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The Correlation between the Endometrial Integrins and Osteopontin Expression with Pinopodes Development in Ovariectomized Mice in Response to Exogenous Steroids Hormones

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ABSTRACT

Background: The ovariectomized animals are good models to evaluate the effect of different steroid hormone treatments on implantation events and the pattern of integrin expression. Therefore, this study was performed to compare the expression of integrins and osteopontin (OPN) in correlation with pinopode development in ovariectomized mice endometrium which was subjected to steroid hormones. **Methods:** Ovariectomized mice were subjected to estrogen, progesterone and estrogen-progesterone hormones. Their uterine horns were evaluated for integrin expression by immunohistochemistry and real-time RT-PCR and for pinopode development by transmission and scanning electron microscopic studies. **Results:** No immunostaining for integrin and OPN molecules were detected in the endometrium of non-ovariectomized mice except in metestrus phase. The α4 and β1 integrin genes were expressed in all phases of estrous cycle. In ovariectomized mice, no reaction was detected in the endometrium of control, sham and estrogen-treated groups, but in both progesterone-treated groups, all examined genes were expressed. There was not any correlation between pinopodes and integrin expression in ovariectomized mice. **Conclusion:** The progesterone is more effective on endometrial integrin expression than estrogen and differences in the expression pattern of integrins reflect their important and different roles in embryo implantation. The pinopodes may have minor effect in mice implantation or have some delay in their expressions in ovariectomized mice which were subjected to exogenous hormones. *Iran Biomed. J. 14 (3): 109-119, 2010*

Keywords: Endometrium, Estrogen, Integrin, Ovariectomized mice, Osteopontin (OPN)

INTRODUCTION

Implantation is a dynamic physiological and biochemical event. It requires not only synchrony between the maternal environment and the embryo but also requires establishment of tight contact between the trophoblasts and endometrial epithelial cells. This contact involves integrin molecules, large cell surface glycoproteins, which belong to the cell adhesion molecules and have composed of two α and β subunits [1, 2]. These subunits form different heterodimers which serve as receptors for different types of extracellular matrix proteins including fibronectin, vitronectin, laminin, collagen and osteopontin (OPN) [3].

The interaction between integrins and their ligands can promotes different functions such as cell adhesion, migration, proliferation, differentiation and apoptosis. In addition, it plays an important role in contact between the embryo and endometrium by interaction with a variety of extracellular proteins during implantation period [4-6].

Integrin molecules like other parameters in endometrium are expressed in a cyclic manner and they have shown different patterns of distribution during the mammalian pregnancy [7-13].

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Among different types of integrins, the heterodimers of $\alpha_v\beta_3$ and $\alpha_4\beta_1$ are considered for uterine receptivity due to co-expression of these molecules with their ligands during implantation period and they may have important roles in decidualization [14, 15]. Thus, it is suggested that the integrin expression may be controlled by steroid hormones; however, there were some controversial reports in this regard [10, 11, 16-20].

Despite the studies which demonstrated that integrin expression does not vary with externally administered steroids or different serum levels of ovarian steroids [10, 11], the study by Lessey and Arnold [16] noted that the expression of avb3 integrin increased with administration of estrogen and progesterone during the implantation window. In addition, Kimmins *et al.* [17] results indicated that in bovine endometrium, the expression of αvβ3 integrin was influenced by estrogen [17].

Somkuti *et al.* [18] and Widra *et al.* [19] have shown that estrogen alone or in combination with progesterone down-regulate αvβ3 expression, but no effect was observed for αv or β1 integrin subunit. Moreover, Basak *et al.* [7] demonstrated that α4β1 integrin expression was induced by estradiol and down-regulated by progesterone in mice during implantation.

Moreover, the *in vitro* experiments have also demonstrated different results [18, 21]. Somkuti et al. [18] showed that cultured endometrial Ishikawa cells, which were exposed to estrogen or pretreated with estradiol and followed by progesterone exposure, down-regulate αvβ3 [18], but Grosskinsky *et al.* [21] did not observe any alteration in the integrin expression in cultured endometrial stromal cells treated with estradiol or progesterone.

OPN is an extracellular matrix molecule and a member of the integrin-binding ligand glycoprotein which influences the uterine microenvironment. It binds to several types of integrin heterodimers as receptors including αvβ1, αvβ3, αvβ5, α4β1 and α9β1, [22, 23].

Uterine OPN expression has been detected during the peri-implantation and implantation period in several mammalian species [23-26] and it indicated an important role in implantation [23].

Similar to the integrins, the expression of OPN may be controlled by steroid hormones and expressed in the glandular epithelium in response to progesterone [25].

Pinopode is another endometrial receptivity marker which expresses for a short time at implantation window [26-29]. The αvβ3 integrin expression and pinopode formation in human endometrial epithelium were both estrogen and progesterone dependent [28-31]. Although the pattern of integrins and their ligand expression during mouse pregnancy were shown [12], but the correlation between the expressions of different types of integrin molecules and OPN with pinopode expression in mouse was not evaluated. The ovariectomized mice are good models to evaluate the effect of different steroid hormone treatment on implantation events and the pattern of integrin expression. Therefore, this study was performed, for the first time, using the real-time RT-PCR and immunohistochemistry to evaluate the distribution and expression of several types of integrins and OPN in combination with the pinopode development using electron microscopy in the ovariectomized mice which were subjected to estrogen and/or progesterone and in normal estrous cycle.

