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## **EXPRESSION OF PINOPODIES IN WOMEN WITH ENDOMETRY HYPERPLASIA WITHOUT ATIPY AND FERTILITY DISORDERS**

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### **Abstract**

Non-atypical endometrial hyperplasia (EH) most often occurs against a background of relative or absolute hyperestrogenism, responds to abnormal hormonal effects. In the reproductive age, the presence of EH can be a factor in the development of fertility disorders. The aim of the study was to learn the expression of pinopodes in ovulatory infertile women with non-atypical EH on the day of the implantation window. Under observation there were 196 women of reproductive age, of which 166 had been treated for infertility, and 30 conditionally gynecologically and somatically healthy, fertile women of the control group. A special examination of women included the determination in the endometrium by scanning electron microscopy of pinopodes' expression. It was found that in women with non-atypical hyperplasia of the endometrium, areas without pinopodia were recorded in 47.59% of cases, pinopodes of small size - in 79.52% and abundant, small, thick microvilli - in 37.95%; the decrease in the incidence of developed pineapods in comparison with healthy prolific women was 2.72 times, that of pinopodes of large size was 3.39 and the small number of microvilli was 3.64, an increase in the occurrence of pinopodes of medium size 3.65, microvilli in moderate amounts - at 3.74. The authors conclude that the characteristic feature of endometrial pinopodes in EH is the presence of mosaicism of their shape (60.24%), size

(64.46%) and the presence of numerous areas of absence of pinopodes (47.59%), which plays a significant role in disorders fertility.

**Key words: impaired fertility, non-atypical endometrial hyperplasia, scanning electron microscopy, implantation window, endometrial receptivity, pinopodia, microvilli.**

**Introduction** Pinopodes are apical epithelial cellular protrusions of the endometrium of the uterus into its lumen [1]. In rats pinopodes appear on the 4<sup>th</sup> day, become developed on the 5<sup>th</sup> day and rapidly regress on the 6<sup>th</sup> day of early pregnancy, which coincides with the acceptability of the endometrium or period of implantation window [2]. Due to its existence for a limited period of time pinopodes can be used as a marker of the uterus' receptivity [3]. Pinopodes appearance coincides with the other receptive markers expression such as leukaemia inhibitory factor (LIF) [4], mucin, L-selectin, integrin  $\alpha\beta 3$ , and epidermal growth factor of epidermis [5, 6], which ties up heparin. However, in humans, their role as a marker of receptivity remains controversial [7].

In the endometrium of the uterus pinopodes have several functions. In rodents they participate in pinocytosis, which promotes the removal of fluid from the lumen of the uterus, initiates its closure and precedes the attachment of blastocyst [8]. It is reported that pinopodes are involved in the interaction of the embryo-endometrium via the L-selectin [9] ligand, which contains the carbohydrate epitope MECA-79 [10]. In humans pinopodes release secretory vesicles containing LIF into the lumen of the uterus [11]. Pinopodes' exposure depends on progesterone (P4) [12]. In the uterus of humans [12], rats [1] and mice [13] a strong correlation between pinopodes and blood plasma P4 and expression of P4 receptors has been registered. Estrogens cause loss of pinopodes' expression [1]. It has been found that there is a large number of pinopodes in mice with ovarian removal, and this allows to suggest that the absence of estradiol is most likely to increase pinopodes' expression [14].

Non-typical EH is a precancerous nonphysiological noninvasive proliferation of endometrium with an increase in the tissue volume of the normal polyclonal endometrium with changes in glandular architecture and the ratio of endometrial glands to stroma greater than 1: 1 [15]. Non-atypical hyperplasia of the endometrium often occurs at the background of relative or absolute hyperestrogenia responds to abnormal hormonal effects. In reproductive age, the presence of endometrium hyperplasia may be a factor causing the development of fertility disorders.

**The aim** of the research - to study the expression of pinopodes in ovulatory infertile women with non-atypical EH on the day of the predicted implantation window.

### **Material and methods**

196 women of reproductive age have been examined. Among them 166 patients applied for infertility treatment constituted group I, and 30 conditionally gynecologically and somatically healthy, fertile women formed group C.

Instrumental examination of the women included echography, hysterosalpingography, hysteroscopy. All the women under examination had uterine tubes patency..

The research material was The endometrium scrapings obtained during hysteroscopy on the 22<sup>nd</sup> day of the menstrual cycle prior to treatment, as well as the biopsy from group C pregnant women with changes in endometrium corresponding the criteria of R.W. Noyes [16] have been investigated.

Pinopodes' expression was investigated by scanning electron microscopy. The latter was carried out after washing the endometrium samples from blood in physiological solution, fixing it in a 1.25% solution of glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), dehydration with acetone solution on distilled water at increasing concentrations (from 60 to 100%), drying in carbon dioxide using the method of transition of a critical point, spraying gold (150-200 A) to eliminate charges and sufficient contrast. Scanning electron microscopy was performed with microscope "JEOL Superprobe 733" with an increase  $\times 2000$ .

