

Effect of Endometrial Compaction, Sub Endometrial Blood Flow and Serum Progesterone Levels on Pregnancy Rate in Frozen Embryo Cycle Regimes.

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ABSTRACT

Background: Successful results of frozen embryo transfer (FET) cycles depend on optimal endometrial receptivity. Endometrial compaction, sub-endometrial blood flow, and adequate serum progesterone levels have been offered as probable predictors of implantation and pregnancy success; however, their combined impact remains controversial.

Objective: To assess the impact of endometrial compaction, sub-endometrial blood flow, and serum progesterone levels on pregnancy effects in FET cycles.

Methods: This prospective observational study included women experiencing FET cycles. Endometrial thickness was measured before start of progesterone therapy and on the embryo transfer day to calculate the endometrial compaction ratio. Sub-endometrial blood flow was assessed using transvaginal color Doppler ultrasound. Serum progesterone levels were measured on the day of embryo transfer. The primary result was clinical pregnancy rate. Associations between study parameters and pregnancy outcomes were analyzed

Results: Clinical pregnancy rates were significantly superior among women who demonstrated favorable endometrial compaction and adequate sub-endometrial blood flow. In addition, optimal serum progesterone levels on the day of embryo transfer were positively related to pregnancy achievement. Combined measurement of endometrial compaction, sub-endometrial blood flow, and progesterone levels showed improved predictive value for pregnancy outcomes compared to individual parameters.

Conclusion: Endometrial compaction, sub-endometrial blood flow and serum progesterone levels are important determinants of pregnancy success in FET cycles. The pooled assessment of them may give a more robust evaluation of endometrial receptivity and help to optimise outcomes of FET..

Keywords: Frozen embryo transfer; Endometrial compaction; Sub-endometrial blood flow; Progesterone; Pregnancy outcome.

How to cite this article: Ghorab HAE, Aboufreikha AH, Mustafa MZM, Youssif MAA, Ageez MN., Effect of Endometrial Compaction, Sub Endometrial Blood Flow and Serum Progesterone Levels on Pregnancy Rate in Frozen Embryo Cycle Regimes.. Int J Drug Deliv Technol. 2026;16(83s): 1830-1839; DOI: 10.25258/ijddt.16.83s.199

Source of support: Nil.

Conflict of interest: Nil.

INTRODUCTION

Frozen embryo transfer (FET) has become an integral part of assisted reproductive technology (ART) and accounts for an increasing proportion of embryo transfer cycles worldwide (1). Improvements in embryo vitrification and the widespread use of freeze-all have improved laboratory outcomes and provided greater flexibility in the timing of embryo transfer (2, 3). The effect of endometrial preparation and timing on pregnancy results in FET cycles has been highlighted by recent studies published in Journal of Obstetrics, Gynaecology and Cancer Research (JOGCR), emphasising the clinical importance of assessing endometrial measurements. Frozen embryo transfer (FET) has been a major part of assisted reproductive technology

(ART) and is being used in an increasing number of embryo transfer cycles worldwide (1). Improvements in embryo vitrification and the widespread use of freeze-all have improved laboratory outcomes and provided greater flexibility in the timing of embryo transfer (2, 3). Recent studies published in Journal of Obstetrics, Gynaecology and Cancer Research (JOGCR) have shown the effect of endometrial preparation and timing on pregnancy outcomes in FET cycles, stressing on the clinical importance of assessing endometrial parameters . (4,5).

Despite the technological improvements, implantation failure is still a major issue in ART. Successful implantation is a complex process that involves a coordinated interaction of a receptive endometrium and a competent embryo during

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the implantation window (6). Embryo quality is undoubtedly important, but endometrial receptivity has been increasingly recognised as a critical determinant of pregnancy success especially in hormonally prepared FET cycles (7,8).

Endometrial preparation protocols in FET cycles include natural, modified natural, hormone replacement therapy (HRT) and stimulated cycles (9). However, there is no agreement on the best advancement and individual variability still affects implantation rates. The association of endometrial thickness and morphological pattern with FET outcomes has been demonstrated in a previous study published in JOGCR recommending careful endometrial assessment (4,5).