MATERIALS AND METHODS

Animals and experimental design. Female virgin NMRI mice (n = 72), aged 8-10 weeks old, were cared for and used according to the Tarbiat Modares University Guide for the Care and Use of Laboratory Animals (Tehran, Iran). The mice were considered as two main groups: non-ovariectomized and ovariectomized mice. In both groups of the study after preparation of uterine samples, the expression of integrin molecules was assessed by immunohistochemistry and real-time RT-PCR analyses. In addition, the ultrastructure of endometrium in ovariectomized mice was studied using transmission and scanning electron microscopy (SEM). Stages of the estrous cycle were determined in non-ovariectomized mice (n =12) by analysis of vaginal smears. About 20 ml of phosphate buffered saline (PBS) at room temperature was used to slowly lavage the vagina using a micropipette. A wet mount was prepared using a glass slide and coverslip. The stages of the estrous cycle were determined by examining the proportions and morphology of leukocytes as well as epithelial cells in the smear under a light microscope. The subgroups of proestrus, estrus, metestrus and diestrus were studied in non-ovariectomized mice. The other mice were bilaterally ovariectomized (n = 48) via a dorsal incision under anesthesia using ketamine (100 mg/kg) and xylazine (10 mg/kg). After two weeks [32], they were used for the following experimental

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Table 1. The list of primers.

Genes	Forward	Reverse	bp
β-Actin	5' TCCCTGGAGAAGAGCTACG 3'	5' GTAGTTTCGTGGATGCCACA 3'	131
αv	5' AAC TGC TCG CTG GTC TTC 3'	5' CTG GAC TTG AAA CTC CTC TT 3'	177
β3	5'GTG GAA GAG CCT GAG TGT C 3'	5' CGG TAG GTG ATA TTG GTG A 3'	236
α4	5' AAA TGG CTC TAT CGT GAC TT 3'	5' TTT CCT ATC TGT TCG TGT TG 3'	429
β1	5' TGC CTA CAA CTC TCT TTC TTC 3'	5' TGG TTT CAG ACT CCT TAT TTG 3'	209
OPN	5'AAG CAA GAA ACT CTT CCA AG 3'	5'TTG ACC TCA GTC CAT AAG C 3'	259

groups. Control group, ovariectomized mice without any injection; sham group, the ovariectomized mice which were received two daily injections of 0.1ml/mouse of oil solvent; estrogen-treated ovary-ectomized mice, which were received two daily dosage of 50 ng/mouse of 17 β-estradiol; progesterone-treated ovariectomized mice, which were received two daily dosage of 10 mg/mouse of progesterone and estrogen-progesterone-treated ovariectomized mice, which were received 50 ng/mouse 17 β-estradiol on the first day of treatment and 10 mg/mouse progesterone on the second day of treatment [33].

Tissue preparation. The sampling was carried out in every stage of estrous cycles in non-ovariectomized mice and one day after the last injection in ovariectomized mice. The mice were sacrificed by cervical dislocation and the tissues were obtained from the middle 1/3 part(s) of their uterine horns and processed separately for further studies.

Immunohistochemistry. The endometrial cryo-sections with 5μ thickness were prepared by cryostat, and then the samples were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 20 min and permeabilized by 0.3% Triton X100 for 30 min. Specific binding was blocked with 10% normal serum in PBS. The fixed samples were incubated at 4ºC overnight with primary antibodies for αv, α4, β1, and β3 integrins and for OPN diluted in PBS. The FITC-conjugated secondary antibodies was diluted with PBS and applied for 2 h. Afterwards, the samples were studied under a fluorescence microscope with appropriate filters and all experiments were replicated at least three times for each group.

Real-time RT-PCR. Total RNA was extracted using Qiagen kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The extracted RNA integrity was confirmed using gel electrophoresis and concentration of each sample was determined by ultraviolet spectrophotometry. Total RNA was stored at -80°C and at least triplicates were prepared for each group. After extraction of total RNA, one-step real-time RT-PCR was performed on Rotor-gene 3000 Real Time Thermal Cycler (Corbett Research, Australia) according to QuantiTect SYBR Green RT-PCR kit (Qiagen, Catalog no.204243, Germany). For target sequence amplifications, 500 ng of RNA was used per 25 μL reaction volume. After completing the PCR run, melt curve analysis was used to confirm the amplified product and the real-time PCR products were further verified by agarose gel electrophoresis. For each sample, the reference gene (β-actin) and the target genes αv, α4, β1, β3 and OPN were amplified in the same run. Standard curves were obtained by using the logarithmic dilution series of total RNA. Relative quantization of target gene was determined using the Pfaffl method. The primers sequences for detecting the integrin expression and OPN genes are listed in the Table 1.

