Journal of Medical and Surgical Practice (JMSP)
Vol. 07, No. 2, April-June, 2021

Comparison of E-Cadherin Expression Between Fertile Females and Patients Having Primary Infertility With Failed in Vitro Fertilization and Intrauterine Insemination

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Original Article

Summary

Successful implantation requires a receptive endometrium, E-cadherin is a cell surface glycoprotein responsible for adhesion between epithelial cells. Its role in the implantation of embryo remains controversial. Endometrial biopsy samples can be used to identify molecules associated with uterine receptivity to obtain a better insight into human implantation. Therefore, this study aimed to investigate E-cadherin expression in the endometrium throughout the menstrual cycle of fertile females and to compare the positivity index (PI) in the proliferative phase with patient having primary infertility with failed in vitro fertilization (IVF) or intra uterine insemination (IUI). Hence, fractional endometrial biopsies were taken from anterior, posterior, fundal walls, and the cervix of 32 fertile women as control group compared to 75 patients with primary infertility; 33 had failed IVF and 42 patients had failed IUI . We found that the PI of E-cadherin in the glandular epithelium of the anterior wall is significantly higher than that of posterior and fundal walls in the control group (P=0.037). PI of E-cadherin of the endocervical epithelium is significantly higher than that of the walls of the endometrial cavity in control, failed IVF group (P=0.004, 0.002 respectively). The PI of E-cadherin expression in the stromal epithelium of the fundal wall is significantly higher than that of anterior and posterior walls in failed IVF group (P=0.001). In conclusion, E- cadherin might have a role in implantation.

Keywords: *E-Cadherin expression, fertile females, in vitro fertilization, intrauterine insemination*

Article information: Received: February , 2021, Accepted and Published April, 2021

How to cite this article*: Al-Jubouri F.A.H., Al-Ani F.S., Skheel H.Dh.. Comparison of E-Cadherin Expression Between Fertile Females and Patients Having Primary Infertility With Failed in Vitro Fertilization and Intrauterine Insemination. JMSP, 7 (2): 255-70*

1. INTRODUCTION

Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to the embryonic implantation (1). It is the window of time when the uterine environment is conductive to blastocyst acceptance and subsequent implantation (2). Successful implantation requires a receptive endometrium, a normal and functional embryo at the blastocyst developmental stage and a synchronized dialogue between maternal and embryonic tissues (3). The process of implantation may be classified into three stages: apposition, adhesion and invasion (4). Even though the blastocyst can implant in different human tissues, surprisingly in the endometrium, this phenomenon can only occur during a self-limited period spanning between days 20 and 24 of a regular menstrual cycle. Throughout this period, namely the window of implantation (5), the human endometrium is primed for blastocyst attachment, given that it has acquired an accurate morphological and functional state initiated by ovarian steroid hormones (6-8). Implantation involves a complex sequence of signaling events, consisting in the acquisition of adhesion ligands together with the loss of inhibitory components, which are crucial to the establishment of pregnancy (9). Histological evaluation, now considered to add little clinically significant information, should be replaced by functional assessment of endometrial receptivity. A large number of molecular mediators have been identified to date, including adhesion molecules, cytokines, growth factors, lipids and others. Thus, endometrial biopsy samples can be used to identify molecules associated with uterine receptivity to obtain a better insight into human implantation (9). Implantation failure remains an unsolved obstacle in reproductive medicine and is a major cause of infertility in otherwise healthy women(10). In the field of assisted reproductive technologies (ART), embryo implantation remains the bottle neck of ART. In contrast to all the steps that take place earlier in the ART measures such as notably multiple follicular stimulation, oocyte retrieval, fertilization and cleavage rates, which have an efficacy largely more than 50 percent, embryo implantation rates continue to lag behind (11). Inadequate uterine receptivity is responsible for approximately two-thirds of implantation failures, whereas the embryo itself accounts for only one-third of failures. Therefore, the management of repeated implantation failure (RIF) is one of the most difficult issues in assisted reproduction (12).