Transvaginal ultrasonography is the main tool for assessing endometrial receptivity before embryo transfer. Endometrial thickness and morphology have been used traditionally as markers, but these parameters may not be representative of functional competence (10,11). Endometrial compaction, the dynamic changes in endometrial thickness following progesterone exposure, has been proposed as a marker for secretory transformation. Conflicting data have been reported; some studies have suggested compaction may be representative of adequate endometrial maturation, while others have not found any significant association with pregnancy outcomes (12–14). Another major factor influencing implantation is sub-endometrial blood flow. Doppler assessment of vascularity has been proposed as a marker of receptivity but its predictive value is inconsistent (15,16). Similar to this, serum progesterone levels on the day of embryo transfer are considered critical in HRT-FET cycles and evidence suggests that both low and suboptimal levels can reduce implantation success (17–19).

Given the multifactorial nature of implantation, the predictive power of any single parameter alone may be limited. Combined evaluation of structural changes of the endometrium, vascular perfusion and hormonal milieu can give a more comprehensive assessment of endometrial receptivity. Therefore, the current study purposed to investigate the impact of endometrial compaction, sub-endometrial blood flow, and serum progesterone levels on pregnancy results in FET cycles

Aim of the work

The purpose of this work is to assess the effect of Endometrial Compaction, sub endometrial blood flow and Serum progesterone levels on Pregnancy Rate in FET Regimes.

Patients and Methods

Study design: prospective Cohort study

Setting: Obstetrics and Gynecology departments of Tanta university hospitals and Kasr Al-Einy university hospitals

Number of cases: 60 patients.

Timing of the study: from April , 1, 2022 to september,1, 2024.

Cases selection: The cases were selected from infertile women recruited from infertility outpatient clinics of Gynecological departments of Tanta and Kasr Al-Einy university hospitals

Criteria of patient selection:

The Inclusion criteria were as follows

female aged 18 years to 39 years with regular menses (26-34 days)

Suffering In-vitro fertilization (IVF) treatment and ICSI.

Normal uterine cavity confirmed by 2D or 3D transvaginal ultrasound.

First frozen-thawed transfer cycle.

Exclusion criteria were as follows:

Poor ovarian answerer in line with Bologna criteria

Hydrosalpinx unless tubal disconnection is done

Asherman syndrome

Any proven contraindications or allergies to oral progesterone or estradiol.

Discontinuation of HRT medication (medication error in research HRT cycle)

Duration of estradiol exposure $\geq$ seventeen days and endometrium < 7 mm at day of start of progesterone therapy.

Patients with history of recurring implantation failure.

Sample size calculation: The sample size for this study was calculated based on the following statistical parameters: 95% confidence level, 80% power of study, and an expected difference in the predictive sensitivity of clinical pregnancy rates between favourable and less favourable endometrial parameters (90% vs 65%). Based on these criteria, a minimum of 50 participants were recruited to ensure adequate statistical reliability. To take into account possible dropouts and missing data, the sample size was increased to 60 participants, which will ensure robust and reliable outcomes of the study..

Materials and Methods

Study Participants and Data Collection

Written informed consent was obtained from all participants before enrolment. Detailed data were collected, including demographic data (age, parity, occupation, residency, lifestyle, medical and surgical history, drug therapy or allergies, and specific data on previous IVF/ICSI cycles). Menstrual history was taken (age at menarche, regularity, duration, volume of cycle and associated dysmenorrhoea).

A thorough general and pelvic examination was performed to rule out any systemic or local pathology. Infertility workup included pelvic ultrasonography and hormonal profiling (day 2 FSH, LH, AMH, TSH, and prolactin) to exclude newly developed conditions that could affect implantation outcomes.