Transmission electron microscopy (TEM). The uterine horn samples were obtained from ovariectomized mice and fixed in 2.5% gluteraldehyde in PBS (pH 7.4) for 2 h and post-fixed with 1% osmium tetroxide in the same buffer for 2 h. After dehydration in a graded ethanol series, specimens were placed in propylene oxide and embedded in Epon 812 (TAAB, Berkshire, UK). Semi-thin sections (0.5 μm) were stained with toluidine blue for light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by a transmission electron microscope (Zeiss, Gottingen, Germany).

Scanning Electron Microscopy (SEM). After collection of uterine horns in ovariectomized mice, their lumens were washed several times by flushing with buffer phosphate to remove mucus secretion. Then, the samples were fixed, dehydrated (as previously described in TEM study), dried, mounted and coated with gold particles (Bal-Tec,

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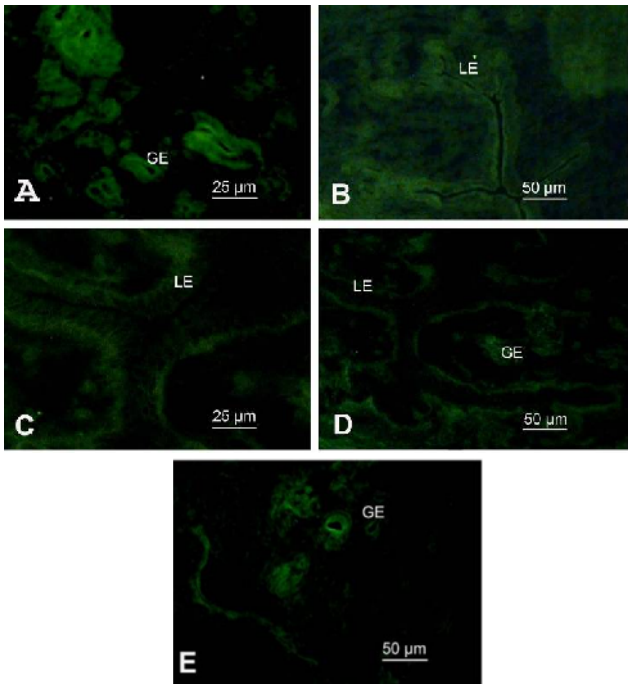

Fig. 1. Immunohistochemical localization of integrins αv, β3, α4, β1 and their ligand osteopontin in cryosections of mouse endometrial tissue at the metestrus phase of the estrous cycle. **(A)** integrin αv, **(B)** integrin α4, **(C)** integrin β1, **(D)** integrin β3 and **(E)** osteopontin. LE, luminal epithelium; GE, glandular epithelium.

Switzerland) and examined using a scanning electron microscope (Philips XL30, Netherland).

Statistical analysis. The real-time RT-PCR results were compared by one way ANOVA test and Tukey's HSD Post Hoc Test (P<0.05).

RESULTS

Immunohistochemistry of integrin and OPN in endometrium of non-ovariectomized mice during estrous cycle. The αv, α4, β1 and β3 integrins and OPN were not detected in the endometrium of non-ovariectomized mice except in metestrus phase. The expression of integrin molecules was as diffuse pattern in apical and basal part of surface and glandular endometrial epithelium and stroma. The αv subunit was most intense in the apical and basal membranes of the glandular uterine epithelial cells (Fig. 1A) and the α4 subunit showed a diffuse pattern that was intense in the apical and basal membranes in both surface and glandular uterine epithelial cells (Fig. 1B). These two types of α subunit were not detected in the stroma of endometrium. β1 integrin subunit exhibited a diffuse staining pattern, intensely positive in the basal membrane of surface epithelial cells and stroma (Fig. 1C). β3 integrin subunit was positive in the basal membrane of both surface and glandular epithelial cells and it was not detected in the stroma (Fig. 1D). The OPN expression was restricted to the apical membrane of surface and glandular epithelium and was not detected in the stroma (Fig. 1E).

Immunohistochemistry of integrin and OPN in endometrium of ovariectomized mice treated with estrogen and progesterone. No reaction was detected in the endometrium of control, sham and estrogen-treated groups for αv, α4, β1, β3 integrins and OPN, but in progesterone-treated group, the αv subunit showed an intense staining in the apical and basal membranes of the surface uterine epithelial cells and there was not any staining in the glandular epithelium and stroma in this group (Fig. 2A). In contrast, the α4 subunit showed an intense staining in the apical and basal membranes of the glandular