2. PATIENTS and METHODS

Patients, Materials and Methods: Thirty-two fertile females, between 20-40 years of age, participate in the current study as a control (volunteers). They are parous with no history of abortion, gestational trophoblastic disease, preterm labour or ectopic pregnancy. Seventy-five females of comparable age with history of primary infertility who had failed IUI (n=42) and failed IVF-ET (n=33) are enroled as the infertile groups. Control group are collected from patients attend Gynaecological Department in Al- Yarmouk Teaching Hospital who agreed to participate in the study as a volunteers with informed consent form. Cases of failed IVF attempt and failed IUI trials were collected from the Consulation Department of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. Under simple analgesia, fractional endometrial biopsy from anterior wall, posterior wall, fundus, and endocervix were taken for the control group at different days of the menstrual cycle to investigate the changes in the expression of the studied marker (E- cadherin) with days of the cycle and to estimate the differences in the Positivity Index(number of immunostained cells /total number of cells in the examined field) between different walls. For the other groups, fractional endometrial biopsy were taken during late proliferative phase to compare the level of expression with the control group. To quantify immunostained cells objectively, we used a computerized image analysis programme(Aperio Image scope). Several parameters per sample were computed: the percentage of immunostained surface (compared with the counterstained surface), the mean staining intensity, and an immunostained score (percentage of immunostained surface _ mean staining intensity). This programme can provide tools to cut a certain area for isolated analysis. All images are (×400).

Statistical Analysis: Data analysis was computer assisted using SPSS19 (statistical package for social sciences). Frequency distribution for selected variables was done first. The statistical significance of difference in mean of a quantitative variable between 2 groups was tested by independent samples t-test. While, analysis of variance (ANOVA) was used for more than two groups (13).

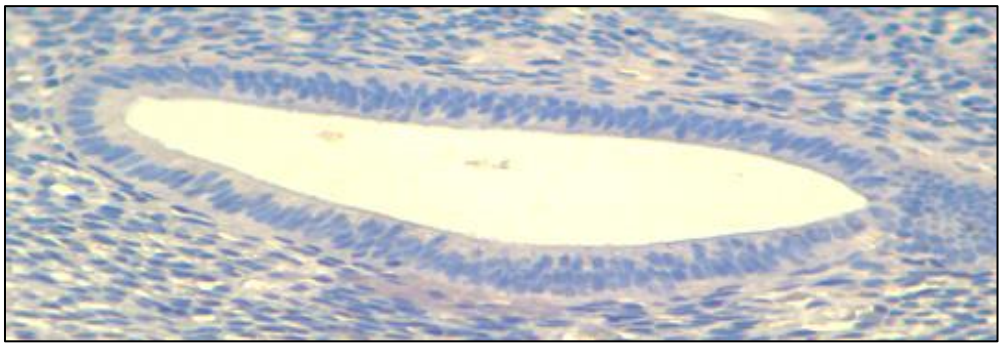

Figure 1. IHC staining of endometrium showing endometrial gland in late proliferative phase.

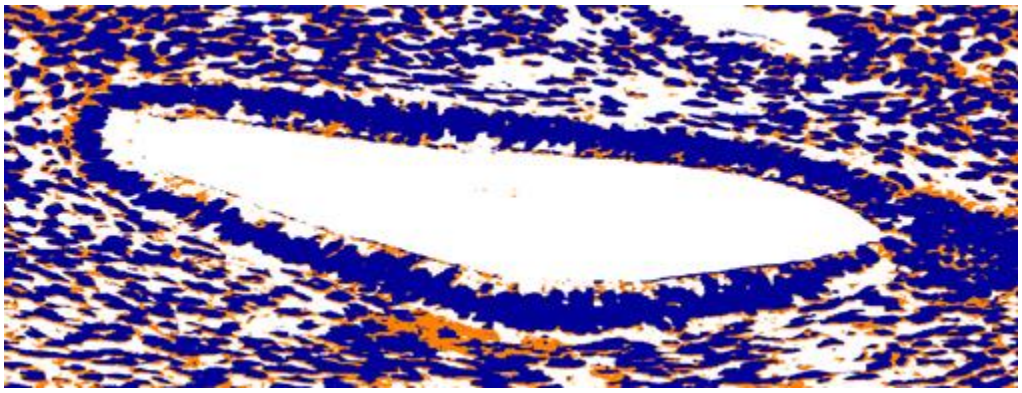

Figure 2. Computerized image analysis for the whole IHC stained section of endometrium showing E-cadherin expression in a light brown color