Endometrial preparation was commenced on day two to three of the menstrual cycle following a baseline ultrasound to confirm the endometrial and ovarian status (endometrial thickness $\leq$ five mm and no dominant follicles). All participants were given a standardised hormone replacement therapy (HRT) protocol for FET. Patients received oral micronised oestradiol (Cyclo progynova®; 4 to 8 mg twice daily) with dose titration based on serial ultrasounds to achieve an endometrial thickness of ≥ 7 mm. Progesterone supplementation was commenced with vaginal suppositories (Prontogest® 400 mg twice daily) once the endometrium had reached a thickness of ≥ 7 mm with a trilaminar pattern. The 1st day of progesterone

administration was noted as P+0, and blastocyst transfer occurred on P+5.

Progesterone and Estradiol supplementation lasting until 12 weeks of gestation in cases of confirmed pregnancy.

Embryo transfer was performed by an experienced practitioner, transferring one or two high-quality blastocysts under standard clinical conditions.

Pregnancy Assessment

Clinical pregnancy was classified as the ultrasonographic visualization of a gestational sac with a fetal heartbeat approximately two weeks after a positive pregnancy test, conducted on day 14 post-transfer.

Diagnostic Assessments During Luteal Phase

Endometrial Thickness and Compaction

Transvaginal 2D ultrasound (TVS) was performed at P+0 and on embryo transfer day. Endometrial thickness (EMT) was determined from one endo-myometrial junction to the opposite junction, one cm below the uterine fundus, utilizing a mid-sagittal plane occupying ~2/3 of the screen. Patients were categorized into compacted (decrease in EMT) and non-compacted (no vary or improve in EMT) groups.

Sub-Endometrial Blood Flow

Evaluated utilizing 2D transvaginal color power Doppler on embryo transfer day.

The uterus was assessed in the longitudinal plane with an empty bladder, visualizing the endometrium and sub-endometrial zone (~10 mm beneath the endometrial-myometrial junction). Blood flow patterns were classified as absent, sparse, or multifocal.

Serum Progesterone Levels

Blood samples were saved on embryo transfer day, assayed in the laboratory, and reported in ng/mL.

Statistical Analysis

All collected data were analyzed utilizing suitable statistical methods to estimate the association of endometrial compaction, sub-endometrial blood flow, and serum progesterone levels with clinical pregnancy outcomes.

Ethical approval and clinical trial registration: This study was accepted by local ethical committee of Tanta University before the start of this work with approval code 35389/3/22, All patients were notified about work design, risks, and interventions. All patients signed written consent. security and Privacy were kept all over the period of work.

There was no unexpected risks during the course of research.

Results:

A total of 60 women undergoing FET cycles were entered in the final analysis. The mean age of the study population was 31.57 ± 4.65 years (range: 17-34 years), with a BMI of 27.85 ± 3.40 kg/m². Primary infertility was present in 68.3% of participants, while 31.7% had secondary infertility. The mean duration of infertility was 4.96 ± 2.55 years. The most common reasons of infertility were male factor (25.0%), tubal factor (21.7%), mixed causes (20.0%), and polycystic ovary syndrome (PCOS) (15.0%), followed by unexplained infertility (10.0%) and endometriosis (8.3%) (Table 1).

Baseline hormonal assessment showed a mean follicle-stimulating hormone (FSH) level of 8.08 ± 2.70 mIU/ml and

a mean luteinizing hormone (LH) level of 6.12 ± 2.40 mIU/ml. The mean anti-Müllerian hormone (AMH) level was 3.34 ± 2.95 ng/ml. Thyroid-stimulating hormone (TSH) and prolactin (PRL) levels were within normal ranges, with mean values of 1.80 ± 1.07 mIU/l and 17.29 ± 7.88 ng/ml, respectively (Table 2). Clinical pregnancy was achieved in 28 women (46.7%), while 32 women (53.3%) did not achieve pregnancy.

There were no statistically significant variations among non-pregnant and pregnant groups according type of infertility, duration of infertility, age, BMI, or causes of infertility. Although women who achieved pregnancy inclined to have a slightly shorter duration of infertility, this variation did not reach statistical significance ($p = 0.08$). Overall, baseline clinical and demographic characteristics were similar among either groups (Table 3).

