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## Beta3 Integrin Expression within Uterine Endometrium and its Relationship with Unexplained Infertility

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**Abstract:** The aim of this study was to investigate whether the endometrium of women with unexplained infertility differs in the presence of the beta 3 ( $\beta 3$ ) integrin molecule from the endometrium of normal fertile women. In a retrospective case-control study 30 endometrial biopsies from hysterectomies with nonendometrial pathology and 30 endometrial samples from women with a history of unexplained infertility were collected during the window of implantation. Immunohistochemically staining with a monoclonal antibody against  $\beta 3$  integrin subunit in endometrial biopsies was performed and then assessed semiquantitatively by microscope on different endometrial compartments including glandular epithelial cells, vessels, lymphocytes, macrophages and stromal cells. Chi-square test was used to compare the expression and defect of  $\beta 3$  integrin subunit between two groups. The results showed that  $\beta 3$  integrin molecules were present in fertile and infertile endometrial uterine tissues. The majority of glandular epithelial cells expressed  $\beta 3$  integrin in fertile endometrium. However, the endometrial expression of  $\beta 3$  integrin was reduced significantly in infertile endometrium during the window of implantation ( $p < 0.05$ ). A few numbers of vessels and stromal cells expressed  $\beta 3$  integrin molecule with no statistical significance between the two groups. In conclusion Abnormal endometrial integrin expression is a frequent finding in women with unexplained infertility. A therapeutic potential approach in improving uterine endometrium receptivity together with up-regulation of  $\beta 3$  integrin in this population of women suggested.

**Key words:** Endometrium, infertility,  $\beta 3$  integrin, secretory phase

### INTRODUCTION

Infertility is defined as 1 year of unprotected coitus without conception (Speroff *et al.*, 1999). It affects approximately 10-15% of women during reproductive age (Randolph, 2000). Endometrial receptivity is established during the mid-secretory phase, between cycle day 20-24 (Wilcox *et al.*, 1999). Women with various gynecologic disorders, appear to exhibit decreased uterine receptivity and abnormal expression of endometrial biomarkers (Donaghy and Lessey, 2007). Numerous cell adhesion molecules particularly integrins are expressed by the endometrium during the menstrual cycle and in pregnancy and appear to be necessary for the successful interaction of the embryo with the endometrium (Lessey *et al.*, 1992, 1994a; Achache and Revel, 2006). Integrins are a family of cell-surface glycoproteins that bind extracellular matrix proteins and regulate different type of cellular activities, including inflammatory response, angiogenesis, cell migration, proliferation, differentiation and gene

expression (Hynes, 1992). Integrins are classified into several groups according to their  $\beta$  subunit.  $\alpha v \beta 3$  integrin is one of the two members of  $\beta 3$  integrin that share in a common  $\beta 3$  (CD61) subunit (Abbas *et al.*, 2007). Data have been accumulated that  $\beta 3$  integrin weakly expressed by epithelial cells in proliferative endometrium but reactivity increased in the mid to late secretory phase, the stage at which implantation could occur (Chen *et al.*, 1998; Gonzalez *et al.*, 1999). Decreased uterine receptivity and abnormal endometrial expression of  $\beta 3$  integrin have been reported in some pathological disorders, including, unexplained infertility, hydrosalpinges, endometriosis and luteal phase defect (Hii and Rogers, 1998; Creus *et al.*, 2002; Savaris *et al.*, 2006). Integrin expression has mostly been studied in endometrial epithelial cells in previous studies. To confirm these observations and extend investigations of endometrial expression, the present study examined  $\beta 3$  integrin in different compartments of uterine endometrium of infertile women in comparison with fertile women.

## MATERIALS AND METHODS

**Participants:** In this prospective case-control study thirty samples of non-pregnant endometrium (Fertile cases) were obtained fresh from hysterectomies performed for nonendometrial pathology from the operating theaters during 2006-2007. The inclusion criteria for fertile women including healthy and proven fertile, normal menstrual cycle and not used contraceptive drugs or intrauterine devices within the last 6 months.