3. RESULTS

For the control group, (56.25%) aged 20-29 years, Among IVF and IUI groups (51.52% and 59.52% IUI respectively aged 30-39 years (**Table 1**). Indication for IVF or IUI included Tubal factor for IVF in (18.18%) but not IUI, while unexplained infertility is the most common indication for both ARTs (50% and 45.45%) for IVF and IUI groups, respectively .There is a significant difference between the IVF, IUI groups in indications for ARTs (P= 0.036). The difference is mainly due to tubal factor (P= 0.001), **(Table 2**)

E-cadherin expression in the control group:

The E-cadherin was measured in the endometrium at three sites: anterior, fundal, and posterior walls in addition to the endocervical epithelium from early proliferative phase to the mid-secretory phase. Three epithelial regions were evaluated for E-cadherin expression:

membranous, stromal, and glandular epithelium. The expression is increasing with time during the proliferative phase (e.g. at anterior stromal epithelium it increases from 4±1) and peak at late proliferative phase (40±6) , then decline gradually towards mid-secretory phase(32±3 at day 20 to 8±2 at day 25) (implantation window). Note that the expression of fundal wall and ;to a lesser extent; the posterior wall continue to rise to the midsecretory phase while the expression of the anterior wall and the cervix decline at the same period, (**Table 3**). PI is evaluated at three regions: stromal, membranous, and glandular epithelium. There is a significant difference between the three walls only in the glandular epithelium (P=0.037), (**Table 4**).

The PI of anterior, posterior, fundal walls of the endometrial cavity and the endocervical epithelium. The endocervical epithelium has a significantly higher PI of E-cadherin expression than the three walls of the endometrium cavity, (P=0.004), (**Table 5**) shows mean

E-cadherin expression in failed IVF group.

The PI of E-cadherin is measured in infertile women with failed IVF trial during the proliferative phase of the menstrual cycle. As in the control group, the PI was measured in the anterior, posterior, and fundal walls at three regions: stromal, membranous, and glanduler epithelium. No significant difference was found between stromal, membranous, and glandular epithelium in the anterior and posterior walls(P= 0.592, 0.077 respectively) while a significant difference was found between the three regions in the fundal wall(P=0.001). From other point of comparison, a significant difference was found between the three walls in PI of E-cadherin expression in the stromal epithelium which was significantly higher in the fundal wall (P=0.001), while no such difference was found between the three walls in membranous and glandular epithelium (P=0.215, 0.183 respectively), (**Table 6**).

Comparison between PI of E-cadherin in anterior, posterior, and fundal walls with the endocervical epithelium in the 10th day of the menstrual cycle revealed a significant between the three walls of the endometrium and the endocervical epithelium which has a significantly higher PI (P=0.002), (**Table 7**).

E-cadherin expression in failed IUI group.

The PI of E-cadherin expression in the anterior, posterior, and fundal walls of the endometrial cavity in failed IUI group at day 10 of the menstrual cycle. A significant

difference was found between stromal, membranous, and glandular regions in the anterior (P=0.001) and fundal (P=0.001) walls respectively. No such significant difference was found in the posterior wall (P=0.149). No significant difference was found between the three walls in stromal and membranous epithelium (P=0.105 and 0.499 respectively) while in glandular epithelium PI was significantly lower in the anterior wall (P=0.009), **(Table 8**). A significant difference between the three walls and endocervical epithelium which has a higher PI of E-cadherin expression(P=0.001), (**Table 9**)

Comparison of PI in the control, IVF, and IUI groups

In the present study, a comparisons was made between the three studied groups taking day 10 as a time point of comparisons .PI of E-cadherin was measured in the anterior, posterior, and fundal walls of each group. A significant difference was found in the anterior wall of the IVF group(≤0.05), (**Table 10**)

Table 1. The women age of the control, failed IVF, and failed IUI groups.

Study groups	Age (year)						
	20-29		30-39		≥ 40		Total
	Count	%	Count	%	Count	%	
Control	18	56.3	10	31.25	4	12.5	32
Failed IVF	12	36.4	17	51.52	4	12.12	33
Failed IUI	14	33.3	25	59.52	3	7.14	42

Table 2. Indication for IVF, IUI in the studied infertile groups

Indication	Group				P. value
	Failed IUI		Failed IVF		
	No.	%	No.	%	
Asthenozoospermia	6	14.29	3	9.09	0.539
Ovulatory	15	35.71	9	27.27	0.436
Tubal	0	0	6	18.18	0.001
Unexplained	21	50	15	45.45	0.696
Total	42	100	33	100	

Table 3. Mean E- cadherin expression from early proliferative phase towards midsecretory phase in the anterior, posterior and fundal walls.

