The Role of E-cadherin in the embryo implantation and its site in the endometrium

Henan Dh.Skheel[1],Fakhir S. Al-Ani[2] and Farkad A. Hassan[2]
1College of Medicine-Al-Mustansyria University
2Department of Clinical Reproductive Physiology ,High Institute of Infertility Diagnosis &Assisted Reproductive Technology-Al-Nahrain University.

Abstract:

Background:
Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to the embryonic implantation. Histological evaluation, now considered to add little clinically significant information, should be replaced by functional assessment of endometrial receptivity. A large number of functional molecular mediators have been identified to date, including adhesion molecules. Thus, endometrial biopsy samples can be used to identify molecules associated with uterine receptivity to obtain a better insight into human embryo implantation.

Objectives:
to investigate E-cadherin expression in the endometrium throughout the menstrual cycle of fertile females

Patients, Materials and Methods:
Under simple analgesia, fractional endometrial biopsies from anterior, posterior, fundal walls, and the cervix of (32) fertile females(age= 29.91± 5.9 year, parity= 3.62.1±) were taken at different days of the menstrual cycle. The immunohistochemical (IHC) stained tissues were analyzed using computerized image analysis for measurement of PI of E-cadherin which is ratio of the number of stained cells to the number of total cells. PI were measured in three regions: stromal, membranous, and glandular epithelium.

Results:
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 (P=0.027). 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 (P=0.004).

Conclusion:
Conclusion: E-cadherin might have a role in embryo implantation

Key Words: Endometrial receptivity; adhesion molecules;E-cadherin.

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)(7)(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).

Cadherins constitute a group of glycoproteins responsible for the calcium-dependent cell-to-cell adhesion mechanism. They are divided into subclasses E- (epithelial), P- (placental), and N- (neuronal) cadherins that are distinct in immunological specificity and tissue distribution. They promote cell adhesion via a homophilic mechanism. In regard to implantation, E-cadherin represents the most studied subclass.E-cadherin is a cell surface transmembrane glycoprotein, which belongs to the family of calcium-dependant CAMs, that mediates cell–cell adhesion through homeotypic binding. E-cadherin is located in the adherents junctions that are specialized regions on the lateral side of the epithelial plasma membrane and is believed to be critical for the establishment and maintenance of these junctions in epithelial cells (10,11). E-cadherin is expressed by a variety of tissues and plays an important role in embryogenesis formation during gastrulation, neurulation and organogenesis (12). Studies on mouse embryo implantation have shown that targeted mutations in the E-cadherin gene result in defective preimplantation development (13). The role of E-cadherin in human embryo implantation is not fully known, but based on its expression pattern; it has been suspected that it is of importance for this process. E-cadherin mRNA levels were shown to be significantly higher during the luteal phase (14). Nevertheless, these menstrual cycle variations were not detected at the protein level by immunohistochemical studies (15).

The regulation of E-cadherin availability at the epithelial cell surface enables cellular adhesion control. Down-regulation of E-cadherin expression correlates with the acquisition of metastatic potential by carcinomatous cells. Subsequently, the tissue architecture is lost resulting in cell dissociation and dispersion (16). Intracellular calcium is essential in the E-cadherin regulation. Indeed, a rise in its concentration activates key signaling pathways that mediate cytoskeletal reorganization and disassembly of E-cadherin at the adherents junctions. Alterations in intracellular calcium concentrations affect epithelial cell adhesiveness and polarity by triggering CAMs redistribution (17). This phenomenon could be of importance in EECs expressing E-cadherin. Interestingly, calcitonin expression is induced by progesterone in the human endometrial epithelium specifically during the mid-secretory phase of the menstrual cycle (18). Indeed, calcitonin is known to be a potential regulator of implantation (19). Progesterone, probably via endometrial calcitonin induction leading to increased intracellular calcium, could regulate E-cadherin expression. Thus, it is possible that E-cadherin possesses a dual function. In the preliminary phases, its expression at the cell surface is required to ensure adhesiveness. In contrast, E-cadherin may be subsequently down-regulated to enable epithelial cells dissociation and blastocyst invasion(9).

Objectives

to investigate E-cadherin expression in the endometrium throughout the menstrual cycle of fertile females

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. 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.

