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Histomorphometric Analysis and Markers of Endometrial Receptivity Embryonic Implantation in Women With Polycystic Ovary Syndrome During the Treatment With Progesterone

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Abstract

Literature data indicate that the infertility in women with polycystic ovary syndrome (PCOS) is not only attributed to anovulation but also to endometrial dysfunction. Endometrial biopsies were performed in the proliferative and secretory phases of women with normal cycle and in women with PCOS before and after oral treatment with micronized progesterone. After the treatment, the endometrium of the women with PCOS exhibited a lower number of glands and thicker luminal epithelium compared to the normal women in the secretory phase. In addition, the PCOS group exhibited reduced integrin and MECA-79 immunoexpression during the secretory phase. The expression of E-cadherin was higher in the PCOS and the expression of intercellular adhesion molecule 1 was lower in PCOS, during the secretory and proliferative phases, respectively. Also, there is a negative correlation with MECA-79 and integrin expression and body mass index. Conventional doses of progesterone may not be enough to correct the changes of endometrial histomorphology and the receptive markers of PCOS-bearing women. The obesity may be a factor that interferes with this response.

Keywords

polycystic ovary syndrome, endometrium, cell adhesion molecules

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women.[1,2] Its clinical expression is variable and usually includes oligoovulation or anovulation, clinical and/or biochemical hyperandrogenism, and polycystic ovaries on ultrasound.[3] Polycystic ovary syndrome -associated infertility was attributed to the effects of obesity and/or metabolic disorders, including inflammatory cytokines, growth factors, increased androgen production, and partial decreases in follicle-stimulating hormone (FSH), which result in anomalous ovulatory growth and inadequate oocyte quality.[4] In addition to those factors, some data indicate that the reduced fertility is apparently not only due to anovulation but also due to endometrial dysfunction in patients with PCOS.[5] In addition, in affected woman the infertility can be caused by failure of blastocyst implantation in the endometrium.[6]

Although ovulation can succeed upon proper drug treatment, the procedures of in vitro fertilization usually fail because the implantation rates remain very low, although the quality of the transferred embryos is very high.[6,7] Therefore, anovulation may not be the only cause of infertility[8]; endometrial receptivity may play a crucial role in the establishment and development of pregnancy in women with PCOS.[8] Nevertheless, data on the

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endometrium of women with PCOS, especially during the implantation window, are scarce.

Some evidence suggests deregulated expression of known endometrial receptivity markers in women with PCOS. A large number of molecular mediators influenced by the ovarian hormones was postulated to intervene in the implantation, especially during the implantation window, which is the most critical period of the maternal–fetal interaction, such as adhesion molecules including integrins, L-selectins, E-cadherins, and intercellular adhesion molecule 1 (ICAM-1).[9] In fact, $\alpha_v\beta_3$ integrin is reduced in patients with PCOS during the late secretory phase[8,10]; however, other authors did not observe this phenomenon.[11]

L-selectin plays an important role in human implantation.[12] The adhesion system of selectins is well established at the maternal–fetal interface and plays a larger role in the blastocyst apposition stage. L-selectin was observed on the blastocyst side; regarding the maternal side, the expression of the selectin ligand MECA-79 increases during the ''implantation window.'' Margarit et al[13] found lower immunoreactivity of the L-selectin ligands located at the endometrium of patients with PCOS. In addition, there are few studies in the literature on the cell adhesion molecules E-cadherin and ICAM-1 in the endometrium of patients with PCOS. E-cadherin is regulated by calcitonin, which is induced by progesterone.[14] Adhesion interactions are crucial for transendothelial migration of leukocytes and several other immune functions.[15] The leukocyte population expresses ICAM-1 at the endometrium, and ICAM-1 interferes with the immune system. Some evidence suggests that low-grade chronic inflammation and endothelial dysfunction are involved in the pathogenesis of PCOS[16] and insulin resistance.[17]

In this study, we aimed to establish whether progesterone replacement suffices to maintain the endometrium in patients with PCOS in an appropriate state together with the elements that are known to influence the maternal–fetal interaction during the implantation window.

Materials and Methods

Patients

The present study was a prospective case–control study approved by the research ethics committees of Federal University of São Paulo (Comitê de Ética em Pesquisa-CEP no 0164/09) and Getúlio Vargas Hospital of Federal University of Piauí (Universidade Federal de São Paulo-UNIFESP/Hospital Getúlio Vargas-HGV). The study was carried out at the Human Reproduction section of HGV from April 1, 2009, to August 31, 2011.

A total of 80 women, aged 18 to 35 years old, agreed to participate in this study and were thus divided into 2 groups: 40 women bearing PCOS and 40 normal controls.