Site		PI (Mean ± S.D)							
		6th day	7th day	8th day	9th day	10th day	14th day	20th day	25th day
Anterior	Stromal	4±1	7±2	10±1	12±1	20±4	40±6	32±3	8±2
	Membranous	5±1	5±1	6±1	9±1	15±2	29±5	24±6	16±2
	Glandular	4±1	4±1	4±1	5±1	8±1	26±11	26±4	6±3
Posterior	Stromal	8±2	10±1	10±1	18±1	22±1	32±2	27±4	19±8
	Membranous	8±1	6±1	8±1	10±1	16±4	33±3	33±5	20±8
	Glandular	5±1	4±1	7±1	10±1	15±2	27±7	25±5	13±7
Fundal	Stromal	12±1	14±1	14±1	16±1	24±2	44±12	43±8	32±4
	Membranous	8±1	10±1	10±1	11±1	13±0	31±14	40±11	23±11
	Glandular	5±1	6±1	8±1	10±1	14±2	25±6	29±6	18±7

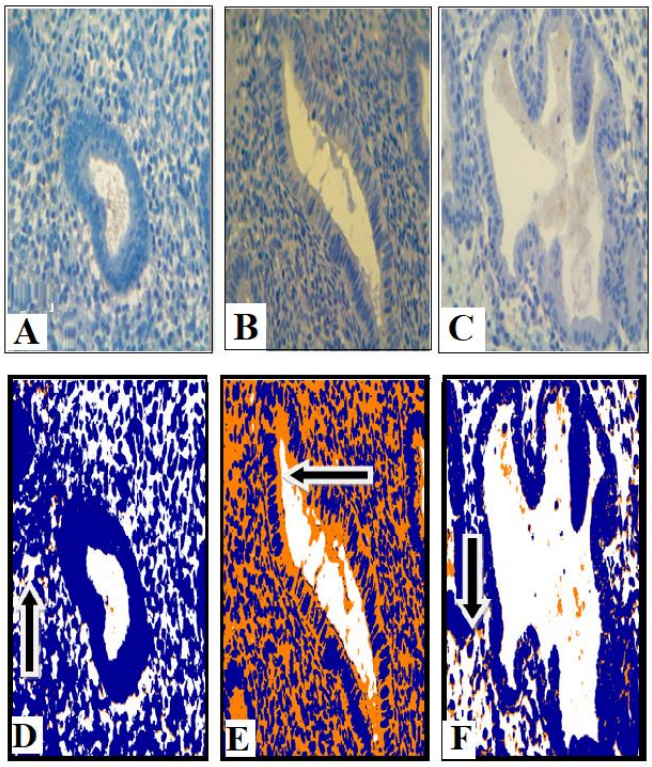

Figure 3. E-cadherin in the glandular epithelium at different times of the menstrual cycle in the control group. IHC staining of endometrial glands at early proliferative phase(A), late proliferative phase(B), and mid secretory phase(C). D, E, and F are computerized image analysis for PI of E-cadherin(orange color) in A, B, and C.

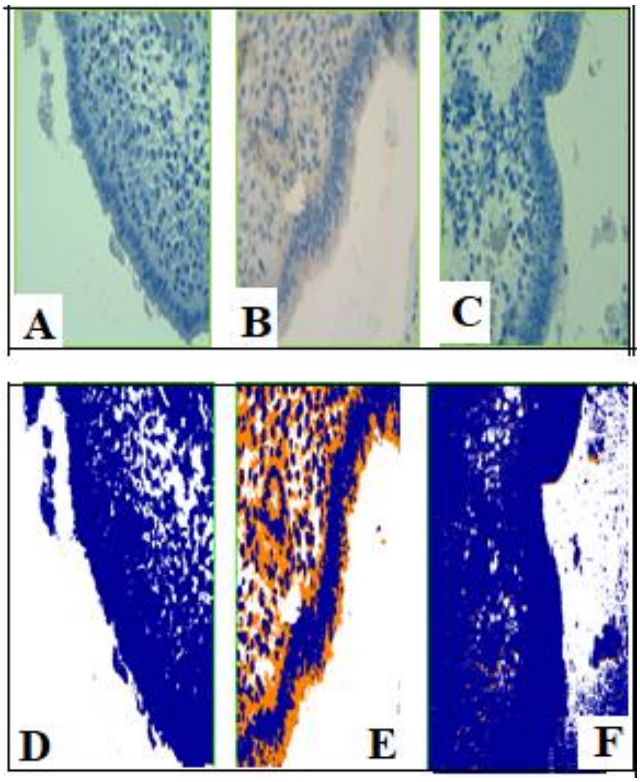

Figure 4. E-cadherin in the membranous epithelium at different times of the menstrual cycle in the control group. IHC staining of endometrial surface epithelium at early proliferative phase(A), late proliferative phase(B), and mid secretory phase(C). D, E, and F are computerized image analysis for PI of E-cadherin(orange color) in A, B, and C.