Under simple analgesia, fractional endometrial biopsies 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) throughout the cycle and to estimate the differences in the level of expression between different walls. The formaldehyde fixed paraffin embedded tissues are stained with immunohistochemical stain. To quantify immunostained cells objectively, a computerized image analysis program (Aperio Image scope) was used. 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).

Results

In table (1) shows that 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 midsecretory 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 (eg. at anterior stromal epithelium it increases from (4 ±1) and peak at late proliferative phase (40± 6), then decline gradually towards midsecretory 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 figuns (1,2,3).

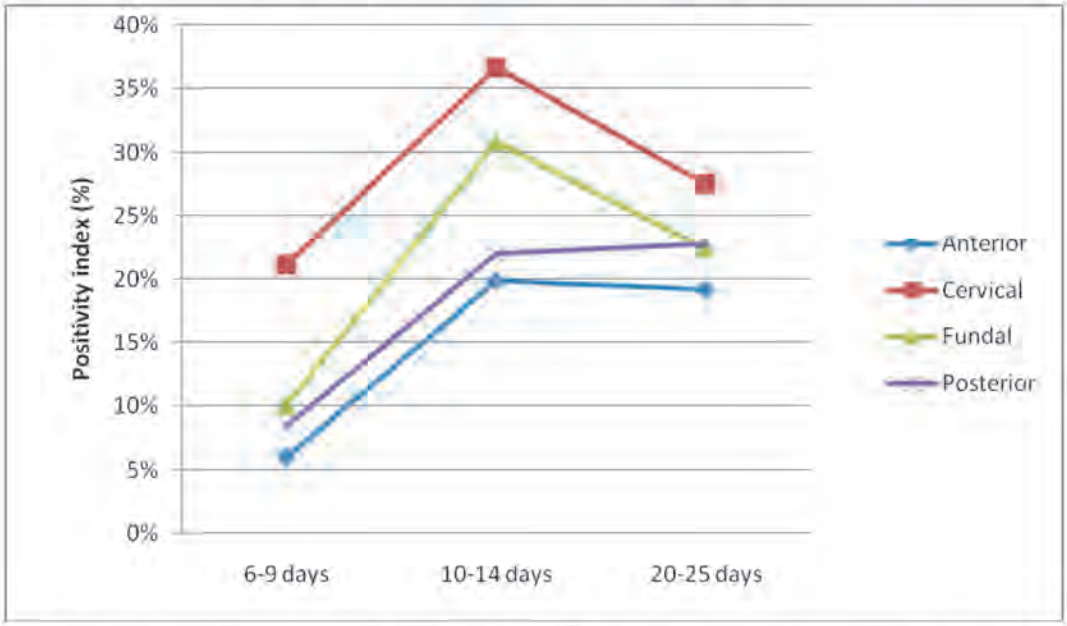
Table(1): Mean E- cadherin expression from early proliferative phase towards midsecretory phase in the anterior, posterior and fundal walls.

Site of endometrium		E- cadherin expression							
		Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Site	Region	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
Posterior	Glandular	4±1	4±1	4±1	5±1	8±1	26±11	26±4	6±3
	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
Fundal	Glandular	5±1	4±1	7±1	10±1	15±2	27±7	25±5	13±7
	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