Thirty endometrial samples were collected by uterine curetting from women with unexplained infertility from IVF Center. The inclusion criteria including infertility of a duration exceeding 1 year; normal quality sperm; normal ovulation; normal anatomical uterus and normal menstrual cycle.

All samples were obtained from women after informed consent at the time of implantation window in secretory phase from Ahvaz Imam Khomani Hospital.

**Antibody and immunohistochemical staining:** We investigated the expression of  $\beta 3$  integrin subunit on 5-6  $\mu$ m thick cryostat sections obtained from endometrial biopsies utilizing immunohistochemical staining with mouse monoclonal antibody against CD61 marker (supplied from Dako Ltd.). Tissue sections were incubated for 30 min with primary antibody and stained with streptavidin-biotin-peroxidase system (Dako LSAB kit system). The sections were then stained by DAB enzymatic produced, counterstained with Mayer's haematoxylin and finally evaluated microscopically. Negative control sections were incubated with TBS or irrelevant mouse monoclonal antibodies instead of primary antibodies. All incubations were carried out in a moist chamber at room temperature.

**Scoring method:** The reactivity of antibody directed against integrin  $\beta 3$  subunit with different compartments of the endometrium (glandular epithelium, vessels, lymphocytes, macrophages, stromal cells) was scored semi-quantitatively according to the degree of positive staining: (-) when there was no reactivity greater than that observed in the negative control; ( $\pm$ ) fewer than 5% of cells were positive; (+) 5-25% of cells were positive, (++) 25-50% of cells were positive; and (+++) more than 50% of cells were positive (Lessey *et al.*, 1994; Tabibzadeh, 1992). Data were analyzed with the program Minitab version 14.0. Two groups were compared by Chi-square analysis test.

## RESULTS AND DISCUSSION

The results showed  $\beta 3$  integrin molecules were present in fertile and infertile endometrium tissues (Table 1). No reactivity with glandular epithelial cells, vessels, lymphocytes, macrophages and stromal cells were detected in negative control (Fig. 1). There were similarities and differences in the expression of  $\beta 3$  integrin in different compartments. The reactivity was detected in glands, vessel and stromal cells in endometrial tissues. The reaction pattern for epithelial cells was significantly different between fertile and infertile endometrial tissues (Fig. 2, 3). In contrast to infertile cases, the reactivity for epithelial cells was significantly positive for majority of fertile cases ( $p = 0.001$ ). A few numbers of vessels and stromal cells expressed  $\beta 3$  integrin molecule with an unaltered pattern of staining in either fertile or infertile women ( $p > 0.05$ ). No reactivity was detected with

Table 1: Reactivity of  $\beta 3$  integrin with different compartments in secretory phase endometrium of fertile and infertile women

Endometrial compartments	-	$\pm$	+	++	+++
<b>Glands</b>					
Fertile	0	0	9	14	7
Infertile	3	9	10	8	0
<b>Vessels</b>					
Fertile	9	14	7	0	0
Infertile	6	19	5	0	0
<b>Lymphocytes</b>					
Fertile	30	0	0	0	0
Infertile	30	0	0	0	0
<b>Macrophages</b>					
Fertile	30	0	0	0	0
Infertile	30	0	0	0	0
<b>Stromal cells</b>					
Fertile	2	13	15	0	0
Infertile	6	10	14	0	0

--: No reactivity,  $\pm$ : <5%, +: 5-25%, ++: 25-50, +++: >50%

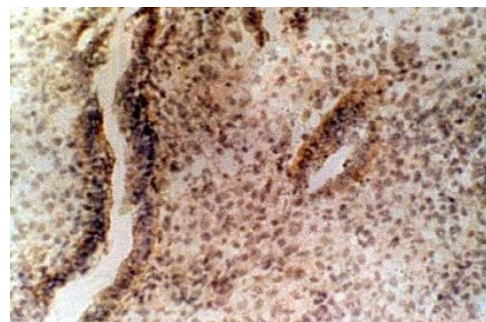


Fig. 1: Section of uterine endometrium tissue of fertile women in secretory phase stained immunohistochemically with irrelevant mouse monoclonal antibody. No reactivity with glandular epithelial cells, vessels, lymphocytes, macrophages and stromal cells were seen. Magnification x200

