$\alpha_v\beta_3$ Integrin Express on Mid-luteal Human Endometrium: An Immunogold and Immunofluorescent Staining Study

Hossein Nikzad[1*], Ali Akbar Taherian[2], Yoshiro Akimoto[3], Mitsutoshi Iwashita[4], Javad Amini Mahabadi[1], Mahdi Salehi[4], Mohsen Nikzad[4]

1. Gametogenesis Research Center, , Kashan University of Medical Sciences, Kashan, Iran.
2. Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.
3. Department of Anatomy, School of Medicine, Kyorin University, Mitaka, Tokyo, Japan.
4. Department of Obstetrics and Gynecology, School of Medicine, Kyorin University, Mitaka, Tokyo, Japan.
5. Student of medicine, Student Research Committee, Kashan University of Medical Science, Kashan, Iran.

I am an academic member (Professor) in Kashan University of Medical Sciences and teach anatomy and embryology courses for medical and paramedical students for 20 years. My interest research field is clinical embryology, IVF, reproduction and androloy. Until now, I published more than 40 manuscript at the national and international journals. Now, I am head of gametogenesis research center in Kashan University of Medical Sciences.

Article info:
Received: 28 Nov 2012
Accepted: 3 Feb 2013

ABSTRACT

Introduction: The implantation is a complex procedure that involves many molecules. One of these molecules is integrin specially $\alpha_v\beta_3$ which serves as receptor for components of extra cellular matrix to act as bridging molecules between the blastocyst and the endometrial surface during the implantation process. By blocking $\alpha_v\beta_3$ interactions, the implantation can be impaired.

Methods: The endometrial biopsies obtained from the anterior wall of the uterine cavity of 12 women. Each biopsy divided into three pieces; one fixed in 10% neutrally buffered formaldehyde for light microscopy and immunofluorescent study. The second fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for immunogold electron microscopy and the third fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for scanning electron microscopy. Afterwards, the biopsies evaluated by immunofluorescent, immunogold and scanning electron microscopy techniques.

Results: Immunofluorescent staining demonstrated that $\alpha_v\beta_3$ integrin express only on luminal surface epithelium and glandular epithelium of mid-luteal phase. Immunogold staining images in mid-luteal phase samples showed that $\alpha_v\beta_3$ integrin express on ciliated, non- ciliated (pinopdes) cells and junctional complexes. While, no reactivity observed on the endometrial surface, using the negative control antibody or in specimens incubated without primary antibody in any of the specimens.

Conclusion: The results showed that $\alpha_v\beta_3$ integrin express only on luminal surface epithelium and glandular epithelium in the mid-luteal phase of human endometrium and it may play a key role during the process of the embryo implantation. Targeting integrins may provide a new avenue for the development of contraceptive technologies, and the loss of this integrin in certain infertility states may signify the presence of implantation defects that reduce cycle fecundity in women.

Key Words:
$\alpha_v\beta_3$ Integrin,
Mid-luteal Phase,
Endometrium,
Immunogold,
Immunofluorescent Staining.

* Corresponding Author:
Hossein Nikzad, PhD
Gametogenesis Research Center, Kashan University of Medical Sciences, Kashan, Iran.
Tel/ Fax: 0098 361 5621158
E-mail: nikzad_h@kaums.ac.ir

1. Introduction

The endometrium remodeled throughout the menstrual cycle, and exhibits only a short period of receptivity, known as the "window of implantation" (1). The endometrium becomes receptive to blastocyst 6-8 days after ovulation and remains receptive for 4 days (cycle days 20-24) (2).

The implantation is a complex procedure that can be divided into three distinct steps: opposition, attachment, and invasion (3). Shortly after the opposition step, an integrin-dependent adhesion occurs. This allows the blastocyst to attach firmly to the uterine wall and trophoblasts transmigrate across the luminal epithelium, burying the embryo beneath the uterine wall (4).