Sample data were evaluated by the quantitative, nominal and ranked scales. The results obtained were processed with IBM PC using the package of programs Statistica 6.0 and Biostate.

### **Results and discussion**

The age of the women under examination ranged from 25 to 35 years old and on average it was: in group I  $30.23 \pm 0.15$  years, in the group C –  $30.37 \pm 0.53$  years ( $p > 0,05$ ).

The distribution of the average duration of menstruations, the duration of the menstrual cycle and the number of menstrual cycles per year in both groups was homogeneous (Table 1).

Table 1

Characteristics of MC in the women under examination (M±m)

Group	Age of menarche, years	Average duration of menstruations, days	Average duration of menstrual cycle, days	Average number of menstrual cycles per year
I, n=166	12.04 ±0.06 <sup>c</sup>	5.25±0.08	28.13±0.20	13.09±0.10
C, n=30	12.73±0.14	5.07±0.17	28.03±0.24	13.05±0.11

Note: <sup>c</sup> is a significant difference with group C ( $p < 0,05$ ).

The significant differences found between groups I and C concern the specificity of menstruation: the amount of blood lost, the severity of the pain syndrome, which corresponds to endometrial hyperplasia clinic (Table 2).

Table 2

Characteristics of menstruations in the women under examination, n (P ±p,%)

Group	Painful	Scanty	Moderate	excessive
I, n = 166	104(62.65±0.61) <sup>c</sup>	14(8.43±0.23)	64(38.55±0.48) <sup>c</sup>	88(53.01±0.57)
C, n = 30	10 (33.33±1.07)	2(6.67±0.48)	25(83.33±1.69)	3(10.00±0.59)

Note: <sup>c</sup> is a significant difference with group C ( $p < 0,05$ ).

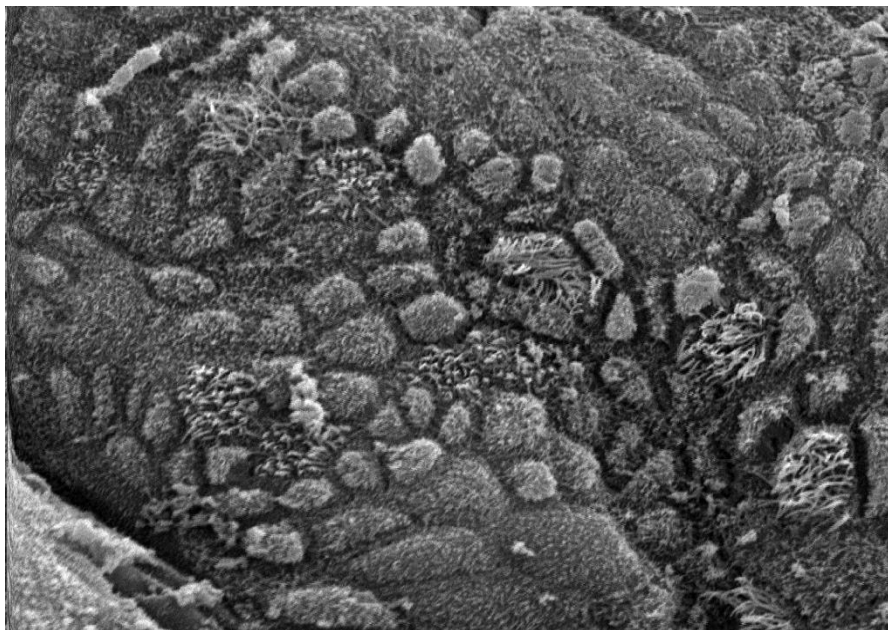
Among Group I patients primary infertility was reported in 126 (75.90 ± 0.93%) cases and secondary in 40 (24.10 ± 0.60%). 11 (13.41 ± 0.41%) of the patients under study had miscarriage.

Scanning electron microscopy of endometrial specimens from non-atypical endometrial hyperplasia women showed violation of endometrium's acceptability which manifested itself as deviations in the development of pinopodes, their microvilli, appearance of numerous ciliary cells during the predicted window of implantation. In subjects with endometrial hyperplasia, in comparison with the control group, in 137 (82.53 ± 0.70%) patients there were developing pinopodes in the day of forecasted implantation window, while they were absent the control group. In atypical endometrial hyperplasia patients a decrease in the incidence of developed pinopodes was recorded in 2.72 times (55 (33.13 ± 0.45%) versus 27 (90.00 ± 1.75%),  $p < 0.01$ ); large-sized pinopodes - 3.39 times (49 (29.52 ± 0.42%) versus 30 (100.00 ± 1.85%),  $p < 0.01$ ); cases of small number of microvilli - in 3,64 times (41 (24,70

$\pm 0,39\%$ ) versus 27 ( $90,00 \pm 1,75\%$ ),  $p < 0,01$ ); the frequent occurrence of pinopodes of average size in 3.65 times ( $101 (60.84 \pm 0.61)$  vs. 5 ( $16.67 \pm 0.76\%$ ),  $p < 0.01$ ) and microvilli in moderate amounts - 3.74 times ( $62 (37.35 \pm 0.47\%)$  vs. 3 ( $10.00 \pm 0.59\%$ ),  $p < 0.01$ ).