Inclusion Criteria

The diagnosis of PCOS was performed according to the criteria proposed by the Androgen Excess and PCOS Society.[18] Women with other causes of hyperandrogenism or anovulation were excluded using appropriate tests, and participants had not used hormonal or other drugs in the 3 months prior to the study. Women with systemic diseases, sexually transmitted diseases, uterine tumors, ovarian cysts or tumors, other endocrine disorders in addition to PCOS, or using statins, corticoids, or infertility drugs in the 3 months prior to the study were excluded. The control group comprised healthy women aged 18 to 35 years with confirmed fertility (1 child at least) and tubal ligation registered at the institution to perform surgical reversal. We excluded patients who were using hormones or herbal substances in the last 6 months.

The material was insufficient in 11 of the 80 participants Therefore, 69 women were included in our study. The PCOS group comprised 34 participants (68 biopsies and the control group comprised 35 fertile women (70 biopsies). Initially, all participants were subjected to a detailed clinical interview, a physical examination, including assessment of the body mass index (BMI), waist circumference (WC), hip circumference (HC), and a gynecological examination including the Papanicolaou test, transvaginal pelvic ultrasound, and hysterosalpingography.

Anthropometric data were collected including height, weight, WC, HC, and BMI. Waist circumference was measured at the narrowest area between the last rib and the iliac crest and the HC at the most prominent point of the gluteal area. Clinical hyperandrogenism was established by the presence of hirsutism, which was established by the score suggested for by Ferriman and Gallwey; the patients with scores equal to or higher than 8 were considered to be hirsute.[19]

The serum levels of thyroid-stimulating hormone, free thyroxine, FSH/luteinizing hormone, free and total testosterone, androstenedione, cortisol, prolactin, dehydroepiandrosterone sulfate, 17α-hydroxyprogesterone, fasting insulin, fasting glycemia, 2-hour oral glucose tolerance test, β-human chorionic gonadotropin (β-hCG), and sex hormone-binding globulin were measured in both the groups.

Two biopsies were performed for each group, like most of the participants in the PCOS group were anovulatory, and thus, the first endometrial biopsy was performed without regard for the cycle phase after a negative β-hCG result and a transvaginal ultrasound. After this procedure, micronized progesterone (UTROGESTAN, FMQ, 200 mg/caps/d) was administered by oral route during 10 consecutive days, and a second biopsy was performed at the end, which corresponds to implantation window. The biopsy in the control group was scheduled for cycle day ninth (proliferative phase) and the second biopsy was performed on cycle day 23, on the midsecretory phase of the menstrual cycle (implantation window).

In both the groups of participants, we used the Pipelle de Cornier catheter (Laboratoire CCD, Paris, France) to collect endometrial fragments from the uterine body (the first biopsy was from the anterior wall and the second one from the posterior wall). On the morning of the day, the second biopsy was performed, and a sample of peripheral venous blood was collected to measure the serum levels of progesterone (for confirming the ovulatory cycle of the control group).

The endometrial samples after 24 hours in 10% formaldehyde were dehydrated in increasing concentrations of ethyl alcohol and diaphonized in xylene. Samples were then processed for paraffin inclusion. Ten 4μm-thick slices were prepared for every sample, 2 of them being for hematoxylin and eosin staining for confirming the menstrual cycle phase (proliferative and secretory) using the criteria suggested by Noyes. The slices were used for histological and morphometrical evaluations. The remaining 8 slices were processed for immunohistochemical analyses.

Immunohistochemical Analysis

To analyze adhesion molecules, sections of endometrium were incubated with the primary antibody $\alpha_v\beta_3$ integrin, L-selectin ligand (MECA 79), E-cadherin, and ICAM-1 (IMUNY, Rheabiotech Ltda, Brazil), incubated with the second antibody, chicken antirabbit immunoglobulin G (DakoCytomation, Glostrup, Denmark), and visualized using 3,3′-diaminobenzidine (Sigma Chemical Co). The negative control was prepared by incubating sections of rabbit nonspecific immunoglobulin fraction (DAKO-Cytomation) instead of the primary antibody and staining the sections with hematoxylin. After that, the image was analyzed through AxionVision (Rel 4.6; Carl Zeiss, Oberkochen, Baden-Württemberg), and the expression was quantified according to the color intensity in the field. The intensity of the immunoreaction was classified using criteria proposed by Panzan et al[20] as negative (0), weak (1), moderate (2), or strong/intense (3), and intense (4). Three experienced observers who were blinded to the purpose of the slides performed all of the assessments. After completion of the study, the same observers reexamined the slides to ensure reproducibility of the semiquantitative assessment.

Morphometric Measurements

The 10 images of each slice were acquired using a light microscope (AxioLab-Carl Zeiss) coupled to high-resolution video camera (AxioCamMRC-Carl Zeiss) and an image analysis software (Axio-Vision REL 4.6-Carl Zeiss).