To achieve implantation, many molecules (hormones, cytokines, integrins, enzymes, etc) involve in the dialogue between the human blastocyst and the maternal endometrium (5). Integrins are cell-surface adhesion receptors that play key role in mediating numerous physiological processes, including inflammation, migration, adhesion, and proliferation (6). Integrins composed of an alpha and a beta subunit. Each subunit comprises an extracellular domain, a transmembrane region and an intracellular domain (7). Integrins serve as receptors for components of extra cellular matrix such as osteopontin, fibronectin and collagens.These components have the capacity to act as bridging molecules between the blastocyst and the endometrial surface during the adhesion phase of the implantation process (8-11). The role of integrins in implantation has been widely reviewed (12-20). The extensive work of Lessey et al showed that three integrins ($\alpha_1\beta_1$, $\alpha_4\beta_1$, and $\alpha_v\beta_3$) express in uterine epithelium during implantation window (12-15). In other studies, it was reported that the best characterized cell adhesion molecules on the luminal surface of the endometrium are $\alpha_v\beta_3$ integrin and its ligand osteopontin, repeatedly found in genome-wide studies of human receptive endometrium (21-24). Blocking $\alpha_v\beta_3$ interactions in mouse or rabbit models impairs implantation (25, 26).

The purpose of this study was to establish the expression of $\alpha_v\beta_3$ integrin as a marker of endometrial receptivity in human by immunogold and immunofluorescent staining study and prove its role in implantation.

2. Materials & Methods

2.1. Endometrial Specimens

Endometrial biopsies obtained from the anterior wall of the uterine cavity of 12 women. The samples divided into three equal groups; prolifrative, early luteal and mid-luteal phases. The design of the study approved by the ethics committee of Kyorin University and informed consent obtained from all participating women. All women were fertile with regular menstrual periods (25-35 days). The mean age was 37 years (range 25 – 45) and none of them used steroidal contraceptive or an intrauterine device for at least 3 months before sampling. Each biopsy divided into three pieces; one fixed in 10% neutrally buffered formaldehyde for light microscopy and immunohistochemistry. The second portion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for immunogold electron microscopy and the third in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for scanning electron microscopy.

For endometrial dating, according to the histopathological criteria of Noyes, et al., (27), the paraffin-embedded biopsies stained with hematoxylin and eosin and evaluated by an experienced observer who was blind to the study.

2.2. Scanning Electron Microscopy

For scanning electron microscopy preparation, endometrial tissues fixed for at least 24 hr in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and post fixed for 1hr in 1% osmium tetroxide in 0.1 M phosphate buffer (pH 7.4). The samples dehydrated in a graded series of ethanol (50%, 70%, 90%, 99.5% and 100%), critical-point-dried with carbon dioxide by using a freeze drying device (JFD–300, JEOL, Tokyo, Japan), mounted and coated with gold in a sputter coater (JFC-1300 Auto Fine Coater, JEOL, Tokyo, Japan). Finally, using a scanning electron microscope (JSM- 5600 LV SEM, JEOL, Tokyo, Japan), the samples were examined. The specimens in phase showed pinopodes but no pathological features divided into two experimental groups: early luteal (days 15-19) and mid-luteal (days 20-24).

2.3. Immunofluorescent Staining

For immunofluorescent study, the paraffin sections (4 µm) dewaxed in xylen and rehydrated in decreasing concentrations of ethanol and, finally distilled water. Endogenous peroxidase blocked by 0.3% hydrogen peroxide in methanol for 10 min and nonspecific antibody binding

blocked by incubation in 5% BSA in PBS for 30 min. After this treatment, the sections washed three times (5 min each) with PBS and incubated over night at 4 ºC with the appropriate primary antibodies diluted in PBS (anti- $\alpha_v\beta_3$ integrin IgG 1:100). For control, the sections incubated over night at 4ºC with the same concentration of mentioned antibody, normal mouse serum (substituted for mouse anti--$\alpha_v\beta_3$ integrin) primary antibody. The sections rinsed in PBS extensively and counter-stained with proper fluorescent-labeled secondary antibody (Alexa 568-labeled goat anti-mouse IgG 1:250) appropriately and incubated for 1 hr at room temperature. Finally, the sections washed with PBS, rinsed in deionized water, mounted and were observed under an AX-80 fluorescence microscope (Olympus Optical, Tokyo, Japan). These experiments were repeated four times in different endometrial samples taken from proliferative and luteal phases.