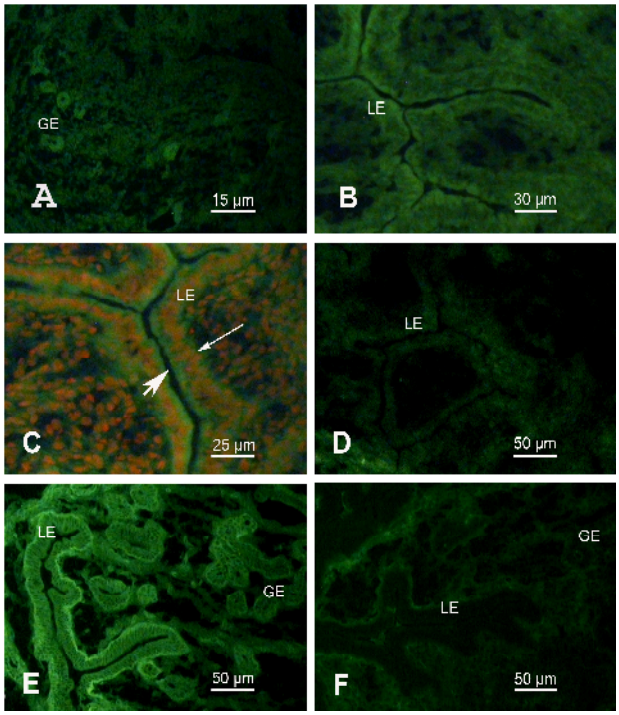

Fig. 2. Immunohistochemical localization of integrins αv, β3, α4, β1 and their ligand osteopontin in cryosections of endometrial tissue of ovariectomized mice which subjected to progesterone. **(A)** integrin αv, **(B)** integrin α4, **(C)** integrin α4 with higher magnification and counterstaining, **(D)** integrin β1, **(E)** integrin β3 and **(F)** osteopontin. LE, luminal epithelium; GE, glandular epithelium. The long arrow shows the basal membrane and the arrowhead shows the apical membrane of surface epithelium.

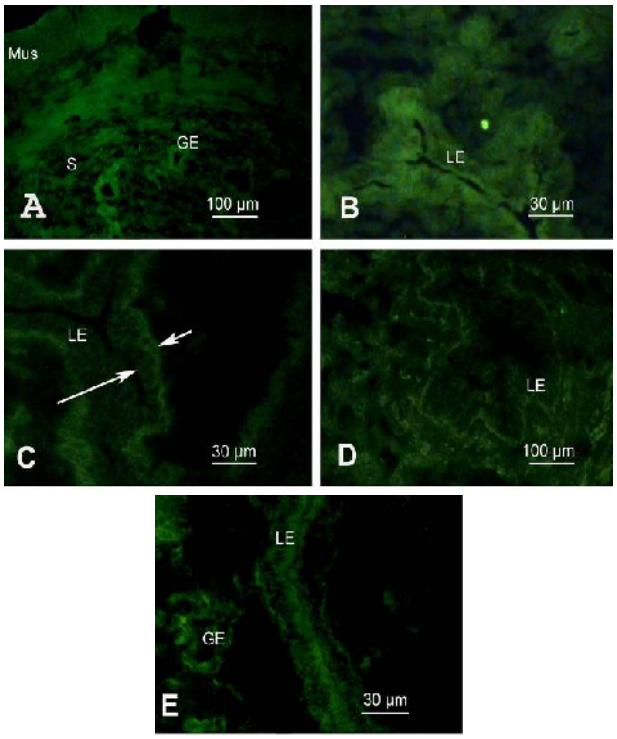

Fig. 3. The immunohistochemical staining of integrins αv, β3, α4, β1 and their ligand osteopontin in cryosections of endometrial tissue of ovariectomized mouse which subjected to estrogen and progesterone. **(A)** integrin αv, **(B)** integrin α4, **(C)** integrin β1, **(D)** integrin β3 and **(E)** osteopontin. LE, luminal epithelium; GE, glandular epithelium; Mus, muscle layer and S, stroma. The small arrow shows the basal membrane and the large arrow showed the apical membrane of surface epithelium.

epithelial cells and stroma and it was not detected in the surface of epithelium (Figs. 2B and C). The expression of β1 and β3 was similar and was detected in basal membrane of both surface and glandular epithelia of endometrium and was not demonstrated in stroma (Figs. 2D and E). OPN was expressed only in the stroma of this group (Fig. 2F). In estrogen-progesterone-treated ovariectomized mice, the immunostaining for *αv* antibody was seen in the basal and apical membranes of glandular epithelium and was not detected in the other parts of endometrium (Fig. 3A). The α4 subunit showed a diffuse pattern that was intense in the apical and basal membranes of the surface and glandular uterine epithelial cells, but was not detected in the stroma (Fig. 3B). β1 integrin subunit exhibited intensely positive in the basal part of both surface and glandular epithelial cells and was not detected in the stroma (Fig. 3C). The expression of β3 integrin subunit was seen as a diffuse pattern in apical and basal parts of the surface and glandular endometrial epithelium and stroma (Fig. 3D). OPN was observed in both epithelials of endometrium, but there was not seen in the stroma of this group of study (Fig. 3E).

Real-time RT-PCR. The mRNA levels of several integrins (αv, α4, β1 and β3) and OPN genes were evaluated by real-time RT-PCR in endometrial tissue in different phases of estrous cycle in normal mice and ovariectomized mice which were subjected to estrogen and or progesterone. The examined genes were expressed differently in normal estrous cycle phases (Fig. 4) and also in ovariectomized mice (Fig. 5). The expression of target genes was compared with the housekeeping gene (β-actin) in all samples and data were showed as Log of this ratio.