Table 4.The mean PI of E-cadherin expression at anterior, posterior, and fundal walls of the control group

Histological layer	Anterior		Fundal		Posterior		P value
	Mean	SD	Mean	SD	Mean	SD	
Stromal	20%	4%	24%	2%	22%	1%	0.461
Membranous	15%	2%	13%	0%	16%	4%	0.689
Glandular	8%	1%	14%	2%	15%	2%	0.037
P value	0.027		0.001		0.171		

Table 5. The mean PI of E-cadherin expression in the anterior, posterior, and fundal walls of the endometrial cavity and the endocervical epithelium.

Wall	Mean	S.D.
Anterior	14.17%	6.94%
Fundal	17.08%	5.92%
Posterior	17.75%	5.94%
Cervical	29.00%	7.79%
P. value = 0.004, Significant		

Table 6. PI of E-cadherin expression in anterior, posterior, and fundal walls of the endometrial cavity of the failed IVF group at day 10 of the menstrual cycle.

	Anterior		Fundal		Posterior		p value
Layer	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Stromal	11%	3%	27%	2%	15%	0%	0.001*
membranous	10%	0%	12%	1%	10%	2%	0.215
Glandular	12%	1%	16%	2%	21%	5%	0.183
p. value	0.592		0.001*		0.077		
*Significant P<0.05							

Table 7. Mean PI of E-cadherin expression in the anterior, posterior, and fundal walls of the endometrial cavity and the endocervical epithelium of failed IVF group at day 10 of menstrual cycle

Wall	Mean	S.D.
Anterior	14.33%	4.81%
Fundal	16.67%	6.11%
Posterior	17.33%	4.54%
Cervical	26.75%	4.57%
P. value = 0.002, Significant		

Table 8. PI of E-cadherin expression in the anterior, posterior, and fundal walls of the endometrial cavity in failed IUI group at day 10 of the menstrual cycle.

	Anterior		Fundal		Posterior		p value
Layer	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Stromal	20%	1%	25%	1%	21%	2%	0.105
membranous	12%	1%	12%	1%	14%	2%	0.499
Glandular	11%	0%	13%	0%	17%	2%	0.009
p value	0.001		0.001		0.149		

Table 9. PI of E-cadherin expression in anterior, posterior, and fundal walls of the endometrial cavity with endocervical epithelium of failed IUI group at day 10 of the menstrual cycle.

Wall	Mean	S.D.
Anterior	10.89%	4.11%
Fundal	18.50%	7.74%
Posterior	15.44%	8.62%
Cervical	29.00%	8.29%
P. value = 0.001, Significant		

Table 10. E-cadherin expression at different sites in the control, IVF, and IUI groups at day 10 of the menstrual cycle

Site of sample	Study groups		
	Control	IVF failed	IUI failed
Anterior	14.17±2.33	10.89±1.33*	14.33±0.95
Posterior	17.75±2.29	15.44±2.28	17.33±1.96
Fundal	17.08±1.27	18.50±1.52	16.67±0.53
Significantly lower < 0.005			

