18 mm diameter, hCG or decapeptyl or dual trigger was given. Oocyte retrieval was done 35 hours later. Embryo cryopreservation was done by vitrification. Endometrial preparation was started on Day 2/3 of cycle after baseline scan. Once endometrial thickness is ≥ 8 mm, Progesterone was started. On the day of starting the progesterone, Uterine Scoring System for Reproduction (USSR), assessment of endometrial volume and the direction of peristalsis was done. Embryo transfer was done on Day3/4/5 after thawing the embryos and full luteal phase support is given. After FET patients were started on full luteal support and pregnancy confirmed after 2 weeks by serum beta hCG. If positive, transvaginal USG was done two weeks after biochemical evaluation to confirm intrauterine pregnancy.

Table 1: Applebaum Uterine Scoring System

PARAMETER	SCORING
ENDOMETRIAL THICKNESS (in mm)	<7mm = 0 7-9mm = 2 10-14mm = 3 >14mm = 1
ENDOMETRIAL LAYERING	NO LAYERING = 0 HAZY 5- LINE =1 DISTINCT 5- LINE = 3
MYOMETRIAL ECHOGENICITY	COARSE, INHOMOGENOUS = 1 RELATIVELY HOMOGENOUS =2
ENDOMETRIAL MOTION IN 2 MINS	< 3 CONTRACTIONS =0 > 3 CONTRACTIONS = 3
ENDOMETRIAL BLOOD FLOW WITHIN ZONE III	ABSENT = 0 SPARSE BLOOD FLOW =2 MULTIFOCAL BLOOD FLOW =5
UTERINEARTERY BLOOD FLOW EVALUATED BY PI	>3 =0 2.5- 2.99 =0 2.2- 2.49 =1 < 2.19 =2
MYOMETRIAL BLOOD FLOW ON EXAMINATION	ABSENT =0 PRESENT =2

Ultrasound Parameters:
1) Endometrial thickness: Maximum distance between echogenic interface of myometrium and endometrium, measured along longitudinal axis of uterus
2) Endometrial layering: Five distinct layers/ trilaminar pattern
3) Endometrial motion: The presence or absence of endometrial peristalsis and the direction of movement. The direction of endometrial motion was described as Cervicofundal (from the cervix to the fundus); fundocervical (from the fundus to the cervix); indeterminate; and nil/ absent (no contraction/direction).
4) Myometrial echogenicity
5) Uterine artery Doppler flow: Pulsatility index (PI)
6) Endometrial blood flow within zone III
7) Myometrial blood flow
8) Endometrial volume using 3D transvaginal probe: The sector of interest covering the endometrial cavity in a longitudinal plane of the uterus was adjusted, and the sweep angle was set to 90 to ensure that a complete uterine volume was obtained.3D volume was acquired keeping the patient and the 3D transvaginal probe still during the volume acquisition. Using the manual mode, EV was calculated by the VOCAL program automatically and given in cubic centimetres.

Statistical Analysis:
The sample size was calculated using the Statistical Analysis Software:

$$SS = Z^2 \times P \times Q / E^2 \longrightarrow 3.84 \times 4.5 \times (100-4.5) / 25 = 65$$

Z is the value from the standard normal distribution reflecting the confidence level that will be used (Z = 1.96 for 95%), P= Estimated population proportion, Q= Complement of P, which is equal to 100 –P, E= Margin of error, ie., Absolute Precision (5%)

At 95% confidence level, 80% power of the study, absolute precision of 5% and the prevalence of clinical pregnancy outcomes in previous studies among subfertile women as 4.5% according to study by Zhang et. al. The calculated sample size was 65

Outcome Measures:
The primary outcome of the study was the pregnancy rate. Clinical pregnancy was defined as ultrasound confirmation of gestational sac and fetal heart rate four weeks after embryo transfer. Ongoing pregnancy was defined as pregnancy with a detectable heart rate after 12 weeks of gestation. Miscarriage was defined as spontaneous pregnancy loss after a clinical pregnancy with a gestational sac visible in the uterine cavity. Ectopic pregnancy was defined as extra - uterine visualization of the gestation sac.

3. Results

Our study encompassed 65 patients, aged between 23 and 46. Among the 65 women who underwent Frozen embryo transfer 20 achieved conception, resulting in a pregnancy rate of 30.8%.