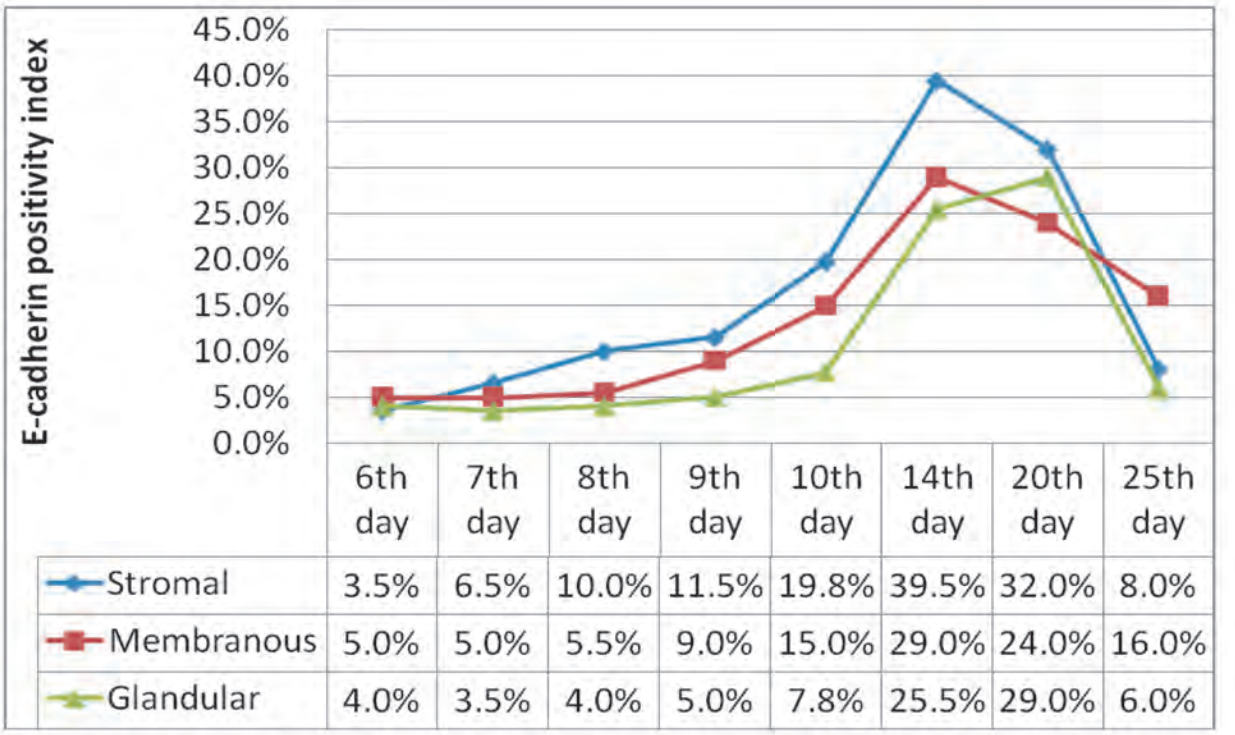
Figure (1)shows PI of E-cadherin in the three walls of endometrial cavity. Note that the cervix has the highest level at peaks (37%) while the anterior wall has the lowest level at peaks(20%).

Figure (2) shows that the PI of epithelial E-cadherin is increasing steadily at early proliferative phase (day 6 to day 9) then peak sharply at late proliferative phase(day 10 to day 14) then decrease in the secretory phase. The glandular epithelium shows the lowest level of PI at peaks (up to 29%) compared with 39.5% in the stromal epithelium while the decrement is sharp in the glandular epithelium (down to 6%) and more steadily in the membranous (down to 16%) and to lesser extent stromal epithelium(down to 8%).As in the anterior wall, the increase starts steadily at early proliferative phase and peaks at late proliferative phase then decrease at early and midsecretory phase. The membranous epithelium shows the highest peak of PI followed by stromal epithelium. The glandular epithelium has the lowest PI level at peaks and the most sharp decrement in the secretory phase. Similar to the anterior wall, steady early followed by late sharp in increase is noticed. Again, the glandular epithelium has the lowest level at peaks.

Figure (2) shows that the PI of E-cadherin is increasing during the proliferative phase, peaks at late proliferative phase and ovulation, and decreasing in the secretory phase .The same results are shown in figure (3) steady elevation in stromal, membranous, and glandular regions in the three walls during day 6,7,8, and 9, respectively.