No statistically significant alterations were observed among non-pregnant and pregnant women in terms of baseline FSH, LH, AMH, TSH, or PRL levels. However, AMH levels pointed a non-significant trend toward higher values in the pregnant group matched with the non-pregnant group ($p = 0.091$) (Table 4).

Regarding the type of ovarian stimulation protocol, agonist protocols were more frequently used in the pregnant group linked with the failed group (64.3 vs. 46.8 percent), although this deviation was not statistically significant ($p = 0.176$) (Table 4). Likewise, the number of retrieved oocytes was slightly greater in women who achieved pregnancy, but the deviation among groups did not achieve statistical significance ($p = 0.076$) (Table 5).

Endometrial compaction was observed more normally in the pregnant group than in the failed group (57.1% vs. 37.5%); however, this deviation was not statistically significant ($p = 0.128$). Sub-endometrial blood flow patterns and progesterone levels on FET day also did not vary significantly among non-pregnant and pregnant groups. Most women in both groups had progesterone levels ≥ 10 ng/ml (Table 6).

Spearman correlation analysis demonstrated a weak positive correlation between sub-endometrial blood flow and serum progesterone levels on FET day; however, this correlation was not statistically significant. (Table 7)

Regarding the duration of estrogen administration prior to progesterone initiation, no statistically significant variation was perceived among women who achieved pregnancy and those with failed outcomes. The mean duration of estrogen-only treatment was comparable in both groups (11.3 ± 1.2 days in the pregnant group versus 11.2 ± 1.3 days in the failed group; $P = 0.823$). Median duration and interquartile range were similarly distributed between both groups (Table 8).

Preprogesterone endometrial thickness was not significantly different between pregnant and failed groups. The mean endometrial thickness was 9.5 ± 0.7 mm in pregnant women and 9.5 ± 0.9 mm in those with failed cycles ($P = 0.858$). Median values and confidence intervals also supported that there was no important difference between both outcomes (Table 8).

Serum progesterone levels were analysed and there was no statistically significant correlation with the outcome of

pregnancy. The mean progesterone level was slightly higher in the pregnant group than in the failed group (18.78 ± 6.94 ng/ml versus 16.69 ± 6.69 ng/ml) but this was not statistically significant ($P = 0.486$). The median values and ranges were highly overlapped in the two groups, indicating that progesterone profiles were similar regardless of pregnancy outcome (Table 8).

However, significant relationship was found between the levels of serum progesterone and the compaction of endometrium. All cases with progesterone levels below 10 ng/ml were in the non-compacted endometrial group, while more than half of women with progesterone levels $\geq$ ten ng/ml showed endometrial compaction (53.8%). This association was statistically significant ($P = 0.001$, $\chi^2 =$

13.061). Moreover, sub-endometrial blood flow patterns were significantly different between compacted and non-compacted endometrium. The compacted group showed predominantly multifocal blood flow, whereas non-compacted cases showed absent or sparse blood flow patterns ($P = 0.004$) (Table 9).

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the predictive value of progesterone level for pregnancy outcome. The AUC was 0.552 showing poor discriminatory power. A progesterone cutoff value of > 17.32 ng/ml yielded a sensitivity of 46.3% and a specificity of 50%, with no statistically significant predictive value for pregnancy outcome ($P = 0.486$) (Figure 1).

Table 1: Basic characteristics of study participants.

	Study participants N=60	
Age (years)		
Mean $\pm$ SD	31.57 $\pm$ 4.65	
Range	17 – 34	
Median (IQR)	32 (27-36)	
95% Confidence Interval	30.36 - 32.77	
BMI (kg/m ²)		
Mean $\pm$ SD	27.85 $\pm$ 3.4	
Range	19 – 34	
Median (IQR)	28 (26 - 30)	
95% Confidence Interval	26.97 - 28.73	
Type of infertility	N	%
Primary causes	41	68.3
Secondary causes	19	31.7
Duration of infertility		
Mean $\pm$ SD	4.96 $\pm$ 2.55	
Range	1 – 12	
Median (IQR)	5 (3-6)	
95% Confidence Interval	4.30 - 5.62	
cause of infertility	N	%
Endometriosis	5	8.3
Male factor	15	25.0
Mixed causes	12	20.0
PCO	9	15.0
Tubal factor	13	21.7
Unexplained	6	10.0

Table 2: Hormonal assay of study participants.