4. DISCUSSION

Only few, small human studies on E-cadherin in normal cycling human endometrium have been reported (14), less for repeated implantation failure following IVF-ET cycle. Endometrial biopsy samples can be used to identify molecules associated with uterine receptivity and E-cadherin is one of these molecules. There is controversy about cyclical changes in E-cadherin expression in endometrium, so the current descriptive study is conducted to investigate the positivity index(PI) of E-cadherin expression in the endometrium of normal fertile females and its changes during menstrual cycle as well as to evaluate the site of highest expression and to compare with infertile females. The current knowledge of pre-implantation and implantation physiology is the result of observations and descriptive studies conducted by many researchers and physicians through these years. The PI of E-cadherin was measured in the superficial layer of the endometrium at three sites: anterior, fundal, and posterior walls in addition to the endo-cervical epithelium from early proliferative phase to the midsecretory phase. Three epithelial regions were evaluated for E-cadherin expression: membranous, stromal, and glandular epithelium(15). The PI of E-cadherin day 3, 4, and 5 were excluded from the statistical analysis because the value of PI was either very low (near zero) at the time of shedding that might affect the statistical results or very high indicating that the sample was from the basalis layer and not from the functionalis (15). PI of E-cadherin is increasing during the proliferative phase, peaks at late proliferative phase and ovulation, and decreasing in the secretory phase. The increment is characterized by steady elevation in stromal, membranous, and glandular regions in the three walls during day 6, 7, 8, and 9. This may reflect the relatively steady slow elevation of estradiol during early follicular phase (16,17).

The PI is higher in the stromal epithelium than membranous and glandular epithelium. The elevation in stromal E-cadherin more than other region may be related to the higher level of mitotic activity in this region (18). It is well known today that the follicular phase is of variable length in part because of the uncertainty about when its functional onset , the intercycle FSH elevation, truly takes place(11). This may be indicated by the sharp increament that starts at day 10 of the cycle and reaches peaks afterward. Yet, this variability appears not to affect the endometrial priming and has no practical consequences

on the endometrial responsiveness in the ensuing luteal phase (11). If peaks of the PI in the three walls are inspected, it can be observed that the peaks are reached at day 14 in most cases , but it is reached at day 20 in the glandular epithelium of the anterior wall(29%), membranous(40%) and glandular epithelium(29%) of the fundal wall and still , they are lower than other peaks. This discrepancy in reaching the peak might have a role in determining of the site of implantation. Moreover, the peak of the stromal epithelium (44%) is superior to the other peaks in anterior and fundal walls while the peak of the membranous (33%)epithelium(which remain platue between day 14-20 of the menstrual cycle.) is the highest in the posterior wall. The persistence of relatively elevated level of membranous E-cadherin might be a detrimental factor(mostly preventive) in the process of implantation. The peak of the glandular epithelium is lower than stromal and membranous epithelium in anterior, posterior, and fundal walls. The peak of glandular and membranous epithelium was more or less equal in the anterior wall while there is discrepancy between the two peaks in other walls. Delay of the glandular epithelium in reaching peaks until the secretory phase is due to continuation of gland mitosis in that phase as observed by Noyes (19). Since the adhesiveness is positively correlated with E-cadherin level (20) and that glandular secretion requires lowering of this adhesiveness, this may explain the continuously observed low level for E-cadherin in the glandular epithelium in all walls. Low level of peaks for membranous and glandular epithelium in the anterior wall might point to a suitable site of implantation. Concerning the style of decrease in PI of E-cadherin expression after peak, it is obvious that the decrease in the PI of the anterior wall is sharp in glandular epithelium and more steady in the stromal and membranous epithelium. This may reflect the rapid changes during the implantation window in the favorable sites since loose adhesiveness in the glandular epithelium enables the secretion from the endometrial glands which is the main source of uterine endometrial secretion (uterine milk)(16) that is the nutrient to the developing embryo. In the posterior wall, the decrease in PI is steady in all regions with obvious delay in the membranous epithelium, while in the fundal wall, the decrease in PI is steady in stromal epithelium, delayed and sharp in the glandular and membranous epithelium. These two walls may show high level compared with anterior wall to maintain integrity of the endometrium while the embryo is invading the anterior wall. The lowest level of PI of the membranous epithelium remains higher than that of other

regions except for the fundal wall where the lower level of E-cadherin PI in the stromal epithelium (32%) is larger than other region. This may be necessary to maintain integrity of the endometrial epithelium. In some aspects these result are consistent with Shih et al. who found that the expression of E-cadherin in glandular cells was observed mainly in the proliferative phase. The mean PI in the early proliferative phase was 15.2 in functionalis layer and increased in the late proliferative phase to 39.2. The E-cadherin expression decreased significantly in the secretory phase both in the functionalis and in the basalis compared proliferative phase. Stromal cells showed positive staining for E-cadherin in the proliferative and early secretory phases, but the positive staining disappeared in the mid- and late secretory phases. Surface epithelial cells showed positive staining for Ecadherin throughout the menstrual cycle with a slight predominance in the secretory phase (10). No predominance of E-cadherin in surface epithelium were noticed in this study., the same observation was found by Matsuzaki et al. (21) who observed very low or no protein expression of E-cadherin, in luminal and glandular epithelial cell in the mid-secretory endometrium of healthy fertile controls. These findings suggest that temporal down-regulation or loss of E-cadherin expression during the window of implantation might be necessary to enable epithelial cell dissociation and blastocyst invasion, in agreement with the results of several recent animal studies (15).