2.4. Immunostaining for Electron Microscopy

Immunogold labeling performed to ultrastructural distribution of $\alpha_v\beta_3$ integrin molecules according to the previous reports (28). Briefly, the specimens divided into blocks (sized 2 mm^3) and fixed in 4% paraformaldehyde in o.1 M phosphate buffer (pH 7.4) for at least 24 hr at 4 C. After dehydration in a graded series of ethanol (50%, 70%, 90%, 99.5%, 100%), they were embedded in Lowicryle White Resin (London Resin company Ltd, London, UK) and ultrathin sections were cut. Then ultrathin sections washed with PBS and pretreated with 5% BSA for 10 min at room temperature. After a PBS rinse, they incubated with mouse anti- $\alpha_v\beta_3$ integrin IgG (1:100) or with normal mouse serum as control for overnight at 4ºC. Following washing with PBS 5 times (5 min each), the sections incubated with the colloidal gold ((12 nm in diameter))-conjugated goat anti-mouse colloidal gold-conjugated IgG (Jackson Immuno Research Laboratories Inc., West Grove, PA, USA) for 1 hr at room temperature (1:20 dilution), the sections washed with PBS for 5 times and then distilled water 3 times (5min each). The ultrathin sections stained with uranyle acetate and then examined with a transmission electron microscope (JEM- 1010; JEOL, Tokyo, Japan). Using above technique, four different samples from each experimental group were examined.

3. Results

Regarding the images from scanning electron microscopy (SEM), endometrial epithelium in secretory phase showed two different types of cells: ciliated and non-ciliated cells, that the latter cover the majority of luminal surface (Figure 1). Membrane projections on the apical pole of non-ciliated cells appear as fine microvilli and dome-like projections defined as progressing, developed and regressing pinopodes (4). Under scanning electron microscopy, in the early luteal phase (dating 15-19) progressing pinopodes and in mid luteal phase (dating 20-24) fully developed pinopodes were seen (Figure 1 B, C) while in proliferative phase no pinopodes were detected (Figure 1A)

Immunofluorescent staining demonstrated that -$\alpha_v\beta_3$ integrin express only on luminal surface epithelium and glandular epithelium of mid-luteal phase. During the three phases, $\alpha_v\beta_3$ integrin was present in the stroma (Figure 2).

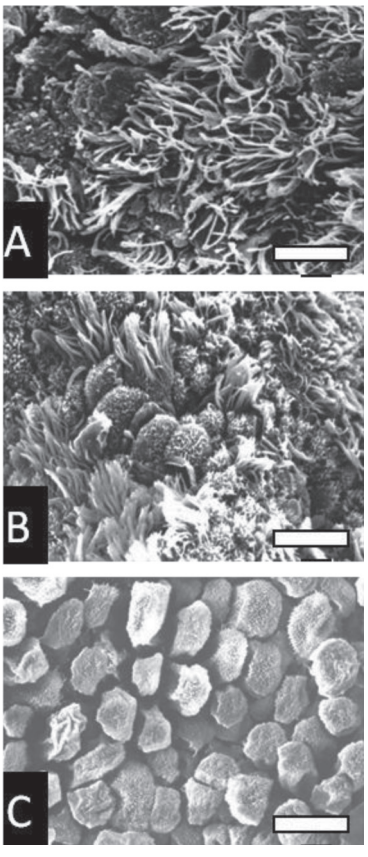

Figure 1. Scanning electron microscopy (SEM) photomicrographs of luminal surface of human endometrial biopsies taken from proliferative (A), early (B) and mid- luteal (C) phases of normal menstrual cycle to identify developmental stage of pinopodes. Notice that there are not any pinopodes in proliferative phase, few isolated pinopodes are detectable in specimens from early luteal phase, numerous developed pinopodes in the mid-luteal phase. Scale bars = 10 µm.

Immunogold staining images in the mid-luteal phase samples showed that $\alpha_v\beta_3$ integrin express on ciliated, non- ciliated (pinopdes) cells and junctional complexes. However, no reactivity was observed on the endometrial surface using the negative control antibody or in specimens incubated without primary antibody in any of the specimens (Figure 3).