The expression of integrins and OPN genes in endometrium of normal mice during estrous cycle. The αv, β3 and OPN mRNA were detected only in the metestrus phase (Figs. 4a and 4d) and these genes were not expressed in other phases of estrous cycle. The α4 and β1 integrin genes were expressed in all phases of estrous cycle (Figs. 4b and 4c). As the results demonstrate, the higher ratio of expression belongs to β1 integrin in proestrus phase than estrous and diestrus phases (P<0.05). There were significantly differences between the expression of α4 vs. αv and β3 and β1 vs. β3 integrins in metestrus phase (P<0.05).

The expression of integrins and OPN genes in endometrium of ovariectomized mice. As the data of real-time RT-PCR showed, there was not any integrin expression in control sham and estrogen-treated groups except the β1 integrin that was expressed in estrogen-treated group (Fig. 5c). In both progesterone and estrogen-progesterone-treated groups, all the examined genes were expressed, but in different pattern. The comparison of the ratio of different integrin gene expression and the housekeeping gene revealed that the maximum expression of integrin mRNA related to the α4 in both progesterone and estrogen-progesterone-treated groups (P<0.05); however in later group, there was not significant different between the expression of α4 vs. αv.

Ultrastructural observation. The TEM electron micrograph showed a similar ultrastructure in the endometrium of control and sham-ovariectomized mice (Fig. 6A). The epithelial cells have round euchromatin nucleus and the organelles, distributed

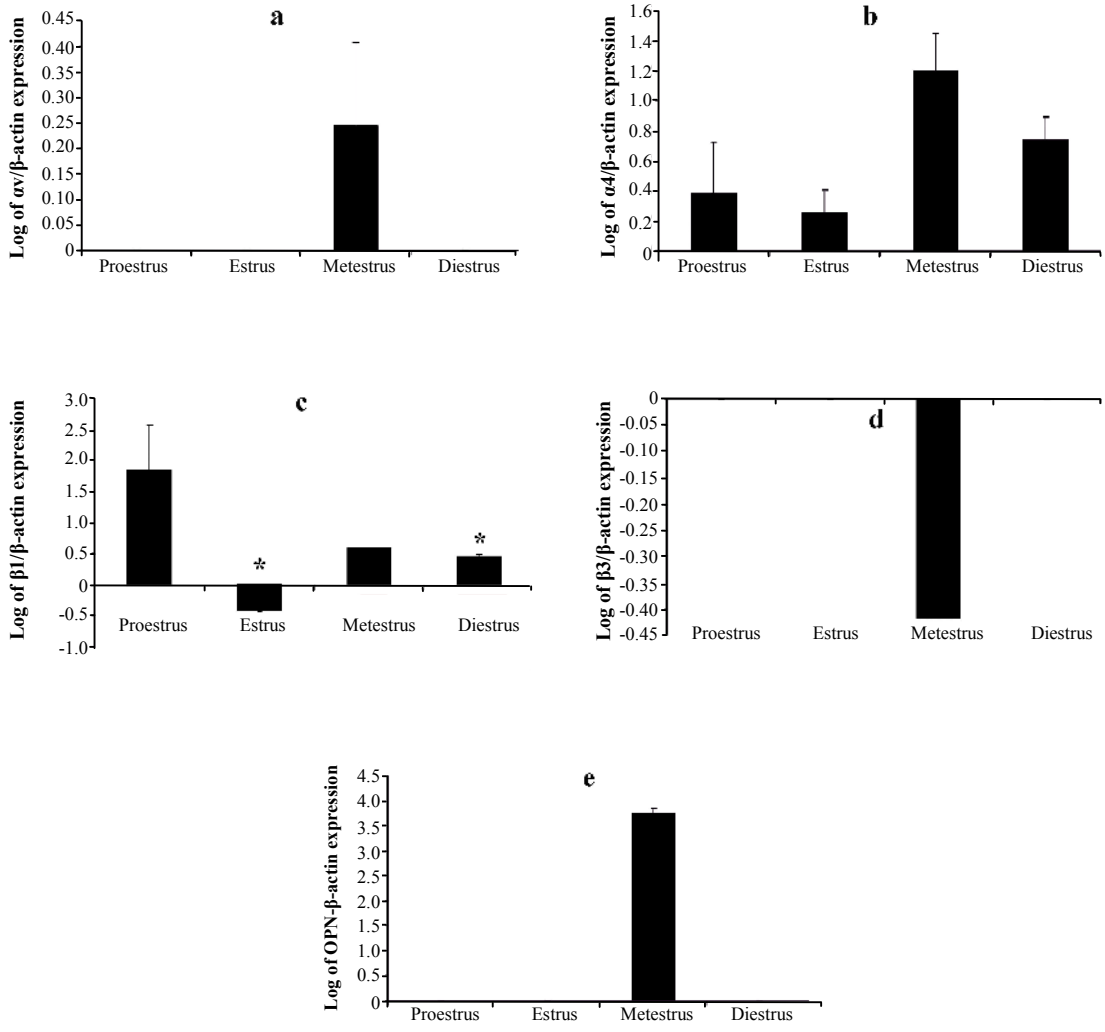

Fig. 4. The ratio of gene expression of integrins αv **(a)** α4 **(b)**, β1 **(c)**, β3 **(d)** and osteopontin **(e)** in endometrial tissue of mice at different phases of estrous cycle (proestrus, estrus, metestrus and diestrus).(*) There were significant differences with proestrus phase (P<0.05).