Initially, the area present (μm^2) in each histological section of the endometrium was determined. The program automatically calculated the total area of the tissue enclosed in the contour. The number of endometrial glands and the number of blood vessels in this area were then counted. The same method was used to measure the thickness of the surface epithelium from the basal membrane until the apical border of epithelial cells, 20 measures for every slice.

A blood vessel was considered to be any vascular structure circumscribed by a vascular wall with all of its layers complete and identified. A count was done for the number of endometrial glands in histological section of the endometrium by area delimited (total area for group = 299 644.00 μm^2). An endometrial gland was deemed any glandular structure circumscribed by glandular epithelium with all of its layers identified and complete. For statistical analysis, we used the means of 10 measures for every specimens.

Table 1. Clinical and Ultrasound Characteristics of the Investigated Women in the Control and PCOS Groups (mean and standard deviation).

	Groups		
	Ctrl (n = 35)	PCOS (n = 34)	*P* Value
Age, years[a]	30.81 ± 2.56	28.03 ± 3.19	.31
Menarche, years[a]	12.03 ± 0.98	11.18 ± 0.83	.21
Parity[b]	2.57 ± 0.69	–	–
N of menstrual cycles per year (n/year)[a]	12.7 ± 0.55	4.23 ± 1.34*	<.01
Ferriman and Galley (score ≥8—Hirsutism)[a]	2.06 ± 1.62	9.35 ± 1.22*	<.01
Body mass index[a], kg/m^2	25.49 ± 2.45	30.01 ± 2.78*	<.05
Waist circumference[c], cm	80.06 ± 5.20	93.05 ± 3.51*	<.05
Hip[c], cm	112.06 ± 11.97	100.05 ± 8.07	.41
Ultrasound (follicular phase)			
R ovary volume[c], cm^3	5.98 ± 1.18	11.53 ± 2.77*	<.01
L ovary volume[c], cm^3	6.01 ± 1.08	11.96 ± 2.96*	<.01
Means of ovarian volume[c], cm^3	5.99 ± 1.12	11.75 ± 2.85*	<.01

Abbreviations: N, number of woman; Ctrl, control; PCOS, polycystic ovary syndrome; R, right; L, left.
[a] Unpaired Student t test.
[b] Statistical testing was not performed due to the absence of pregnancies in the PCOS group.
[c] Mann-Whitney test.
*$P < 0.05$

Statistical Analysis

Data were expressed as mean ± standard error of the mean and were analyzed using 2-way analysis of variance and Tukey-Kramer multiple comparisons and Mann-Whitney tests for immunohistochemical results. The Spearman's Rank correlation was used as a measure of the association between BMI and the following variables: integrin, L-selectin ligand (MECA 79), E-cadherin, and ICAM-1. A significance level of 5% (α) was adopted for all tests.

Results

The demographic and clinical characteristics of the investigated women are described in Table 1. There were no statistical differences between the groups regarding age, age at menarche, or socioeconomic level. The participants with PCOS exhibited lower number of menstrual cycles per year and the BMI, Ferriman and Gallwey score, and WC were higher than the control ($P < .05$).

Histomorphometric analyses of the endometrium showed that the participants with PCOS exhibited increased thickness of the luminal epithelium ($P < .01$), stromal and glandular development similar to the ovulating women in the first biopsy (proliferative phase), and a slightly greater number of endometrial glands, which was not statistically significant ($P = .36$; Table 2). Following the administration of progesterone, the participants with PCOS exhibited presence of secretory

Table 2. Mean and Standard Error of the Histomorphometric Findings and Immunostaining of $\alpha_v\beta_3$ Integrin, L-selectin (MECA-79), E-cadherin, and ICAM-1 in the Endometrial Luminal Epithelium of the Participants in the Control Group and the Women With PCOS Before and During the Use of Micronized P.[a]

	Control (Proliferative)	PCOS		Ctrl (Secretory)	PCOS (During the Use of P)	
	First Biopsy	First Biopsy	*P* Value	Second Biopsy	Second Biopsy	*P* Value
Luminal epithelium thickness, μm	20.29 ± 0.04	26.81 ± 0.03*	<.01	22.01 ± 0.17	27.37 ± 0.33*	<.01
Glands, 10^{-2} mm^2	12.40 ± 0.54	13.30 ± 0.81	.36	39.33 ± 1.36	22.60 ± 1.38*	<.01
Gland/stroma ratio	0.14 ± 0.16	0.15 ± 0.17	.96	0.64 ± 0.16	0.29 ± 0.17*	<.01
Number vessels, 10^{-3} mm^2	1.80 ± 0.07	2.12 ± 0.11*	<.01	2.0 ± 0.11	2.17 ± 0.07	.21
$\alpha_v\beta_3$ integrin	0.50 ± 0.15	0.70 ± 0.21	.44	5.80 ± 0.74	3.20 ± 0.53*	<.01
L-selectin (MECA-79)	0.58 ± 0.13	0.50 ± 0.14	.68	8.18 ± 0.42	2.80 ± 0.32*	<.01
E-cadherin	3.60 ± 0.16	2.80 ± 0.24*	<.05	0.80 ± 0.13	1.40 ± 0.16*	<.05
ICAM-1	2.71 ± 0.12	1.57 ± 0.13*	<.05	2.14 ± 0.17	1.71 ± 0.19	.55