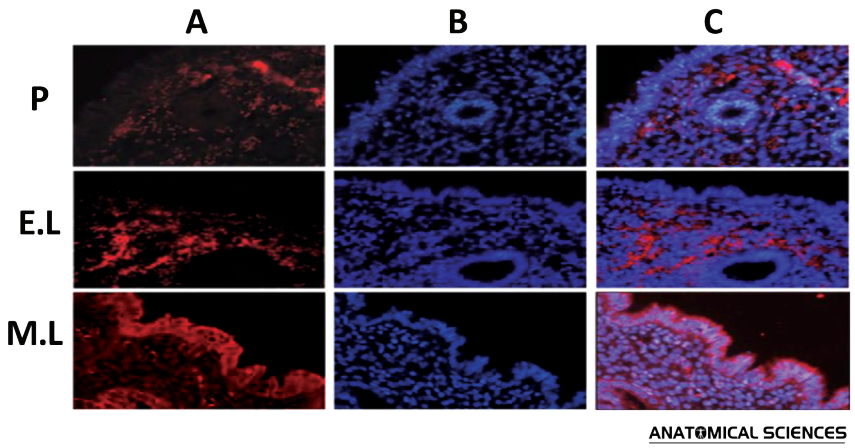

Figure 2. Immunofluorescent staining for $\alpha_v\beta_3$ integrin in proliferative (p), early luteal (E.L), and mid-luteal (M.L) phases of human endometrium. Lane 1 show specific staining for $\alpha_v\beta_3$ integrin; lane 2, DAPI staining and lane 3, Merged (magnification ×400). Notice that $\alpha_v\beta_3$ integrin express on stromal tissue in the three phases but, it expressed only on luminal surface epithelium and glandular epithelium of mid-luteal phase.

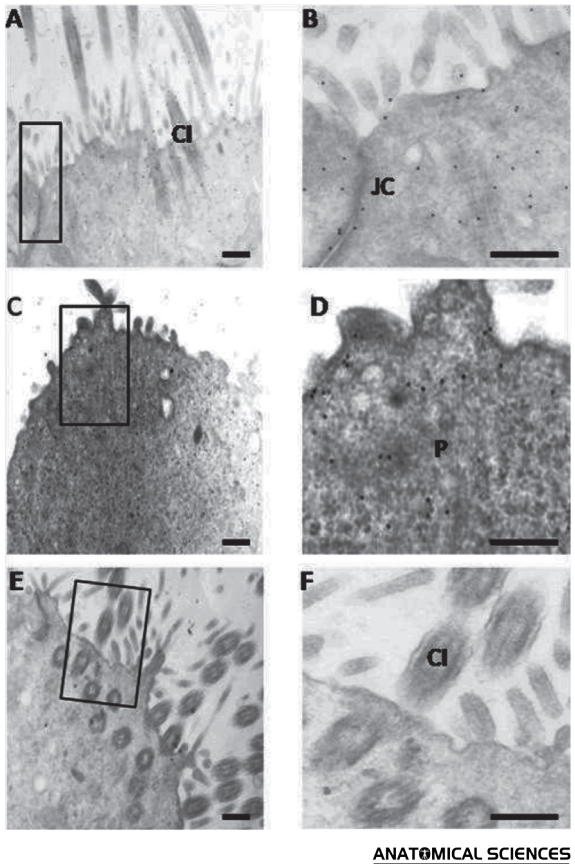

Figure 3. Immunogold labeling showed the expression of $\alpha_v\beta_3$ integrin on ciliated (A, B) and non- ciliated (pinopod) cells (C, D) in the mid-luteal phase of human endometrium samples. Expression of $\alpha_v\beta_3$ integrin was seen on cilia (CI), pinopod (P) and junctional complex (JC).

No reactivity observed on the endometrial surface in the control samples (E, F). Scale bars = 5µm

4. Discussion

Embryo implantation is a part of mammalian reproduction (29). Initial step of embryo implantation is cell adhesion of trophectoderm of blastocyst and endometrial luminal epithelial cells of uterus, at their respective apical cell surfaces. This occurs despite generally the non-adherent nature of apical cell surfaces of epithelial cells; thus, integrin plays a key role in this adhesion (30).