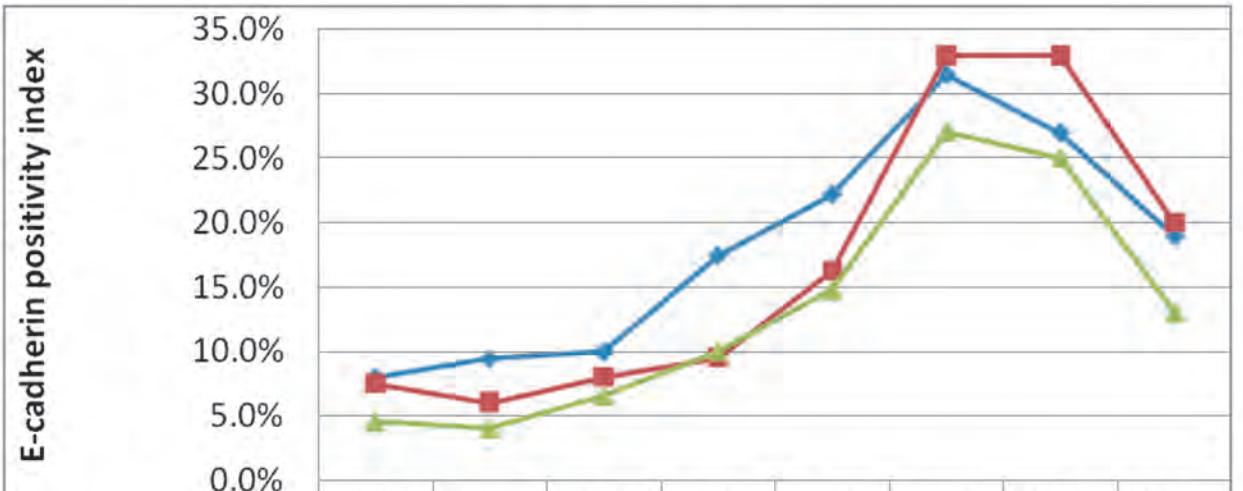


Figure(1): E-cadherin expression in different endometrial walls and the cervix at different days of the menstrual cycles.



	6th day	7th day	8th day	9th day	10th day	14th day	20th day	25th day
Stromal	3.5%	6.5%	10.0%	11.5%	19.8%	39.5%	32.0%	8.0%
Membranous	5.0%	5.0%	5.5%	9.0%	15.0%	29.0%	24.0%	16.0%
Glandular	4.0%	3.5%	4.0%	5.0%	7.8%	25.5%	29.0%	6.0%

Figure (2): E-cadherin positivity index in the anterior wall of the endometrium.



	6th day	7th day	8th day	9th day	10th day	14th day	20th day	25th day
Stromal	8.0%	9.5%	10.0%	17.5%	22.3%	31.5%	27.0%	19.0%
Membranous	7.5%	6.0%	8.0%	9.5%	16.3%	33.0%	33.0%	20.0%
Glandular	4.5%	4.0%	6.5%	10.0%	14.8%	27.0%	25.0%	13.0%

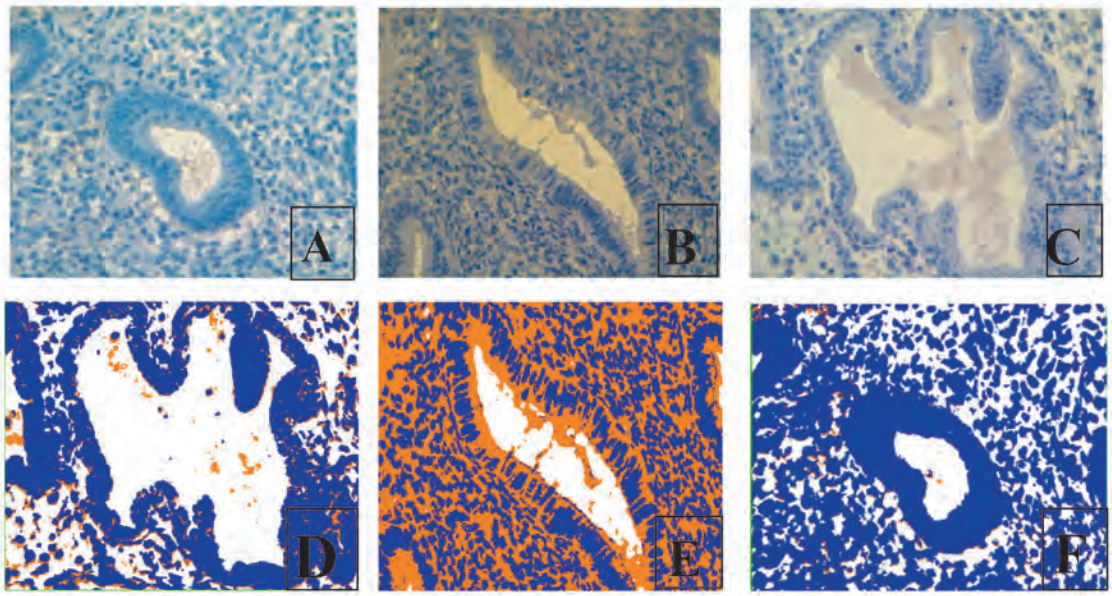
Figure (3): PI of E- cadherin expression in the posterior wall of the endometrium.