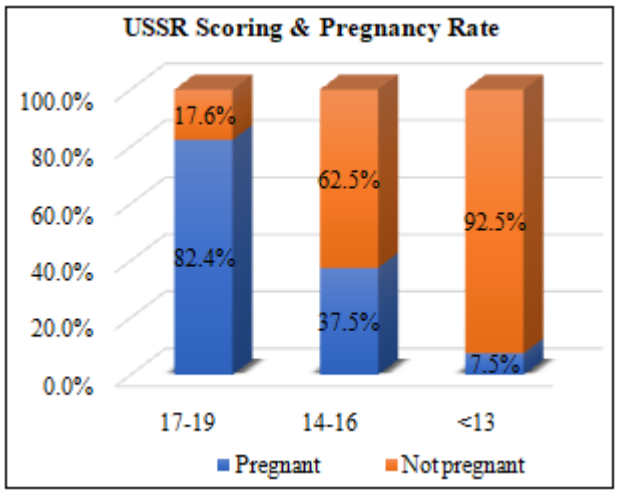
Table 2: Baseline characteristics in women according to whether pregnancy was achieved

	Pregnant (n=20) (Mean ±SD)	Non- Pregnant (n=45) (Mean ±SD)	p - value
Age	33.4±6	34±6	0.72
BMI	28±6	28.1±5	0.92
Baseline FSH (IU/L)	8.2±3.10	8.15 ±4.6	0.085
Baseline LH (IU/L)	4.39 ±3.15	4.44±3.18	0.176
Baseline E2 (pg/ml)	36.31±20.18	36.22±20.03	0.56
AMH (ng/ml)	2.93±2.6	3.02±2.67	0.78
Type of subfertility (n)			
Primary	13 (65%)	26 (58%)	0.89
Secondary	7 (35%)	19 (42%)	
Duration of Infertility (years)	6.65±3.87	7.7±4.62	0.63
Infertiity Diagnosis (n)			
Ovulation disorder	2 (10%)	6 (13.3%)	
Tubal Factor	4 (20%)	6 (13.3%)	
Male Factor	7 (35%)	15 (33.2%)	
Unexplained	0	5 (11.1%)	
Diminished	2 (10%)	5 (11.1%)	
Multiple	5 (25%)	8 (18%)	

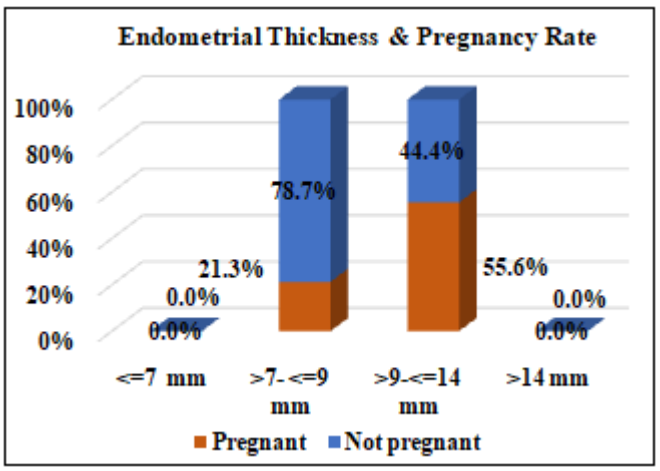
Table 3: Comparison of ultrasound parameters of endometrial receptivity between pregnant group and non - pregnant group

	Pregnant (n=20) (Mean ±SD)	Non - Pregnant (n=45) (Mean ±SD)	p - value
Endometrial thickness (in mm)	9.22±0.9	8.6±0.7	<0.001
Uterine artery blood flow evaluated by PI	2.4±0.4	2.6±0.3	0.06
Endometrial volume (ml)	4.2±1	3.8±0.9	0.1252
USSR Score	16.7±3	10.2±3.4	<0.001

USSR Scoring:
Seventeen patients scored between 17 and 19 on the USSR scale, with 14 of them achieving conception, resulting in a pregnancy rate of 82.4%. Additionally, eight patients scored between 14 and 16, and three of them conceived, yielding a pregnancy rate of 37.5%. For the 37 patients with a score below 13, only three conceived, resulting in a pregnancy rate of 7.5%. Notably, none of the patients attained a perfect score of 20. The p - value, calculated to be less than 0.001, signifies statistical significance.



Endometrial Thickness: The majority of patients (n=47) exhibited an endometrial thickness in the range of 7 - 9mm, with 10 patients demonstrating a pregnancy rate of 21.3%. The highest observed pregnancy rate, 55.6%, was associated with an endometrial thickness in the range of 10 - 14mm. Notably, pregnancy rates dropped to 0% when the endometrial thickness exceeded 14mm. The calculated p - valueis < 0.001, indicating statistical significance.