The mean age of the IVF and IUI groups were comparable (32±4.03 , 31.0±4.86 years for IVF,IUI groups respectively). In addition to age (P=0.499) there were no significant difference in duration of infertility(P=0.291), but there was a significant difference in the indication for ARTs (0.036) which may be attributed to the tubal factor that is excluded in IUI cases. A significant difference was also found in the number of trials (0.004) which is due to the high cost of IVF-ET cycle compared with IUI. For both groups, the PI was measured at day 10 of the menstrual cycle .

The following finding were noticed:

For IVF group and unlike the control group, there were no significant difference between stromal (11%), membranous (10%), and glandular (12%) epithelium in PI of E-cadherin in the anterior wall (P=0.592).The PI of the glandular epithelium was higher than other regions; on the contrary, it has the lowest PI in the control group. Similarly, there is no significant difference between the three histological layers in the posterior wall (P=0.077).

Significant difference were found between the three histological layers in the PI of E-cadherin in the fundal (P=0.001) wall of the IVF group. This might indicate unreceptively anterior and posterior walls, at the same time it might explain the increased incidence of cornual ectopic pregnancy in IVF group.

For IUI group, the result was more or less similar to the control group. A significant difference between the three histological layers were found in the anterior (P=0.001) and fundal(P=0.001) walls while no significant difference was found in the posterior wall(0.149). Moreover, there was a significant difference (P=0.009) in the PI of E-cadherin in the glandular epithelium between the three walls.

Similar to control group, in both IVF and IUI groups, there were a significant difference between the three walls and the cervix which shows the highest PI.

E-cadherin PI was higher in anterior wall of the control and IUI groups than IVF group. The PI in both control and IUI groups were higher in the posterior wall followed by fundal , and anterior walls respectively while in IVF group, the PI were higher in fundal wall followed by posterior and anterior walls respectively. This disarrangement may be attributed to the effect of ovulation induction programs and the high level of E2 on the endometrium.

The IUI group is more or less similar to the control groups. This similarity may be explained and supported by the following:

There is uncertainty that unreceptive endometrium is the cause of infertility in failed IUI since fertilization is not guaranteed. The ovulation induction programs; if indicated, is simple, so it will not disrupt the endometrial environment caused by heavy induction programs. Two of cases in failed IUI group got pregnant after failure, one of them spontaneously and the other following IVF-ET indicating that the endometrium in both is competent. Taking in consideration that this comparison is made in day 10 of the menstrual cycle during which the endometrium shows differences in E-cadherin PI between the three histological layers, it is concluded that two factors are important first, there should be no significant difference in mean PI of the three walls. Second the glandular epithelium show the lower level of PI followed by the membranous epithelium while the stromal epithelium shows the highest level. If the low level of PI of E-cadherin is merely considered essential for implantation, then anterior wall of the IVF group should shows the highest receptivity which is not the truth, so the ratio between the three walls should also be considered as well.

5. CONCLUSIONS

E-cadherin might have a role in implantation. E-cadherin expression in the endometrium is up-regulated in the proliferative phase and down-regulated in the secretory phase of the menstrual cycle. The PI of E-cadherin in the glandular epithelium of the anterior wall of the endometrium was significantly lower than that of posterior and fundal walls in the control group. The PI of E-cadherin in the endocervical epithelium was significantly higher than that in the anterior, posterior, and fundal walls of the endometrial cavity and in control, failed IVF and IUI groups. The PI of E-cadherin in the stromal epithelium of failed IVF group was significantly higher in the fundal wall than that anterior and posterior walls.

Ethical Clearance: Ethical clearance and approval of the study are ascertained by the authors. All ethical issues and data collection were in accordance with the World Medical Association Declaration of Helsinki 2013 for ethical principles for medical research involving human subjects, informed consent obtained from all patients. Data and privacy of patients were kept confidentially.

Conflict of interest: Authors declared none

Funding: None, self-funded by the authors

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