among the epithelial cells. The ultrastructure of the surface epithelial cells in estrogen-treated group was similar to the control group (Fig. 6B). In progesterone-treated group, the epithelial cells were more cylindrical and had elongated nucleus (Fig. 6C) and on the apical cell surface, there were some irregular short microvilli. In the estrogen-progesterone-treated group the apical cell surface projections were more prominent than the other groups (Fig. 6D). Observation by SEM showed some similarity between the morphology of the surface endometrial epithelium in control (Fig. 7A) and sham (Fig. 7B) groups. The luminal epithelial surface was covered by flat apical border cells. No signs of pinopodes or normal microvillus were seen in these groups. In estrogen-treated groups on the surface of the epithelial cells, there were a lot of projections which resemble to irregular short microvillus (Fig. 7C). In the progesterone-treated group on the apical cell surface, the number of short projections was decreased and some fungi-shaped projections (pinopodes like) were seen (Fig. 7D). In addition, the lateral borders of the cells were well defined. In estrogen-progesterone-treated group, the apical cell surface was dome-shaped and the borders of the cells were well defined, the number of cell projection was decreased and similar to the progesterone-treated group and there were some pinopodes like projections on the surface of the cells (Figs. 7E and 7F).

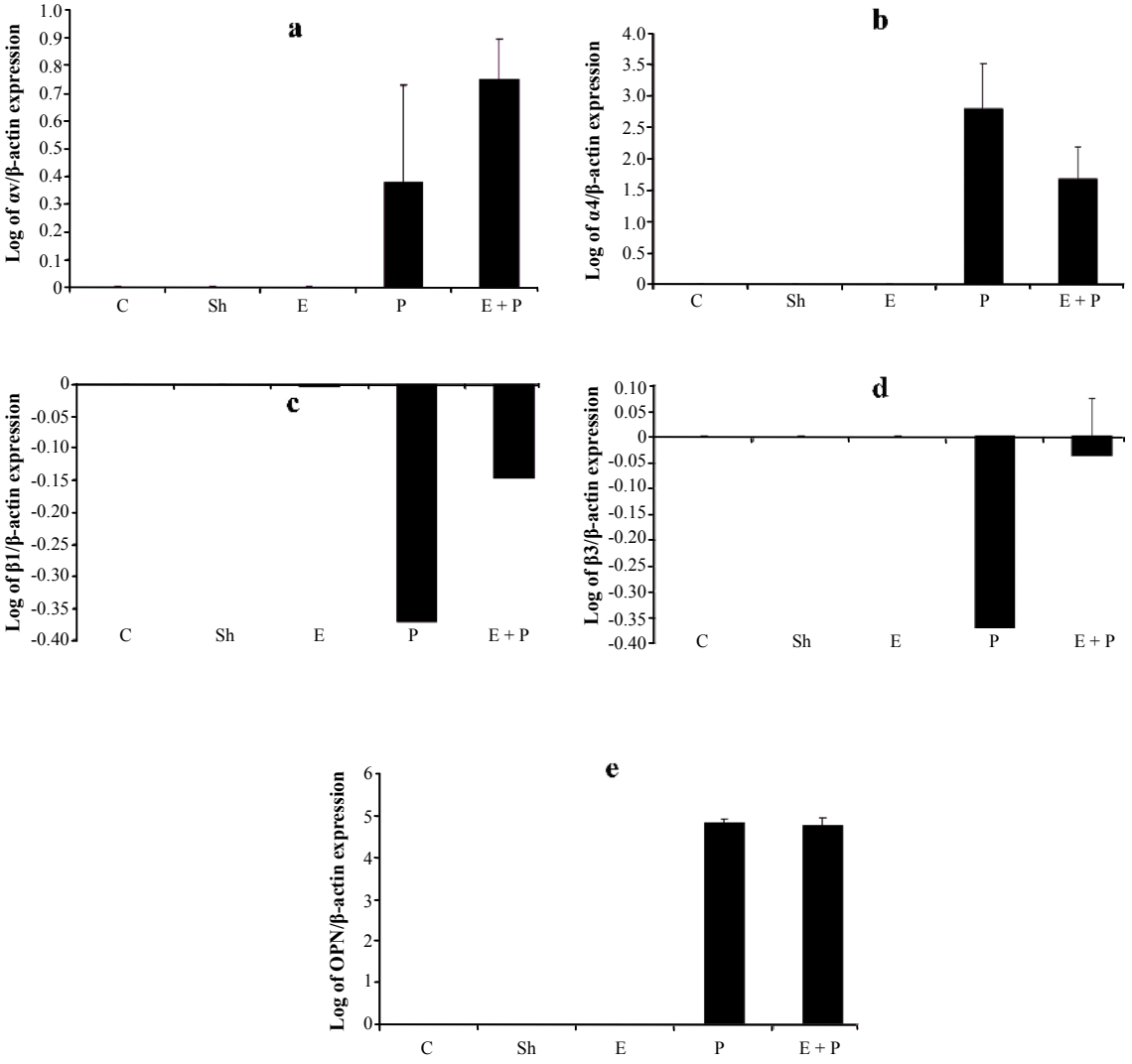

Fig. 5. The ratio of gene expression of integrins αv **(a)**, α4 **(b)**, β1 **(c)**, β3 **(d)** and osteopontin **(e)** in endometrial tissue of ovariectomized mice in non-treated control and sham groups, and estrogen, progesterone and estrogen-progesterone-treated groups. As Figure shows, there was not any sign of expression in control and sham groups. C, control; Sh, sham; E, estrogen; P, progesterone treated; E + P, estrogen and progesterone-treated groups.

DISCUSSION

We have demonstrated, for the first time, the expression of several integrins and the OPN molecules in endometrium of ovariectomized mice subjected to estrogen, progesterone or both of these hormones as well as in different phases of estrous cycle using real-time RT-PCR in combination with immunohistochemistry techniques.