Abbreviations: ICAM, intercellular adhesion molecule 1; PCOS, polycystic ovary syndrome.
[a] Unpaired Student t test; P, progesterone.
*$P < 0.05$

endometrium with histological delay according to Noyes criteria,[21] less developed glands, and higher concentration of stromal component ($P < .01$) than that of the control group (Table 2). With regard to the blood vessels, the PCOS group exhibited greater concentration in the proliferative phase; however, following the administration of progesterone, the percentage of blood vessels was similar to the control group.

The intensity of immunohistochemical reaction to $\alpha_v\beta_3$ integrin exhibited similar behavior in PCOS and control groups in the proliferative phase ($P = .44$), with weak reactivity in the luminal epithelium (Figure 1A and B). During the midsecretory phase (second biopsy), the expression of $\alpha_v\beta_3$ integrin changed but the intensity increased in the luminal and glandular epithelial compartment. Greater concentrations of $\alpha_v\beta_3$ integrin were located at the cell surface and intercellular membrane in the control group, and the difference was statistically significant compared with the PCOS group ($P < .01$). Following the administration of progesterone, immunoexpression was lower in the PCOS group than that of the control group (Figure 2A and B).

The immunoreaction of MECA-79 (L-selectin ligand) was present in all biopsy specimens from both the groups and was immunolocated to a greater degree at the endometrial luminal surface and glandular epithelium, predominantly at the cell membrane as compared with the cytoplasm. During the proliferative phase, very little MECA-79 reactivity was observed in both the groups and was slightly lower in the endometrium of the women with PCOS, but the difference was not statistically significant ($P = .68$; Table 2 and Figure 1C and D). In the PCOS group, following progesterone administration, the immunoreaction of MECA-79 was more evident at the endometrial luminal and glandular epithelia. Although the reaction was more intense compared with the first biopsy, it was less intense compared with the participants from the control group (Figure 2C and D). The MECA-79 scores were significantly higher in the second biopsy of the control group compared with the PCOS group ($P < .01$).

The intensity of immunoreactivity to E-cadherin in the first biopsy specimens was higher in the control group than in the PCOS group ($P < .05$; Figure 1E and F). Following the use of progesterone, the behavior of the PCOS group was different compared with the control and did not exhibit a significant reduction, as shown in Table 2 ($P < .05$) and Figure 2E and F. Upon comparison of the immunoexpression of ICAM-1 in the endometrial luminal and glandular epithelium, the specimens from the first biopsy (proliferative phase) exhibited significant differences between the PCOS group and the control, while the PCOS group exhibited lower scores (Table 2 and Figure 1G, H and Figure 2G, H). No immunoreactivity was detected in the negative controls of $\alpha_v\beta_3$ integrin, MECA-79, E-cadherin, or ICAM-1 (Figures 1 and 2 numbers 1, 2, 3, 4).

Spearman's Rank Correlation

To assess the influence of the BMI on the results from endometrial immunoexpression for postprogestin-treated values, the Spearman Rank correlation coefficient was calculated using the following variables: integrin, L-selectin ligand (MECA 79), E-cadherin, and ICAM-1. The BMI was negatively correlated with integrin and MECA 79 expression, ($\rho = -0.73$ and $\rho = -0.79$, respectively, $P < .01$; Figure 3A and B). No significant association was found with other immunoreactions (Figure 3C and D). Also, there was no correlation between the control group and BMI during the secretory phase.

Discussion

Despite the advances in human reproduction techniques, pregnancy rates are still low among women with PCOS,[6] and the factors explaining such rates are subject of much research. In the present study, we investigated whether the endometrium might be a limiting factor for embryo implantation and development of pregnancy even when it is previously prepared through administration of natural progesterone. It is know that the vaginal route is superior to oral route for reducing the abortion ratio after in vitro fertilization (IVF); however, this route is