	Mean $\pm$ SD	Median (IQR)	95% Confidence Interval	
			Lower	Upper
FSH (mIU/ml)	8.08 $\pm$ 2.7	7.7 (6.4 – 9.3)	7.37	8.79
LH (mIU/ml)	6.12 $\pm$ 2.4	6.2 (4.5 – 7.6)	5.50	6.75

AMH (ng/ml)	3.34 ±2.95	2.8 (1.7 – 4.3)	2.57	4.10
TSH (miu/l)	1.8 ± 1.07	1.4 (1.2 – 2.5)	1.54	2.09
PRL (ng/ml)	17.29 ± 7.88	16.1(12.9–22.1)	15.25	19.32

Table 3: The relationship between basic characteristics and outcomes among the studied group.

	Pregnant N=28		Failed N=32		Test significance	of P value
Age (years)					U= 377.500	0.294
Mean ±SD	30.8 ±4.4		32.2 ± 4.9			
Range	22 – 38		20 – 39			
Median (IQR)	31 (28-33)		33.5 (27-37)			
95% Confidence Interval	29.3 - 32.4		30.6 - 33.74			
BMI (kg/m²)					U=426.000	0.743
Mean ±SD	28.1 ± 2.9		27.6 ± 3.8			
Range	21 – 33		19-34			
Median (IQR)	28 (26.5-31)		28 (25-30)			
95% Confidence Interval	27.04 – 29.2		26.2 – 28.9			
Type of infertility	N	%	N	%	$\chi^2=0.006$	0.941
Primary causes	19	67.9	22	68.8		
Secondary causes	9	32.1	10	31.3		
Duration of infertility					U=331.000	0.08
Mean ±SD	4.5 ± 2.5		5.3 ± 2.6			
Range	1 – 11		1 – 12			
Median (IQR)	4 (3-5)		5 (4-6)			
95% Confidence Interval	3.65 – 5.52		4.45 – 6.26			
cause of infertility	N	%	N	%	$\chi^2=3.202$	0.669
Endometriosis	3	10.7	2	6.3		
Male factor	8	28.6	7	21.9		
Mixed causes	5	17.9	7	21.9		
PCO	5	17.9	4	12.5		
Tubal factor	6	21.4	7	21.9		
Unexplained	1	3.6	5	15.6		

χ^2 for Chi square test. U for Mann Whitney test.

*: Statistically significant at $P \leq 0.05$.

Table 4: Relationship between Hormonal Assay Results and Treatment Outcome among the Studied Group.

	Pregnant N=28	Failed N=32	Mann-Whitney test U	P value
FSH (miu/ml)			413.500	0.609
Mean ±SD	7.8 ± 2.8	8.3 ± 2.8		
Range	2.8 – 16.6	3.5 – 13.9		
Median (IQR)	7.7 (6.5 - 8.8)	7.8 (6.4 - 10)		
95% Confidence Interval	6.89 - 8.87	7.38 - 9.31		
LH (miu/ml)			386.000	