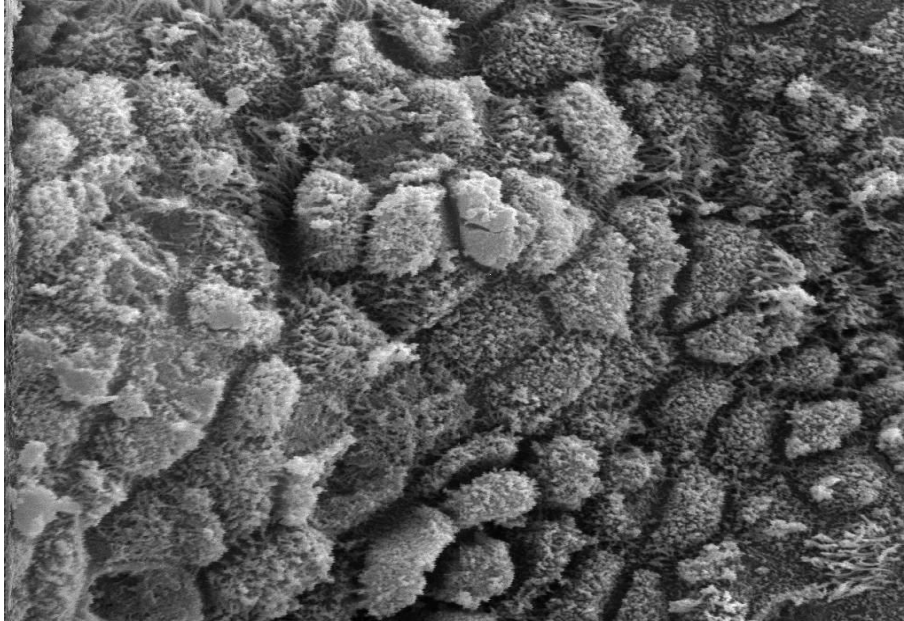
In endometrial hyperplasia patients there were pinopodes of small size in 132 ( $79,52 \pm 0,69\%$ ) cases ( $p < 0,01$ ) and abundant, small, thick microvilli in 63 ( $37,95 \pm 0,48\%$ ) ( $p < 0.01$ ), whereas in the control group they were not observed.

The characteristic feature of endometrial pinopodes in non-atypical endometrial hyperplasia was the presence of their various forms ( $100 (60,24 \pm 0,60\%)$ ), sizes ( $107 (64,46 \pm 0,62\%)$ ) and pinopodes-free sites ( $79 (47, 59 \pm 0,54\%)$ ) (Fig. 1, Fig. 2).



*Fig. 1.* Scanning electron microscopy of endometrium of a patient with non-atypical endometrial hyperplasia without chronologically relevant secretory transformation during the predicted window of implantation: mosaic shape and size, pinopodes-free areas; set of short, thick microvilli; the appearance of ciliary cells. Bar scale = 5 microns.

The peculiarities of pinopodes and microvilli development in women with complex non-atypical EH depended on the presence of chronological correspondence of secretory changes: in patients without appropriate secretory change pinopodes-free areas were met 1.59 times ( $p < 0.01$ ) more often; developing pinopodes were met 1.31 times ( $p < 0.01$ ); pinopodes of middle size were met 1.38 ( $p < 0.01$ ) times often, while developed and large-sized pinopodes were met 3.91 ( $p < 0.031$ ) times and 2.70 times ( $p < 0.01$ ) rarely.



*Fig. 2.* Scanning electron microscopy of the endometrium of a patient with complex non-atypical endometrial hyperplasia with chronologically relevant secretory transformation during the expected implantation window: mosaic shape and size of pinopodes, set of short, thick microvilli; appearance of ciliary cells. Bar scale = 5 microns.

### **Conclusions:**

In women with non-atypical hyperplasia of endometrium, areas of pinopodes absence are recorded in 47.59% of the cases, small pinopodes - in 79.52% and abundant, small, thick microvilli in 37.95% of cases; decrease in the frequency of developed pinopodes in comparison with healthy fertile women in 2.72 times, large-sized pinopodes in 3.39 and small number of microvilli in 3.64, increase of the occurrence of pinopodes of average sizes in 3.65 times, microvilli in moderate numbers - at 3.74. The peculiarities of the development of pinopodes and microvilli in women with hyperplasia depend on the presence of chronological correspondence of secretory changes. The characteristic feature of endometrial pinopodes in EH is the presence of mosaic in their form (60.24%), size (64.46%) and presence of numerous sites of absence of pinopodes (47.59%), which plays a significant role in fertility disorders.

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