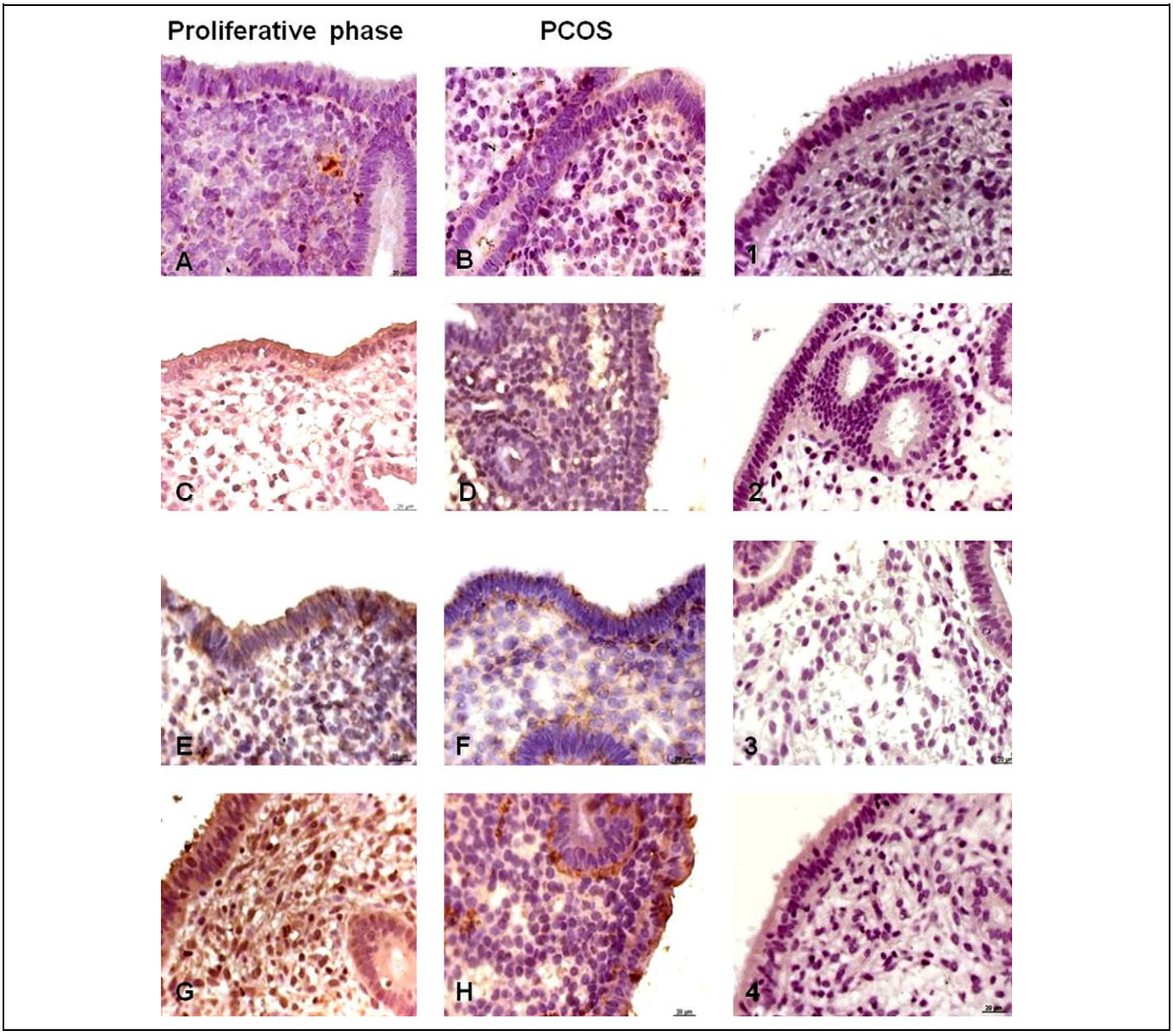

Figure 1. Photomicrographs showing the expression immunohistochemistry of αvβ3 integrin (A and B), L-selectin (MECA-79) (C and D), E-cadherin (E and F), and ICAM-1 (G and H) in the proliferative phase (control group) and woman with PCOS. The numbers represent the respective negative controls (nonspecific goat antibodies) of immunohistochemical reactions: (1) αvβ3 integrin; (2) L-selectin (MECA-79); (3) E-cadherin; and (4) ICAM-1. Bars = 20 μm. ICAM-1 indicates intercellular adhesion molecule 1; PCOS, polycystic ovary syndrome.

not very well accepted by all patients because of side effects such as vaginal irritation and discharge,[22] Then, we chose the oral route to be well accepted by patients. In addition, in India, where 40 000 IVF cycles are performed every year, the oral route is preferred by the majority of women because they find this route of administration more convenient.[23] Our results suggest that the use of micronized progesterone in conventional doses does not suffice to restore the normal endometrial morphology and does not normalize the expression of proteins related to the adhesion of the embryo to the endometrial tissue. These findings may partially account for the difficulties associated with embryonic adhesion in the endometrium of women with PCOS. In this regard, other authors have also been investigating endometrial dysfunction in women with PCOS to develop new therapies to make the endometrium more adequate for implantation.[24]