The discovery of integrins in the human endometrial epithelium and stroma (12, 31, and 32) led to this expectation that these molecules are somehow involved in the procedure leading to successful pregnancy. Recent evidences demonstrate that integrins regulated in the uterus of rodents (33, 34).

Integrins are surface glycoproteins which have α and β subunits. During the adhesion phase of the implantation, these components act as bridge between the blastocyst and the endometrial surface. During implantation, Integrins appear on the blastocyst, surface of glandular and luminal endometrial epithelium (32).

In this study, the interesting finding is the localization of $\alpha_v\beta_3$ integrin in the apical membrane projections in mid-luteal phase of human endometrial epithelium, so called pinopodes.

Pinopodes on the epithelial surface are visible in light microscopy, but other structures may be mistaken for pinopodes (35); using these techniques, it would not be possible to determine their stage. Thus, in this study, scanning electron microscopy used to confirm the presence of pinopodes in the endometrial tissue. Further, using the indirect immunogold technique in this study permitted to evaluate the distribution of $\alpha_v\beta_3$ integrin specifically in pinopodes during opening of the implantation window.

The results obtained from immunogold transmission electron microscopy displayed an increase in expression of $\alpha_v\beta_3$ integrin at uterine pinopodes of mid luteal specimens compared to early luteal phase. Furthermore, the results show that $\alpha_v\beta_3$ integrin distributed with higher density at area near the cell membrane of pinopodes comparing to similar neighboring areas without pinopode. These findings enhance the significance of pinopode formation in preparation for embryo attachment. In photomicrograph from immunogold transmission electron microscopy, $\alpha_v\beta_3$ integrin observed over cytoplasm, nucleus and cell membrane of specimens from mid luteal phase endometrium. However, no reactivity was observed on the endometrial surface using the negative control antibody or in specimens incubated without primary antibody in any of the specimens (Fig.3).

The increase of expression of $\alpha_v\beta_3$ integrin in this study was confirmed by the previous reports (13, 36). Lessey et al reported that the glandular and luminal epithelium affected by independent alterations in integrin expression throughout the menstrual cycle. The expression of one integrin (the $\alpha_v\beta_3$ vitronectin receptor) on both luminal and glandular epithelium coincides with the time of embryo attachment; also they demonstrated that aberrant expression of this integrin is associated with infertility (13). Lessey et al detected one integrin in the glandular epithelium during postovulatory days 5 and 6. Subunit αv is present at the epithelial level during the early and late secretory phase and at the stromal level during the whole cycle; β_3 is present at the epithelium during the late secretory phase and in the stroma during the menstrual cycle. In the luminal epithelium, the expression of $\alpha_v\beta_3$ starts on day 20 of the cycle, continues until the end of the cycle, and persists during early pregnancy (12, 32).

Yelian et al showed that $\alpha_v\beta_3$ integrin appears to be specifically present in the endometrium during the window of implantation in both humans and mice. Ligand binding to $\alpha_v\beta_3$ is dependent on the three-amino acid sequence arg-gly-asp (RGD), which is involved in embryo attachment and outgrowth in vivo (37).

Cheresh et al reported that $\alpha_v\beta_3$ integrin is critical for angiogenesis. Neutralization of this integrin during implantation can reduce embryo survival by preventing new vessel formation at the site of implantation (38).

Mid-secretory phase increase in endometrial epithelial $\alpha_v\beta_3$ resulted from an increase in β_3 after day 19 (15). Aberrant $\alpha_v\beta_3$ integrin associated with unexplained infertility and other endometrial pathologies (12, 32, 39, and 40). Integrins $\alpha_v\beta_3$ and αvβ6 are also present in endometrium (41). Blocking $\alpha_v\beta_3$ interactions in mouse or rabbit models impairs implantation (25, 26).

5. Conclusions

The results showed that $\alpha_v\beta_3$ integrin express only on luminal surface epithelium and glandular epithelium in the mid-luteal phase of human endometrium and it may play key role during the process of the embryo implantation. Targeting integrins may provide a new method for development of contraceptive technologies, and the loss of this integrin in certain infertility states may signify the

presence of implantation defects reducing cycle fecundity in women.