Our observations, obtained by two techniques, demonstrated that there was not any sign of expression for integrins and OPN in sham and control ovariectomized mice. This confirmed that these molecule expressions in mice are induced and controlled by steroid hormones. However, there were some controversially reports in this regard in several species [10, 11, 16-20]. The ovariectomized model would be suitable for evaluation of the effect of the exogenous hormones. Our results demonstrated that all examined genes were expressed on the metestrus phase at the mRNA and protein levels and they were not detected on the other phases. These patterns of expression correlated and synchronous well with the period when serum progesterone concentration was increasing at metestrus phase of estrous cycle. The metestrus phase is similar to the secretory phase of endometrium in human, and in this phase, the

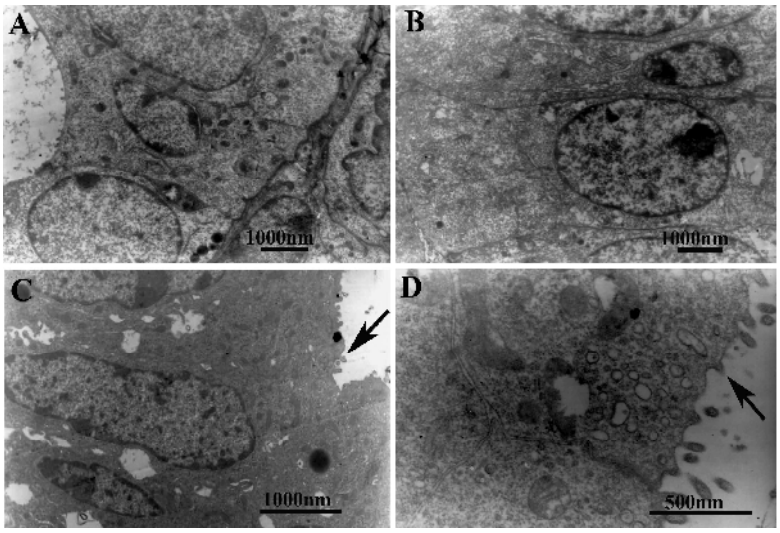

Fig. 6. The transmission electron micrograph of endometrial epithelial cells in ovariectomized mice in the control group **(A)**, estrogen **(B)**, progesterone **(C)** and estrogen-progesterone-treated groups **(D)**. The arrows show some apical cell surface projections in both progesterone-treated groups (C and D).

endometrium under the influence of circulatory progesterone undergoes some changes for reception of embryo, and the implantation takes place in this period.

Both progesterone-treated ovariectomized mice also demonstrated that all the examined integrin molecules were expressed. We concluded that the expression of these molecules was regulated by progesterone whereas the estrogen had minor effect in this regard. The progesterone could affect directly on the integrin expression in epithelial and stromal cells or it could indirectly regulated the expression and synthesis of several growth factors and cytokine such as transforming growth factor-β, basic fibroblast growth factor and prostaglandin that influence the integrin expression [34].

In agreement with our results, Kayisli *et al.* [35] reported the specific regulation of extracellular matrix proteins and integrin expression by *in vitro* treatment of human decidual cells with estrogen and progesterone. Somkuti *et al.* [18] and Widra *et al.* [19] have shown that estrogen down-regulated αvβ3 expression, but no effect was observed for αv or β1 integrin subunit. In contrast to our explanation, Basak *et al.* [7] demonstrated the down-regulation of