Following the administration of micronized progesterone, the endometrium exhibited histological changes compatible with the secretory phase compared with the control group. The endometrial thickness was greater, and the number of endometrial glands was lower than the control group. The endometrium of women with PCOS was shown to be frequently dysfunctional due to increased estrogen levels (not corrected by progesterone), hyperinsulinemia, and decreased insulin-like growth factor (IGF) binding protein 1 (IGFBP-1), which results in increased levels of free IGF-I in the bloodstream to maintain

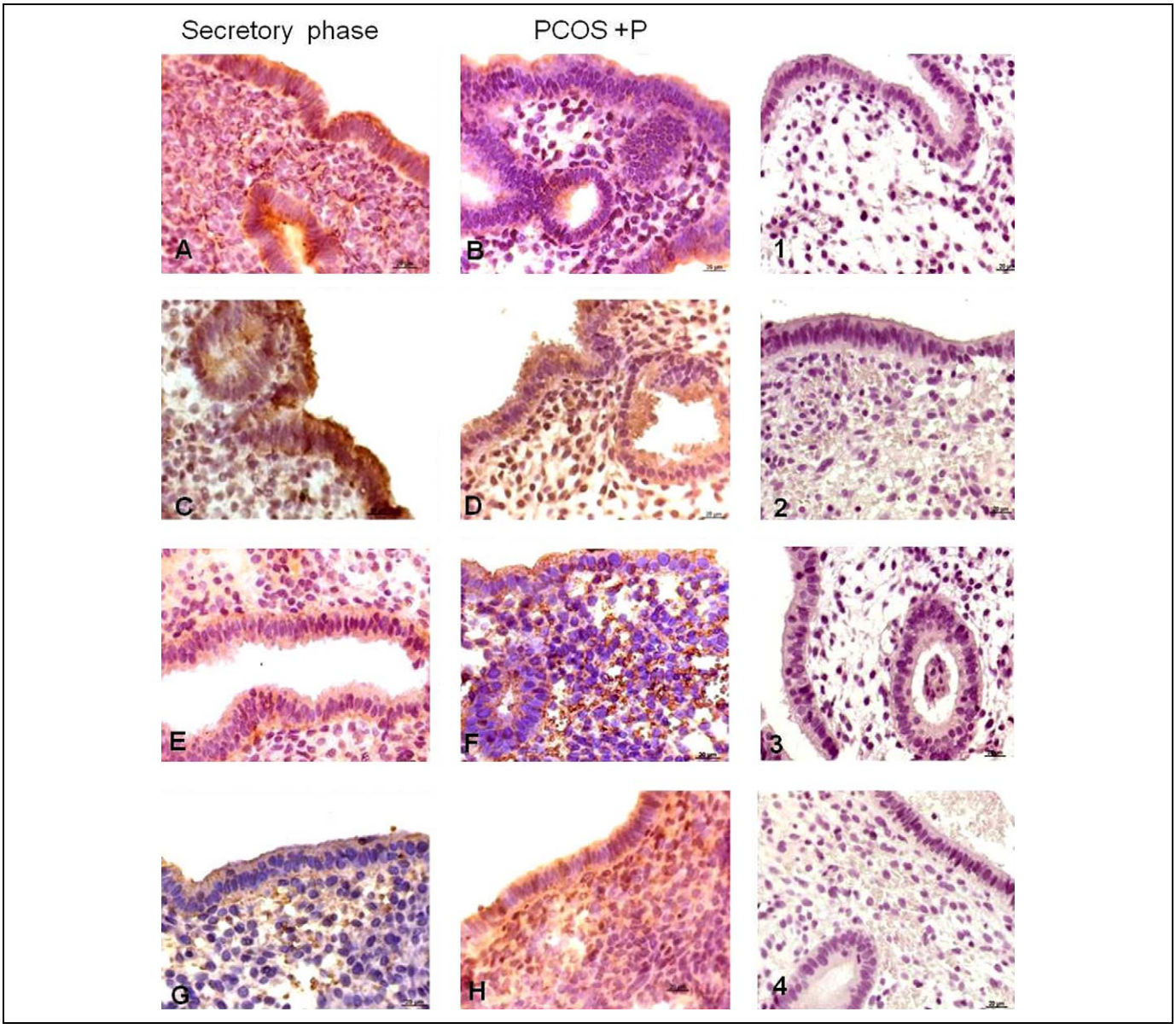

Figure 2. Photomicrographs showing the immunohistochemistry expression of αvβ3 integrin (A and B), L-selectin (MECA-79) (C and D), E-cadherin (E and F), and ICAM-1 (G and H) in the secretory phase (control group) and woman with PCOS treated with progesterone (PCOS + P). The numbers represent the respective negative controls (nonspecific goat antibodies) of immunohistochemical reactions: (1) αvβ3 integrin; (2) L-selectin (MECA-79); (3) E-cadherin; and (4) ICAM-1. Bars = 20 μm. ICAM-1 indicates intercellular adhesion molecule 1; PCOS, polycystic ovary syndrome.