Acknowledgment

The authors would like to appreciate M. Fukuda, T. Shibata and S. Matubara (Department of Anatomy, Kyorin University School of Medicine) for their technical assistance in electron microscopy.

References

1. Croxatto HB, Diaz S, Pavez M. Clinical chemistry in women treated with progestogen implants. Contraception1978 Oct; 18(4):441-50.

2. Bergh PA, Navot D. The impact of embryonic development and endometrial maturity on the timing of implantation. Fertil Steril1992 Sep; 58(3):537-42.

3. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. N Engl J Med2001 Nov 8; 345(19):1400-8.

4. Russell JM, Stephenson GS, Yellowley CE, Benton HP. Adenosine inhibition of lipopolysaccharide-induced interleukin-6 secretion by the osteoblastic cell line MG-63. Calcif Tissue Int2007 Oct; 81(4):316-26.

5. Valles CS, Dominguez F. Embryo-endometrial interaction. Chang Gung Med J2006 Jan-Feb; 29(1):9-14.

6. Borthwick JM, Charnock-Jones DS, Tom BD, Hull ML, Teirney R, Phillips SC, et al. Determination of the transcript profile of human endometrium. Mol Hum Reprod2003 Jan; 9(1):19-33.

7. Singh H and Aplin JD, Adhesion molecules in endometrial epithelium: tissue integrity and embryo implantation J Anat. 2009 July; 215(1): 3–13.

8. Tabibzadeh S, Babaknia A. The signals and molecular pathways involved in implantation a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. Hum reprod 1995; 10: 985-95.

9. Campbell S, Swann HR, Seif MW, Kimber SJ, Aplin JD. Cell adhesion molecules on the oocyte and preimplantation human embryo. Hum Reprod. 1995; 10:1571–1578.

10. Johnson GA, Burghardt RC, Spencer TE, Newton GR, Ott TL, Bazer FW. Ovine osteopontin: II. Osteopontin and alpha (v) beta (3) integrin expression in the uterus and conceptus during the periimplantation period. Biol Reprod. 1999; 61:892–899.

11. Johnson GA, Burghardt RC, Bazer FW, Spencer TE. Osteopontin: roles in implantation and placentation. Biol Reprod. 2003; 69:1458–1471.

12. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, et al. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J Clin Invest. 1992; 90:188–195.

13. Lessey BA, Ilessami AO, Lessey MA, Riben M, Harris JE, Chwalisz K. Luminal and glandular endometrial epithelium express integrins differentially throught the menstrual cycle: Implications for implantation , contraception, and infertility. Am J Reprod Immunol 1998; 35:195-204.

14. Lessey BA, Castelbaum AJ, Wolf L, Greene W, Paulson M, Meyer WR, et al. Use of integrins to date the endometrium. Fertil Steril. 2000; 73:779–787.

15. Lessey BA, Castelbaum AJ. Integrins and implantation in the human. Rev Endocr Metab Disord2002 May; 3(2):107-17.

16. Kimber SJ, Spanswick C. Blastocyst implantation: the adhesion cascade. Semin Cell Dev Biol 2000 Apr; 11(2):77-92.

17. Aplin JD, Spanswick C, Behzad F, Kimber SJ, Vicovac L. Integrins beta 5, beta 3 and alpha v are apically distributed in endometrial epithelium. Mol Hum Reprod1996 Jul; 2(7):527-34.

18. Aplin JD. Adhesion molecules in implantation. Rev Reprod1997 May; 2(2):84-93.

19. Aplin JD, Kimber SJ. Trophoblast-uterine interactions at implantation. Reprod Biol Endocrinol. 2004; 2:48.

20. Nardo LG, Bartoloni G, Di Mercurio S, Nardo F. Expression of alpha (v) beta3 and alpha4beta1 integrins throughout the putative window of implantation in a cohort of healthy fertile women. Acta Obstet Gynecol Scand. 2002 Aug; 81(8):753-8.

21. Casals G, Ordi J, Creus M, Fabregues F, Carmona F, Casamitjana R, et al. Osteopontin and alphavbeta3 integrin as markers of endometrial receptivity: the effect of different hormone therapies. Reproductive Biomedicine Online. 2010 Sep; 21(3):349-59.