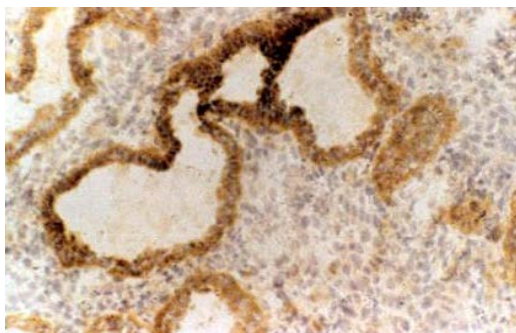


Fig. 2: Frozen section of uterine endometrium tissue of fertile women in secretory phase stained immunohistochemically with anti-CD61 monoclonal antibody. The majority of glandular epithelial cells are positive for  $\beta 3$  integrin. Magnification x200

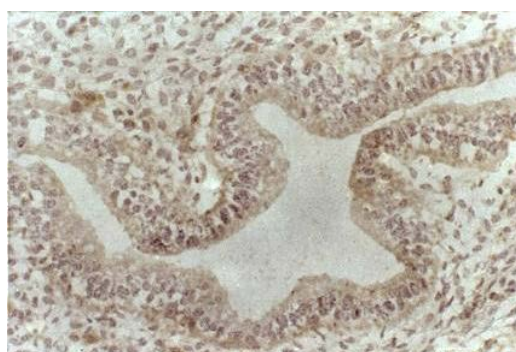


Fig. 3: Section of uterine endometrium tissue of infertile women in secretory phase stained immunohistochemically with anti-CD61 monoclonal antibody.  $\beta 3$  integrin reactivity with glandular epithelial cells reduce significantly in comparison with endometrium tissue of fertile women. Magnification x400

lymphocytes and macrophages in either fertile or infertile endometrium tissues.

Several molecular markers of the window and of uterine receptivity to embryonic implantation have been identified. The present immunohistochemical study showed that the  $\beta 3$  integrin molecules are present in endometrium of fertile and infertile woman. In opposite with fertile endometrium tissues, in most unexplained specimens there were no or low reactivity for  $\beta 3$  integrin in glandular epithelial, which suggests this molecule may participate in the cascade of molecular events leading to successful implantation. Differences in  $\beta 3$  integrin expression between fertile and infertile women have been

shown by Lessey *et al.* (1992, 1994). Although the present study showed no difference reactivity for stromal cells in both groups, Ceydeli *et al.* (2006) reported difference in  $\alpha \nu \beta 3$  integrin expression in endometrial stromal cell in subgroups of women with unexplained infertility. These researchers found that there is a loss synchronous expression of  $\alpha \nu \beta 3$  integrin between the glandular and stromal compartments of the endometrium. In other report, endometrial glandular and stromal cell positivity for  $\beta 3$  integrin subunit have been shown in 9 fertile and 13 infertile women using flow cytometry (Gonzalez *et al.*, 1999). The difference between endometrial stromal and epithelial cell expression in fertile and infertile women did not reach statistical significance, probably due to the insufficient number of subjects in the groups. Differences in integrin expression between in- and out-of-phase endometrial biopsies were also observed for  $\alpha \nu \beta 3$  integrin in glandular epithelial cells expression during the midluteal phase in women with impaired infertility (Creus *et al.*, 2002). In opposite with above studies, other report showed that there is no significant differences in  $\beta 1$  and  $\beta 3$  integrins expression in endometrial biopsies between the IVF group comprising patients with tubal disease, endometriosis and unexplained infertility and the control groups during implantation window. These findings may be related to receiving treatment before IVF, perhaps improving the severity of the disease and therefore, in theory the integrin expression (Thomas *et al.*, 2003).

The integrins we investigated in this study certainly do not represent the complete family of adhesion molecules, which play a role during implantation. Differences in  $\beta 1$  integrin expression have been shown between fertile and infertile women. Similar to  $\beta 3$  integrin, low reactivity for expression of  $\alpha 1 \beta 1$  and  $\alpha 4 \beta 1$  integrins has been found to be associated with endometriosis (Lessey *et al.*, 1994), hydrosalpinges (Meyer *et al.*, 1997) and unexplained infertility (Skrzypczak *et al.*, 2001).