the endometrium out of phase, according to Noyes and Hertig's criteria. We initially hypothesized that the use of progesterone (200 mg/d) for 10 days might suffice to induce appropriate endometrial transformation; however, our histological data suggest that it was not able to correct the existing endometrial alterations, particularly in the luminal epithelium. In addition, women with PCOS may also exhibit partial deficits in the number of endometrial glands, which might strengthen the hypothesis that some important histological alterations are not corrected by progesterone.

The data in the literature are not clear regarding whether the endometrium of women with PCOS is truly dysfunctional despite anovulation and low progesterone levels. Some studies indicated that in women with PCOS, the endometrium is inappropriate for implantation, including abnormal cell proliferation and differentiation, and inappropriate cell response occurs independent of anovulation. Our data suggest that more attention should be paid to endometrial transformation using progesterone in women with PCOS. Other factors may need to be corrected to increase the success of assisted reproduction programs.

During the secretory phase, endometrial differentiation, which is required for implantation, is completed. Molecular and immunohistochemical techniques were able to identify several biomarkers at the surface of the human endometrial luminal

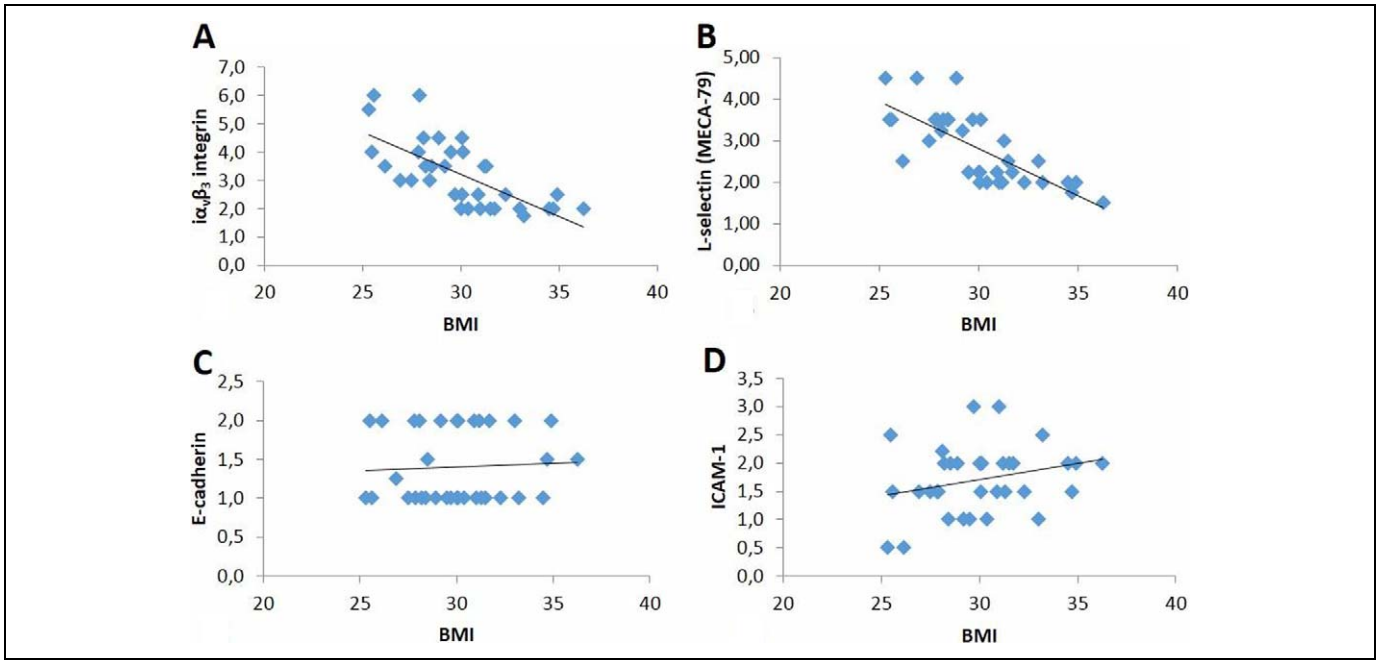

Figure 3. Spearman's Rank correlation analysis of the influence of BMI on endometrial immunoexpression values after progestin treatment. A significant and negative correlation was found between integrin and BMI (A). A similar correlation was found with MECA-79 (B). No significant association was found among other immunoreactions (C and D). 174 × 106 mm (150 × 150 DPI). BMI indicates body mass index.

epithelium that might be involved in the process of embryonic implantation,[25] including $\alpha_v\beta_3$ integrin,[11,26] L-selectin,[9,13] E-cadherin, and ICAM-1.[14] Alterations of these biomarkers might account for some cases of infertility without apparent cause, endometriosis, PCOS, and recurrent miscarriage.[9,10] For that reason, we chose them to investigate endometrial receptivity following treatment with micronized progesterone.