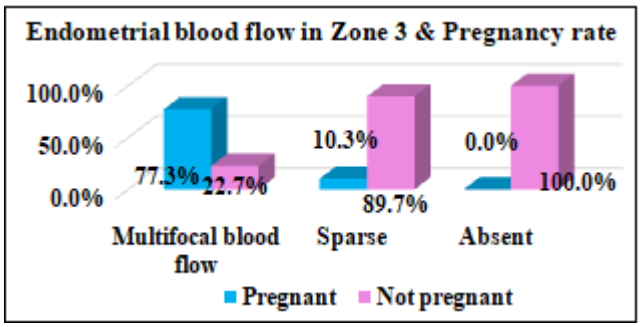


Endometrial Blood Flow: Multifocal endometrial blood flow in zone III showed a significant association with a high conception rate of 77.3% (n=17). Conversely, sparse blood flow was identified in 29 patients, resulting in a notably lower pregnancy rate of 10.3% (n=3), with a significant p - value of <0.01. Furthermore, the absence of endometrial blood flow was observed in 14 patients, correlating with a complete lack of conceptions.

Of the 22 patients displaying multifocal blood flow in zone III, 77.3% (n=17) who successfully conceived demonstrated a homogeneous myometrium, and the association yielded a significant p - value of <0.001.

Myometrial blood flow was present in 17 out of the 20 pregnant patients. Conversely, only one patient achieved pregnancy with absent myometrial blood flow, demonstrating a significant p - value of <0.001.

This observation suggests that a Homogenous Myometrium, combined with good endometrial and myometrial blood flow, is associated with favorable pregnancy outcomes.

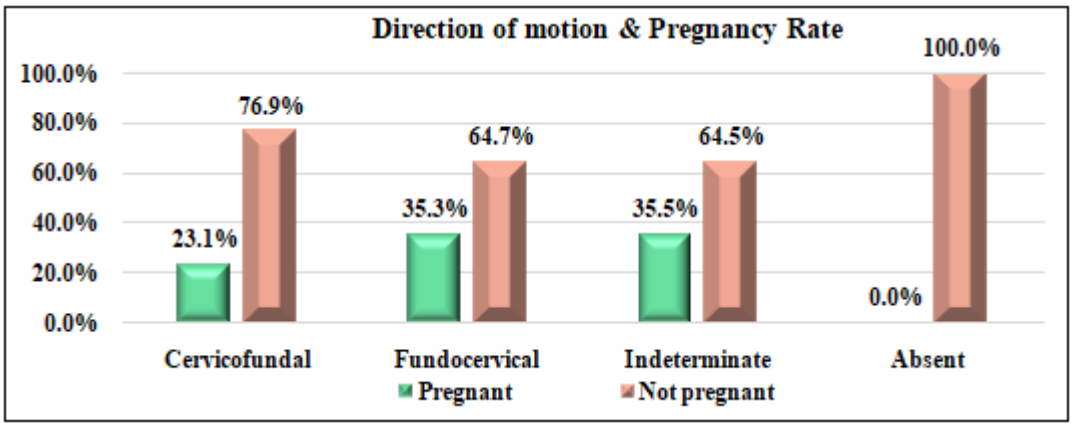


Uterine Artery PI: Among 29 patients with a pulsatility index (PI) between 2.2 - 2.49, the conception rate was 40.7% (n=11). In contrast, patients with PI values ranging from 2.5 - 2.99 exhibited a lower clinical pregnancy rate of 27.6% (n=8). Notably, none of the five patients with a pulsatility index exceeding 3 experienced a pregnancy. A pulsatility index between 2.2 - 2.49 is associated with a favorable pregnancy outcome, yielding a success rate of 40.7%. The calculated p - value for these findings was 0.29.

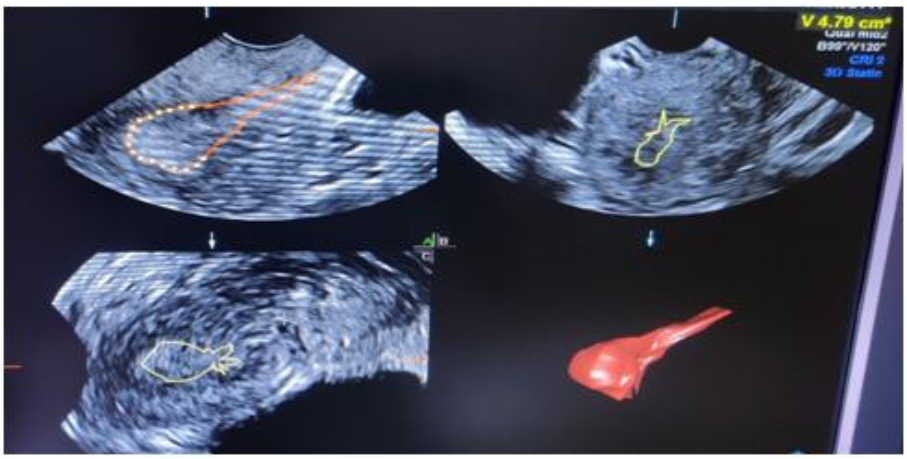
Endometrial Layering: In 32 patients, a distinct 5 - line/trilaminar pattern was identified, with 17 of them achieving conception, resulting in a pregnancy rate of 53.1%. Notably, women lacking any layering pattern in the endometrium did not achieve pregnancies. The associated p - value for these observations was <0.001.