The expression of  $\beta 3$  integrin can be regulated by several factors. Reduced expression of  $\alpha \nu \beta 3$  integrin in the endometrium of unexplained infertility patients with recurrent IVF-ET treated with oral danazol for 12 weeks showed a significant increase in the  $\alpha \nu \beta 3$  staining at the mid-secretory phase (Tei *et al.*, 2003). TGF $\beta$ , GM-CSF, IFN $\gamma$ , TNF $\alpha$  and IL-1 $\beta$  have all been demonstrated to alter the expression of integrin molecules (Nathan and Sporn, 1991). Cervical mucus TNF $\alpha$  concentration was found to be higher in idiopathically infertile women than in fertile controls (Naz *et al.*, 1995). TGF $\beta$  upregulates the expression of almost all the VLA proteins as well as the  $\beta 2$  and  $\beta 3$  integrin heterodimers; this stimulatory effect is induced at the level of the  $\alpha$  chain (Heino *et al.*, 1989;

Ignatz *et al.*, 1989). IL-1, which is potentially involved in the menstrual process, induces production of PGE<sub>2</sub> (Tabibzadeh, 1990). Increased levels of PGE<sub>2</sub> in human endometrium in the secretory phase of the menstrual cycle are seen at the time that the message level of IL-1 increased (Simon *et al.*, 1993). PGE<sub>2</sub> clearly enhances both  $\beta 1$  and  $\beta 3$  integrin subunit expression (Pierro *et al.*, 2003). Hence, cytokines are involved in the induction of changes in the endometrium during the secretory and menstrual phases and consequently these changes affect the expression of adhesion molecules. On the other hand, there is evidence that the function of the cytokine networks in endometrium is controlled by steroid hormones. For instance, positive regulation of the IFN $\gamma$  promoter by oestrogen has been reported (Fox *et al.*, 1991). Ovarian gonadal steroid treatment blocked the release of IL-1 by human blood monocytes (Polan *et al.*, 1988). It has been shown that failure of progesterone receptor was associated with aberrant  $\alpha v\beta 3$  integrin expression (Surrey *et al.*, 2007). In other recent report prolonged GnRH agonist therapy in consecutive infertile patients prior to an IVF cycle resulted in outcomes similar to untreated controls with positive expression of  $\alpha v\beta 3$  (Surrey *et al.*, 2007). Thus, the expression of adhesion molecules may be under the control of cytokines directly and hormones indirectly. It would be interesting to analyse the role of cytokines in the modulation of endometrial adhesion molecule expression.

The ligands for  $\beta 3$  integrin are fibronectin, vitronectin, von Willebrand factor and thrombospondin (Abbas *et al.*, 2007). It has been shown that the integrin,  $\alpha II\beta 3$ , plays a key role in trophoblast adhesion to fibronectin during mouse peri-implantation development (Rout *et al.*, 2004).  $\alpha v\beta 3$ ,  $\alpha 4$  and  $\alpha 5$  integrin subunits, vitronectin and fibronectin are expressed in caprine endometrium and blastocyst and may play a role in the cascade of the implantation process (Garcia *et al.*, 2004).

The existence of vitronectin on the human fetal membrane has been reported by Hayman *et al.* (1983). Oncofetal fibronectin, an alternatively spliced form of fibronectin, has been detected in the human trophoblast unit (Feinberg *et al.*, 1991). The high expression of  $\beta 3$  integrin by glandular epithelial cells in the mid and late secretory phase and early pregnancy suggests an interaction between these two matrix proteins on trophoblast and  $\beta 3$  integrin on the endometrial glands; such an interaction could participate in implantation the establishment of placentation in the very early stages of pregnancy.

## CONCLUSION

The significantly decreased expression of endometrial  $\beta 3$  integrin in unexplained infertility suggests

that  $\beta 3$  integrin molecule may play important role in uterine endometrium receptivity at the time of the implantation. Further studies focusing on improving endometrial receptivity together with up-regulation of  $\beta 3$  integrin recommended.

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