In table (2) PI is measured in three regions: stromal, membranous, and glandular epithelium during day 10 of the cycle .In the anterior wall, there was a significant difference in PI of E-cadherin expression between the stromal (20%), membranous(15%), and glandular(8%) (P=0.027). A significant difference was found between the same regions in the fundal wall(24%, 13%,and 14% for stromal, membranous, and glandular regions respectively)(P=0.001). No such significant difference were found in the posterior wall (P=0.171). In table (2), a significant difference was found between the three walls in the glandular epithelium (P=0.037) while no significant differences were found between the three walls in stromal and membranous epithelium..

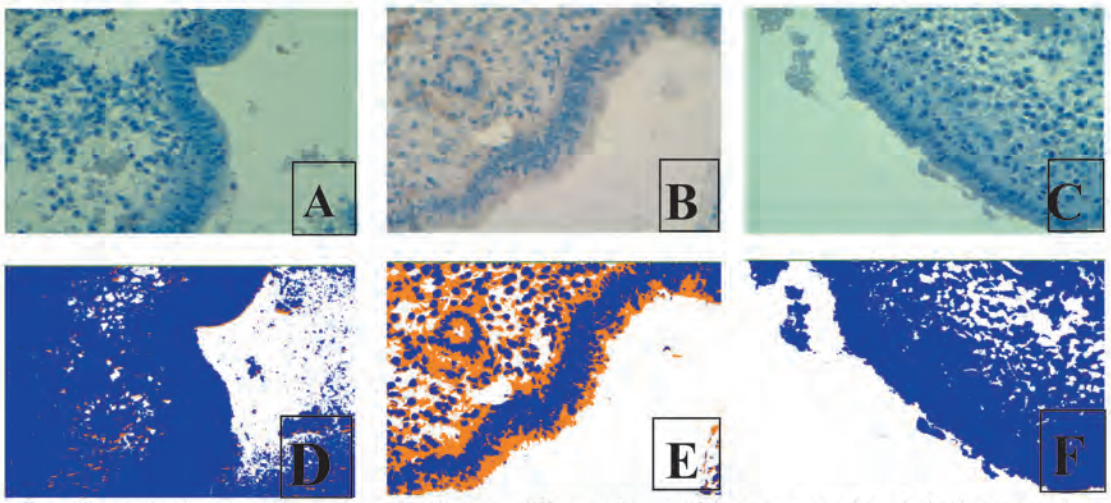
Table (2):The mean PI of E-cadherin expression in the anterior, posterior, and fundal walls of the control group

Control		Anterior		Fundal		Posterior		p value
		Mean	SD	Mean	SD	Mean	SD	
Histological Layer	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		

In figures 4 and 5 ,A more detailed analysis of the PI in the three histological regions, showed that there is a significant difference between stromal, membranous, and glandular epithelium (P=0.027) which may be attributable to the low PI of the glandular epithelium. A more significant difference were noticed in the fundal wall(P=0.001). This may be attributable to the higher PI of the stromal epithelium. No such significant difference were found in the posterior wall (P=0.171). There is no significant(P>0.05) difference between the three walls in mean PI.



Figure(4): 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 in A, B, and C



Figure(5): 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 in A, B, and C.

Discussion

The PI of E-cadherin day 3, 4, and 5 were excluded from the statistical analysis first because the value of PI is very low (near zero) at the time of shedding that might affect the statistical results. Secondly because the value of PI is very high indicating that the sample is from the basalis layer and not from the functionalis(21)

The PI of E-cadherin is increasing during the proliferative phase, peaks at late proliferative phase and ovulation, and decreasing in the secretory phase This may reflect the relatively steady slow elevation of estradiol during early follicular phase (18). 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). It may be indicated by the sharp increment 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)

The PI of E-cadherin 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(19.)

It can be observed that the peak of E-cadherin is reached at day 14 in most cases ,however 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, et al. (22).Since the adhesiveness is positively correlated with E-cadherin level (17) 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) that is the nutrient to the developing embryo(23).

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(23%,18% for membranous and glandular epithelium respectively). This may be necessary to maintain integrity of the endometrial epithelium.

In some aspects these result are consistent with Shih et al.(24) who found that the expression of E-cadherin in glandular cells was observed mainly in the proliferative phase. The E-cadherin expression decreased significantly in the secretory phases both in the functionalis and in the basalis compared to levels in the proliferative phase. Stromal cells showed positive staining for E-cadherin in the proliferative and early secretory phases, but the positive 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 other study that very low or no protein expression of E-cadherin, in luminal and glandular epithelial cell in the mid-secretory endometrium of healthy fertile controls(14). 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 recent animal study (14).