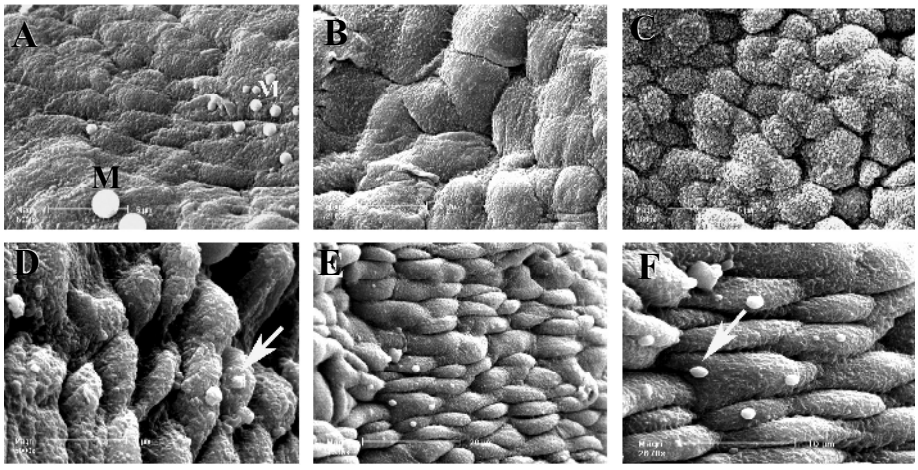

Fig. 7. The scanning electron micrograph of surface epithelial cells in endometrial tissue of ovariectomized mice in the control **(A)** and sham groups **(B)**, estrogen **(C)**, progesterone **(D)** and estrogen-progesterone-treated groups **(E and F)**. The arrows show some pinopodes-like projections in the progesterone-treated group. M, mucous droplets at the surface of epithelium.

α4 integrin expression by progesterone and its up-regulation by estradiol in the mouse blastocysts and the endometrium using a delayed-implantation mouse model. Moreover, some *in vivo* and *in vitro* researches have shown that the estrogen and progesterone decrease the expression of αv β3 integrin heterodimers [8, 16, 23] or its expression not affected by sex steroids *in vivo* [11]. In this regard, Lin *et al.* [10] showed no correlation between the serum levels of steroid hormones and the mRNA expression of αv and β3 integrins, thus they concluded that steroid hormones may play a minor role in the regulation of αv and β3 integrin expression.

These controversially results may be due to the various types of integrin molecules which were evaluated by different protocols and experimental design or these different results related to several types of species which were employed, because there were some diversities in implantation process including invasive and non-invasive types.

The comparison of mRNA expression ratios of different integrins and housekeeping genes revealed that the α4 subunit in endometrium of both ovariectomized mice groups (treated with progesterone and/or estrogen-progesterone) and in different phases of estrous cycles has prominent expression than other molecules and its mRNA was not detected in the estrogen-treated group. This observation reflected the importance of α4 more than other molecules in implantation of embryo. However, our immunohistochemical observations showed that this protein is more expressed and localized at the apical and basal parts of surfaces and glandular endometrial epithelium. The β1 and β3 subunits were almost co-expressed with α4 in surface and glandular epithelium and αv expression was restricted to the glandular epithelium. These observations reflected the roles and importance of different types of integrins heterodimers including α4β3 α4β1, αvβ1, αvβ3 in different site of endometrium not only to establishment of contact between the embryo and endometrium but also for embryo penetration during implantation period. In this regard, Basak *et al.* [7] showed the expression of a4 integrin on the basement membrane of the uterus and suggested that it might help in attachment of the blastocyst to the uterine lumen. In addition, they have blocked the a4 integrin by monoclonal antibodies in the uterus of pregnant mice on the day of implantation and demonstrated the implantation failure in both normal as well as delayed implantation mice [7].

We demonstrated that the OPN, an extracellular ligand for integrin heterodimers were expressed at mRNA and protein levels in endometrium only in metestrus phase and both progesterone-treated groups. Our speculation is that similar to integrin molecules, OPN was controlled by progesterone than estrogen. Cellular localization of OPN by our immunohistochemical observation reveled that OPN mainly restricted to glandular epithelium. In this regard Apparao *et al.* [24] showed that the OPN was expressed in glandular epithelium of pregnant rabbit endometrium. The experiments in human samples have confirmed that OPN expression is upregulated during the receptive phase for implantation [23] and maximal uterine OPN expression was detected at the phase which correlates with the rise in maternal progesterone concentration and it is in agreement with the previous reports in other mammals [36-38]. Our ultrastructural observations by TEM and SEM showed that in spite of the presence some apical projections similar to pinopodes, there was not any correlation between the pinopodes and integrin expression in ovariectomized mice.

In contrast to our results, Nardo *et al.* [31] demonstrated coordination between the pinopode development and strong expression of αvβ3 integrin in human endometrial cells. Our suggestion in this regard is that in contrast to human, the pinopodes may have minor importance in mice implantation or have some delay in expression in ovariectomized mice which were subjected to exogenous hormones. In experiments with ovarian hyperstimulated mice and rats it was shown that the differentiation and development of pinopodes were inhibited by supraphysiological levels of estrogen [39-41] and progesterone cause premature formation of the pinopodes in hyperstimulated mice [42] suggesting that the pinopodes expression may depends on the balance between the estrogen and progesterone.

In conclusion the progesterone more effective on endometrium integrin expression than estrogen and differences in the expression pattern of integrins reflect their important and different roles in embryo implantation.

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