Mean ±SD	6.3 ± 2.4	6.0 ±2.4		0.358
Range	1.4 -10.0	2.8-12.5		
Median (IQR)	6.8 (4.8-8.1)	5.5 (4.2-6.9)		
95% Confidence Interval	5.36 - 7.14	5.14 - 6.91		
AMH (ng/ml)			334.000	0.091
Mean ±SD	4.1 ± 4.0	2.7 ± 1.3		
Range	1.2 - 23.0	1.1 - 5.4		
Median (IQR)	3.5(2.0-4.6)	2.3(1.7-3.6)		
95% Confidence Interval	2.90 - 5.87	2.28 - 3.16		
TSH (miu/l)			407.500	0.548
Mean ±SD	1.7 ± 1.0	1.9 ± 1.1		
Range	0.1-3.9	0.2-5.7		
Median (IQR)	1.4 (1.2-2.4)	1.6 (1.2-2.6)		
95% Confidence Interval	1.35 - 2.09	1.54 - 2.36		
PRL (ng/ml)			433.000	0.824
Mean ±SD	17.8 ± 7.1	16.8 ± 8.6		
Range	7.9 - 34.7	1.2 - 34.7		
Median (IQR)	15.7 (13.6-22.2)	16.7 (12.2-21.9)		
95% Confidence Interval	15.28 - 20.50	13.79 - 20.04		
Type of protocol			1.829	0.176
Agonist	18(64.3%)	15(46.8)		
Antagonist	10(35.7%)	17(53.2%)		

Table 5: The relationship between Number of oocytes retrived with outcome among the studied group.

	Pregnant N=28	Failed N=32	Mann-Whitney test U	P value
Number of oocytes			329.00	0.076
Mean $\pm$ SD	15.7 $\pm$ 3.18	14.56 $\pm$ 3.36		
Range	7 – 21	8 – 22		
Median (IQR)	17(14 – 17.5)	14.5 (13 – 16.5)		
95% Confidence Interval	14.6 – 17.04	13.7 – 15.4		

Table 6: The relationship between Endometrial Compaction and progesterone level and Sub Endometrial Blood Flow with outcome among the studied group.

	Pregnant N=28		Failed N=32		Test significance	P value
	N	%	N	%		
Endometrial Compaction					$\chi^2=2.315$	0.128
Compacted	16	57.1	12	37.5		
Non-compacted	12	42.9	20	62.5		
Sub Endometrial Blood Flow						
Absent blood flow	4	14.3	10	31.3	$\chi^2=3.351$	0.187
Spare blood flow	13	46.4	15	46.9		
Multifocal blood flow	11	39.3	7	21.9		
Progesterone level						

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Above Threshold ($p \geq 10$ ng/ml)	26	92.9	26	81.3	FE	0.187
Below Threshold ($P < 10$ ng/ml)	2	7.1	6	18.8		

χ^2 for Chi-square test. FE for Fisher's exact test.

Table 7: Correlation between Sub Endometrial Blood Flow and progesterone levels.

	Correlation	Progesterone level
Sub Endometrial Blood Flow	r_s P	0.170 0.193

r_s : spearman correlation coefficient

*: statistically significant.

Table 8: Comparison of Estrogen Duration, Endometrial Thickness, and Progesterone Level between Pregnant and Failed Cases

	Pregnant N=28	Failed N=32	Mann-Whitney test U	P value
Duration of estrogen-only (days) before the start of progesterone			433.500	0.823
Mean $\pm$ SD	11.3 $\pm$ 1.2	11.2 $\pm$ 1.3		
Range	10-14	10-15		
Median (IQR)	11 (10 – 12)	11 (10 - 12)		
95% Confidence Interval	10.81 - 11.73	10.81 - 11.69		
Endometrial thickness before start of progesterone (mm)			436.000	0.858
Mean $\pm$ SD	9.5 $\pm$ 0.7	9.5 $\pm$ 0.9		
Range	8 – 11	8 – 11		
Median (IQR)	9.5 (9 - 10)	9.1 (9 - 10)		
95% Confidence Interval	9.21 - 9.73	9.18 - 9.78		
Progesterone level			401.00	0.486
Mean $\pm$ SD	18.78 $\pm$ 6.94	16.69 $\pm$ 6.69		
Range	3.44 – 33.86	4.16 – 29.54		
Median (IQR)	18 (15-21)	17 (13-21)		
95% Confidence Interval	16.32 – 21.27	14.34 - 19.06		

Table 9: The relationship between progesterone level and Sub Endometrial Blood Flow with endometrial compaction.