Endometrial Motion in 2 Mins: Out of the 20 pregnancies observed, 18 were linked to endometrial motion greater than 3, demonstrating a significant p - value of <0.001.

In our study, we conducted an additional analysis of the direction of endometrial peristalsis. Among the seventeen pregnant patients, 35.5% (n=11) exhibited an indeterminate pattern of endometrial motion, while a fundocervical pattern was observed in 35.3% (n=6) of cases. However, no statistical significance was seen in our study, likely due to the limited sample size. Further investigations are necessary to delve into this aspect more comprehensively.



Endometrial Volume: Moreover, we evaluated the endometrial volume to gauge its predictive significance in determining pregnancy rates during frozen embryo transfer cycles. It was categorized into two groups: ≤3 ml and >3 ml, with pregnancy rates of 32.6% and 14.3%, respectively. Notably, nineteen out of the 20 pregnancies were observed in patients with an endometrial volume exceeding 3 ml. Image 1:



Endometrial volume calculation by using three - dimensional ultrasound

However, the associated p - value was found to be insignificant at 0.31

Clinical pregnancy was defined as ultrasound confirmation of gestational sac and fetal heart rate four weeks after embryo transfer – 30.8 % (n=20).

Ongoing pregnancy in our study is characterized by the presence of a detectable heart rate after 12 weeks of gestation, constituting 65% (n=13) of the cases. It's worth mentioning that the data for two patients currently in the early stages of pregnancy has not been incorporated into the ongoing pregnancy rate at this point.

Miscarriage was defined as spontaneous pregnancy loss after a clinical pregnancy with a gestational sac visible in the uterine cavity – 25% (n=5).

No instances of ectopic pregnancy were observed during our study period.

4. Discussion

The role of endometrial receptivity in embryo implantation is pivotal, and assessing endometrial receptivity remains a challenge in clinical practice. In a study conducted by Malhotra et al[15]., involving spontaneous cycles of 222 women, a USSR score of 20 resulted in an 80% pregnancy rate, while a score of 17–19 yielded a 79% conception rate. Conversely, only 7.6% conceived with a score of ≤13. Another study by Nayak et al[16]. revealed that no woman achieved a perfect score of twenty. However, the pregnancy rate for patients with a score of 17–19 was 100%, and for those with a score of 14–16, it was 50%. All women with a score of ≤13 tested negative for pregnancy. Similarly, Shivatre et al. found optimal pregnancy results with a score of 17–19. Additionally, Oliveira JB's[18] study concluded that pregnancies did not occur when the endometrial thickness was less than 7 mm. In our study, we observed outcomes similar toShivatre et al[17]., with a 82.4% conception rate in the 17 - 19 score group. The correlation between a higher USSR score and improved conception outcomes suggests its reliability as a predictive parameter.

According to a study conducted by Strowitzki et al., [19] inadequate endometrium is identified as a significant determinant of fertility. Numerous studies have explored the relationship between various endometrial features such as thickness, length, or pattern and implantation rates, with findings both supporting and refuting this correlation (Bassil, 2001; Kovacs et al., 2003; Richter et al., 2007; Zhang et al., 2005; Rinaldi et al., 1996). In our study, positive pregnancy outcomes were observed when the endometrium measured between 7–14 mm. Specifically, 10 women with an endometrial thickness of 7–9 mm exhibited a pregnancy rate of 21.3%, while 10 women with a thickness of 10–14 mm showed a substantially higher pregnancy rate of 55.6%. Consequently, on an individual basis, endometrial thickness emerges as a reliable parameter for predicting pregnancy showing statistical significance. This aligns with the findings of a study by Shivatre et al., [17] where the highest conception rate (52%) was associated with an endometrial thickness of 10–14 mm.