22. Borthwick J, Charnock-Jones D, Tom B, Hull M, Teirney R, et al. Determination of the transcript profile of human endometrium. Mol Hum Reprod. 2003; 9:19–33.

23. Carson D, Lagow E, Thathiah A, Al-Shami R, Farach-Carson M, et al. Changes in gene expression during the early to mid-luteal (receptive phase) transition in human endometrium detected by high-density microarray screening. Mol Hum Reprod. 2002; 8:871–879.

24. Riesewijk A, Martin J, van Os R, Horcajadas JA, Polman J, et al. Gene expression profiling of human endometrial receptivity on days LH+2 versus LH+7 by microarray technology. Mol Hum Reprod. 2003; 9:253–264.

25. Illera MJ, Cullinan E, Gui Y, Yuan L, Beyler SA, Lessey BA. Blockade of the alpha (v) beta (3) integrin adversely affects implantation in the mouse. Biol Reprod. 2000; 62:1285–1290.

26. Illera MJ, Lorenzo PL, Gui YT, Beyler SA, Apparao KB, Lessey BA. A role for alphavbeta3 integrin during implantation in the rabbit model. Biol Reprod. 2003; 68:766–771.

27. Noyes Rw, Rock j. Dating the endometrial biopsy, Fertil Steril, 1950, 1; 3-9.

28. Philimonenko AA, Janacek J, Hozak P. Statistical evaluation of colocalization patterns in immunogold labeling experiments. J Struct Biol 2000 Dec; 132(3):201-10.

29. Carson DD, Bagchi I, Dey SK, Enders AC, Fazleabas AT, Lessey BA, Yoshinaga K. Embryo implantation. Dev Biol 2000; 223: 217–237.

30. Denker HW. Implantation: a cell biological paradox. J Exp Zool 1993; 266: 541–558.

31. Tabibzadeh S. Patterns of expression of integrin molecules in human endometrium throughout the menstrual cycle. Hum Reprod 1992; 7: 876–882.

32. Lessey BA, Castelbaum AJ, Buck CA, Lei Y, Yowell CW, Sun J. Further characterization of endometrial integrins during the menstrual cycle and in pregnancy. Fertil Steril 1994; 62:497–506.

33. Simo´n C, Gimeno MJ, Mercader A, O'Conner JE, Remohi J, Polan ML, et al. Embryonic regulation of integrins b3, a4, and a1 in human endometrial epithelial cells in vitro. J Clin Endocrinol Metab1997; 82:2607–2616.

34. Illera MJ, Yuan L, Stewart C, Cullinan E, Lessey BA. Effect of peritoneal fluid from women with endometriosis on implantation in the mouse model. Fertil Steril 1999; (in press).

35. Develiglu OH, Nikas G, Hsiu JG, Toner JP, Jones HWJr. Detection of endometrial pinopodes by light microscopy. Fertil Steril 2000; 74: 767-770.

36. Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium: correlation with the normal and abnormal menstrual cycle. J Clin Invest 1998; 90: 88-195.

37. Yelian FD, Yang Y, Hirata JD, Schultz JF, Armant DR. Molecular interactions between fibronectin and integrins during mouse blastocyst outgrowth. Mol Reprod Dev 1995; 41:435–448.

38. Brooks PC, Clark RAF, Cheresh DA. Requirement of vascular integrin av/b3 for angiogenesis. Science 1994; 264:569–571.

39. Apparao KB, Lovely LP, Gui Y, Lininger RA, Lessey BA. Elevated endometrial androgen receptor expression in women with polycystic ovarian syndrome. Biol Reprod 2002; 66: 297–304

40. Tei C, Maruyama T, Kuji N, Miyazaki T, Mikami M, Yoshimura Y. Reduced expression of alphavbeta3 integrin in the endometrium of unexplained infertility patients with recurrent IVF-ET failures: improvement by danazol treatment. J Assist Reprod Genet 2003; 20: 13–20.

41. Aplin JD, Spanswick C, Behzad F, Kimber SJ, Vicovac L. Integrins beta 5, beta 3 and alpha v are apically distributed in endometrial epithelium. Mol Hum Reprod 1996; 2: 527–534.