	Endometrial Compaction				Test of significance	P value
	compacted		non-compacted			
	N	%	N	%		
Progesterone level						
Above Threshold (p ≥10 ng/ml)	28	53.8	24	46.2	$\chi^2=13.061$	0.001*
Below Threshold (P < 10 ng/ml)	0	0.0	8	100.0		
Sub Endometrial Blood Flow						
Absent blood flow	2	14.3	12	85.7		
Spare blood flow	12	42.9	16	57.1	MC	0.004*
Multifocal blood flow	14	77.8	4	22.2		

χ^2 for Chi-square test.

MC for Monte Carlo exact test.

*: Statistically significant at $P \leq 0.05$

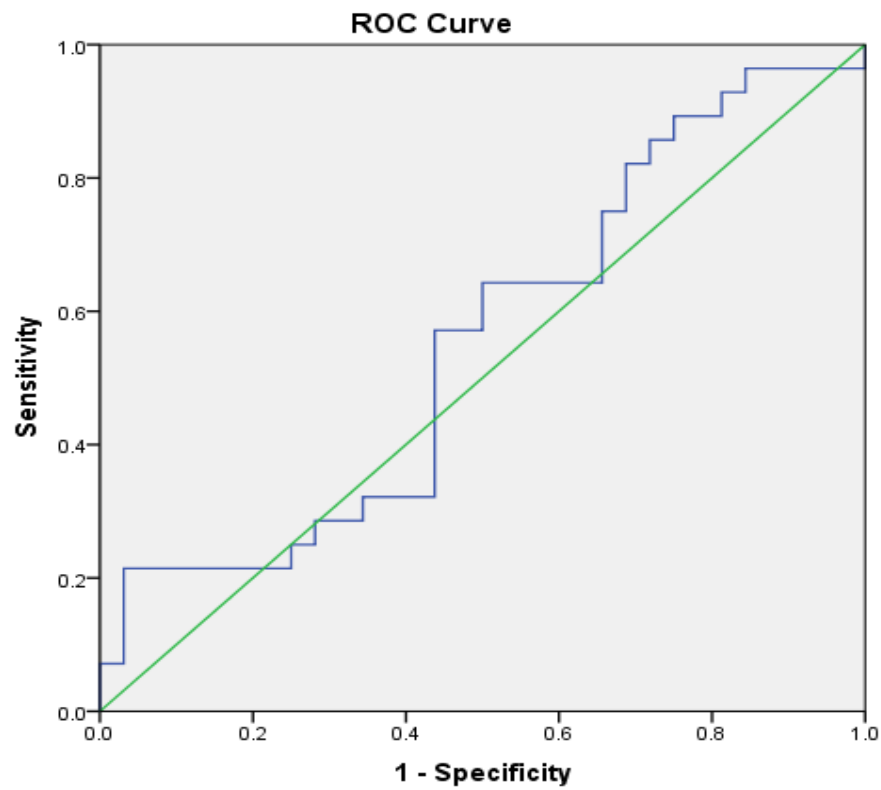


Figure 1: ROC curve for Progesterone level effect on pregnancy outcome

Discussion

The present study aimed to evaluate the effect of endometrial compaction, sub-endometrial blood flow and serum progesterone level on pregnancy outcome in FET cycles. The present work using clinical, ultrasonographic and hormonal parameters in combination, provides insight into the relative contribution of endometrial and luteal factors to successful implantation.

Baseline clinical and demographic characteristics including type and duration of infertility, age, BMI and aetiology of infertility were similar between non-pregnant and pregnant women. These findings are consistent with previous reports by Mackens et al. and Glujovsky et al. who showed that when FET patients are appropriately selected, baseline characteristics have a minor impact on implantation success in comparison with cycle-specific endometrial factors (20,21). The lack of significant differences in these parameters suggests that the results are unlikely to be confounded by baseline patient characteristics. Similar to this, there were no significant differences in the basal hormonal profiles including FSH, LH, AMH, TSH and prolactin between pregnant and non-pregnant groups. This finding is consistent with previous studies by La Marca and Sunkara and Broer et al. that demonstrated that basal hormonal changes within the normal range have little predictive value for pregnancy outcomes in FET cycles,

after excluding ovarian reserve abnormalities and endocrine disorders (22,23). AMH levels were generally higher in the pregnant group but the difference was not significant suggesting that AMH reflects ovarian response rather than endometrial receptivity.