Adequate blood supply in the endometrium is crucial for successful implantation. Our study identified statistical significance in endometrial blood flow as a predictor of pregnancy. Among the 22 women with multifocal endometrial blood flow in Zone 3, 77.3% (n=17) conceived, while 29 women with sparse endometrial blood flow in Zone 3 saw a lower conception rate of 10.3% (n=3). Singh N et al.2[0] reported a higher pregnancy rate in Zone III (51.8%) compared to Zone I (14.8%), mirroring our study findings. Additionally, the study by Ari Kim et al.2[1] emphasized the importance of sub - endometrial flow in determining pregnancy outcomes. Gupta et al.2[2] also noted a higher pregnancy rate when endometrial blood flow was in Zone III (54.5%). These collective results underscore the significance of evaluating endometrial blood flow, particularly in Zone III, as a key factor in predicting pregnancy success. In accordance with study done by Gupta et al[22], the conception rate varied with different zones of endometrial blood flow, with the highest rate observed in Zone 4 at 66.6%, followed by 54.5% in Zone 3. In contrast, patients with endometrial blood flow only extending till Zone 1 had the lowest conception rate, at 11.1%. In a study emphasizing the importance of successful embryo implantation, both genetically and morphologically healthy embryos, a well receptive endometrium, was deemed essential. A prospective study by Serafimi et al[23] (1994) suggested that the multilayered pattern of the endometrium was more predictive of implantation than any other parameter.
In a study done by Shivatre et al, maximum patients had PI between 2.2 - 2.49 with conception rate of 51.42%. PI values of 2.5 - 2.99 had a success rate of only 25%. Pulsatility index of more than 3 was present in 3 patients with no pregnancy. PI less than 2.15 is favourable for pregnancy giving success rate of 66%. In our study we had better pregnancy rates when PI was between 2.2 - 2.49. These findings underscore the intricate interplay of various factors in determining successful pregnancies, emphasizing the significance of both endometrial characteristics and vascular factors.

Various cut - off levels for endometrial volume have been proposed in the literature below which pregnancy is unlikely to occur. Raga et al[24]. reported no conception with an EV < 1 mL, whereas Yaman etal[25] and Zollner et al[26]. found EV of 2.5 mL to be significant for prediction of pregnancy. Ahmed et al. reported that endometrial volume can be a candidate predictor of in - vitro fertilization success to replace the endometrial thickness, because it was significantly difference between pregnant and non - pregnant women[27]. On the contrary, Assen et al. in their study and found that endometrial volume assessed by 3D transvaginal ultrasound was not a useful tool for predicting pregnancy in single blastocyst embryo transfer cycles [28]. Fendo et al had similar findings to our study. In conclusion, uterine volume does not seem to affect live birth rates in women with an unscarred and non - anomalous uterus undergoing a single good quality blastocyst transfer.[10]

In study done by Zhu et al[29] and Fanchin et al the clinical pregnancy rate was much higher in patients with a frequency of <3.0 waves/min, compared with those>3.0 waves /min indicating a beneficial effect of low frequency uterine

peristalsis on pregnancy. Our current findings was contradictory to the previous studies depicting that embryo transfer cycles which ended with successful pregnancy showed more uterine peristalsis before embryo transfer than non - pregnant cycles. In study done by Fendo et al[10], the presence or absence of endometrial peristalsis was not associated with clinical pregnancy rate, while the direction of endometrial peristalsis was associated with clinical pregnancy rate. The negative peristalsis group (fundus to cervix direction) had the highest clinical pregnancy rate, followed by the nondirectional peristalsis group (peristalsis was existed, but the direction was not clear), and the others were lower. In our study the direction of endometrial peristalsis did not have a significant association with pregnancy rate. In study by Chung et al[30] the contraction directions were described as follows: retrograde (from the cervix to the fundus); antegrade (from the fundus to the cervix); indeterminate; and nil/ absent (no contraction/direction). The highest clinical pregnancy rate and LBR found in women with no contractions, followed by indeterminate direction.

5. Conclusion

Applebaum Uterine Scoring System a simple and noninvasive method focuses on the state of the entire uterus. In our study we additionally focused on the shape and function of the endometrium using 3D. Three - dimensional volumetry an objective parameter of the endometrium can give additional information to two - dimensional ultrasound and could be applied in infertile patients in the same manner. The identification of the optimal uterine environment for embryo transfer is extremely important.3D US has been considered as a promising tool for assessing the endometrial receptivity; but the results are still debatable due to multiple techniques differences, inter observer variability. In this context, we believe that the innovative use of 3D markers of endometrial receptivity along with the USSR scoring system will be useful as a effective screening tool for selecting the more suitable cycle for the embryo transfer.

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