In the three histological regions, considering that the anterior wall is more suitable for implantation, showed low PI of the glandular epithelium and higher PI of the stromal epithelium with no difference were found in the posterior wall. Since there is no significant difference between the three walls in mean PI, then it is the relative differences between these histological sites and not the level of PI which is important. Thus ,this study concluded that E-cadherin play an important role in endometrium receptivity.

References

1. Bergh PA and Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. Fertil. Steril. 1992;58:537-542
2. Swierz L and Giudence L. Unexplained infertility and the role of uterine receptivity. Clinics North America. 1997;8:523-543
3. Simon C, Martin J C and Pellicer A. Paracrine regulators of implantation. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000;14,815–820.
4. Enders A. A morphological analysis of the early implantation stages in the rat. Am J Anat. 1967;125,1–29.
5. Psychoyos A. Hormonal control of ovoimplantation. Vit Horm. 1973;31,205–225.
6. Finn C A and Martin L. The control of implantation. J Reprod. Fertil. 1974; 39,195–206.
7. Yoshinaga K. Uterine receptivity for blastocyst implantation. Ann N Y Acad Sci. 1988;541,424–431.
8. Paria B C, Reese J and Das S K et. al. Deciphering the cross-talk of implantation: advances and challenges. Science. 2002; 296,2185–2188.
9. Achache H and Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum. Reprod. Update. 2006;6,731–746 .
10. Jaschevatzky O E, Shalit A and Grunstein S et al Increased decidual prostaglandin E concentration in human abortion. Br J Obstet Gynaecol. 1983;90, 958-960.
11. Gumbiner B M. Cell adhesion: the molecular basis of tissue architecture

and morphogenesis. Cell. 1996;84,345–357.
12. Huber O, Bierkamp C and Kemler R. Cadherins and catenins in development. Curr Opin Cell Biol. 1996;8,685–691.
13. Barth A I, Nathke I S and Nelson W J. Cadherins, catenins and APC protein: interplay between cytoskeletal complexes and signaling pathways. Curr Opin Cell Biol. 1997;9,683–690.
14. Riethmacher D, Brinkmann V and Birchmeier C. A targeted mutation in the mouse E-cadherin gene results in defective preimplantation development. Proc Natl Acad Sci USA. 1995;92,855–859.
15. Fujimoto J, Ichigo S and Hori M et al. Alteration of E-cadherin, alpha- and beta-catenin mRNA expression in human uterine endometrium during the menstrual cycle. Gynecol Endocrinol. 1996;10,187–191.
16. Poncelet C, Leblanc M and Walker-Combrouze F et al. Expression of cadherins and CD44 isoforms in human endometrium and peritoneal endometriosis. Acta Obstet Gynecol Scand. 2002;81,195–203.
17. Comijn J, Berx G and Vermassen P et al. The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol Cell. 2001;7,1267–1278.
18. Gumbiner B, Stevenson B and Grimaldi A (1988) The role of the cell adhesion molecule uvomorulin in the formation and maintenance of the epithelial junctional complex. J Cell Biol 107,1575–1587.
19. Kumar S, Zhu LJ, Polihronis M, Cameron ST, Baird DT, Schatz F, Dua A, Ying YK, Bagchi MK, and Bagchi IC (1998) Progesterone induces calcitonin gene expression in human endometrium within the putative window of implantation. J Clin Endocrinol Metab 83, 4443-4450.
20. Zhu LJ, Cullinan-Bove K, Polihronis M, Bagchi MK, and Bagchi IC . Calcitonin is a progesterone-regulated marker that forecasts the receptive state of endometrium during implantation. Endocrinology.1998; 139: 3923- 3934.
21.Abdulla HI, Wren MR ,Bergh PA, et al. Age of the uterus does not affect pregnancy and implantation rates .A study of egg donation in women of different ages sharing oocytes from the same donor. Hum Reprod.1997;12:79-82.
22.Noyes N,Herting AT. And Rock J.Dating the endometrium biopsy. Fertil Steril.1950;1:3-25.
23.Guyton T and Hall A. Textbook of Medical Physiology.12ed .Saunders Press.2011,Pp:1004.
24. Shih IM and Kurman RJ. Molecular basis of gestantial trophoblastic disease. Curr.Mol. Med.2002;2:1-12.