In the present study, endometrial compaction, defined as a decrease in endometrial thickness after starting progesterone, was not significantly associated with pregnancy outcome. These results are contrary to studies by Bu et al. and Haas et al. who found higher pregnancy rates in cycles with endometrial compaction (24,25), but in agreement with other reports by Yuan et al. and Vaegter et al. who failed to show a clear advantage (26,27). Discrepancies among studies may be due to differences in definitions of compaction, timing of measurements, endometrial preparation protocols and sample size. Thus, endometrial compaction alone might not be a reliable independent marker of endometrial receptivity.

There were also no significant differences in sub-endometrial blood flow patterns between the pregnant and failed cycles. More multifocal blood flow was seen in the pregnant group, but this difference was not statistically significant ($P=0.12$). Previous studies on the predictive value of the sub-endometrial blood flow reported conflicting results; some studies (Chien et al. and Ng et al.) reported an improved implantation with abundant vascularity while others (Alcázar) found limited clinical

utility (15,28,29). The weak and non-significant correlation between progesterone levels and sub-endometrial blood flow in this study, further supports the idea that vascularity alone may not be an appropriate indicator of functional endometrial receptivity.

Serum progesterone concentrations on the day of embryo transfer were not statistically related to pregnancy outcome. The pregnant group had a slightly higher mean progesterone level, but the ROC curve analysis revealed poor discrimination with low sensitivity and specificity. These findings are consistent with reports by Labarta et al., Gaggiotti-Marre et al. and Alsbjerg et al. who reported that a serum progesterone level above a minimum threshold is needed for implantation but higher levels do not necessarily result in better pregnancy outcomes (17,30,31). This supports the concept of a “window effect” for progesterone, beyond which further exposure does not provide further benefit.

Interestingly, a significant correlation was found between progesterone levels and endometrial compaction and also between sub-endometrial blood flow patterns and endometrial compaction. All women with progesterone levels < 10 ng/ml had a non-compacted endometrium and higher progesterone levels were significantly associated with endometrial compaction. This observation is in line with the biological role of progesterone in inducing secretory transformation and stromal decidualisation as described previously by Evans and Salamonsen and Gellersen and Brosens (32,33). Finally, the prevalence of multifocal blood flow in compacted endometrium may be representative of progesterone-induced vascular remodelling during the luteal phase. Nevertheless, these associations did not translate into higher pregnancy rates for either compaction or vascularity, highlighting the multifactorial nature of implantation and endometrial receptivity.

To summarise, the results of this study showed that although progesterone levels and sub-endometrial blood flow are significantly associated with endometrial compaction, they do not independently predict pregnancy effect in FET cycles. This highlights the limitation of single ultrasonographic or hormonal markers of endometrial receptivity and suggests that combined or molecular approaches may be required to identify the optimal window for embryo transfer more accurately.

Conclusion:

Our study showed that in FET cycles, clinical pregnancy rates were not significantly affected by endometrial compaction, serum progesterone levels or sub-endometrial blood flow when these factors were evaluated independently. Thus, the routine measurement of each parameter in isolation during hormone replacement therapy (HRT)-FET cycles may have limited predictive value for implantation success.

Interestingly, we found a significant association between endometrial compaction and serum progesterone levels and subendometrial blood flow. This finding suggests a possible interaction between endometrial structural changes, vascular perfusion and hormonal environment. These

combined factors may have more effect on endometrial receptivity than any single parameter. Further studies are urgently needed to ascertain the clinical implications of the combined assessment of these variables for optimal outcomes of FET cycles.

Conflicts of interest

There was no conflict of interest

Acknowledgement:

Many thanks for dr/ Mohamed Mahmoud Waly (professor / Cairo University/ Egypt) for his guidance, and his great advices.

Funds of the article

There was no funding for this work. Authors declare no competing interests.

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