The endometrial secretory phase is difficult to assess in anovulatory women without the use of drugs, such as clomiphene citrate,[27] gonadotropins, or analogs of gonadotropin-releasing hormone.[28] However, these studies might be criticized for using substances that could modify endometrial receptivity.[28] Thus, we sought a model that could mimic the events in the endometrium when the deficient element, that is progesterone, is replaced. However, our results showed that although the appearance of the endometrium became more similar to the one of the controls, its histomorphology was altered along with the expression of the proteins related to embryonic adhesion.

The $\alpha_v\beta_3$ integrin is one of the most well-characterized endometrial biomarkers related to infertility.[10] Integrins are cell-adhesion molecules that may perform several functions within the endometrial cells, possibly including participation in embryonic implantation.[10,11] The integrins appear at the apex of the luminal surface as subnuclear and glandular secretory granules when the implantation window opens[10] and are expressed until the onset of pregnancy, expanding into the decidua. Some studies found that $\alpha_v\beta_3$ integrin is reduced in the endometrium of women with PCOS during the implantation window compared with the controls.[10] However, the literature is controversial because some authors found different results concerning the expression of $\alpha_v\beta_3$ integrin during the luteal phase.[29,30]

The $\alpha_v\beta_3$ integrin is considered to be a predictor of IVF success; however, very few studies on this subject have been published.[10,31] Some studies observed dynamic variations and cyclic distributions of $\alpha_v\beta_3$ integrin in the endometrium, and these changes might be induced by treatment using gonadotropins and clomiphene.[30] Other studies assessing endometrial cells in vitro suggest that integrins might be regulated by cytokines and growth factors, whereas deciduas formation is regulated by steroid hormones.[32] Therefore, other factors influenced the expression of $\alpha_v\beta_3$ integrin in our samples. Some authors have suggested that IGF-1, which is increased in PCOS, and other factors may contribute to the excess of androgens and/or insulin.[33]

Lessey and Arnold[34] observed increases in $\alpha_v\beta_3$ integrin following exogenous administration of estrogen and progesterone during the implantation window. Other authors, however, reported reduced $\alpha_v\beta_3$ integrin expression in patients treated with ovulation-inducing agents concomitant to rupture of the gland/stroma structural harmony (excessive increase of the stroma) as a function of the supraphysiological estrogen levels.[35] Lacin et al[29] did not find any change in the integrins following administration of clomiphene citrate, hormonal contraceptives, estrogen, or progesterone in patients with infertility by unknown causes. The main criticism of that study is that some patients considered to have no apparent cause might have actually experienced endometriosis or some other alterations associated with differential behavior of the integrins.

Our findings in the control group corroborate data reported by other authors, namely, the expression of MECA-79 is low during the proliferative phase and high during the secretory phase.[9,13] In addition, Foulk et al[36] observed reduced

expression of MECA-79 in patients with repeated implantation failure. Those data suggest that MECA-79 may be related to endometrial receptivity disorders. According to our data, MECA-79 increased following the administration of progesterone in women with PCOS, however, its levels were lower compared with the control. Therefore, the administration of progesterone alone was not able to correct the deficiency of MECA-79 in endometrial receptivity during the implantation window. Similar to the integrins, other factors are likely involved in the expression of MECA-79.

Also, both substances may decrease with the increase in BMI as we noticed by Pearson's correlation analyses. Probably the obesity is an independent factor for changing the expression of integrin and MECA-79 in endometrium of patients with PCOS after progesterone treatment. A collaborative meta-analysis revealed that obesity may exert influence on infertility.[37]

Loss of E-cadherin is necessary for the penetration and invasion of the trophoblast into the endometrium[26] because it plays an important role in the maintenance of cell coaptation.[14] E-cadherin has been scarcely studied in women with PCOS, and our study shows that this integrin may prevent pregnancy in women with chronic anovulation. In the present study, reduced expression of E-cadherin was observed in the women with PCOS compared with the control. However, the reduction was much lower following the administration of progesterone to the point that the calcitonin–E-cadherin ratio was inverted in the second phase. The E-cadherin level was higher in the PCOS group than in the control. These results may indicate that the dose and duration of exogenous progesterone administration were not sufficient to stimulate the endometrial calcitonin and to reduce E-cadherin.[38] Another explanation might be related to the possible influence of other factors, similar to integrins and L-selectins. Also, it is important to emphasis that further studies are necessary to confirm those changes by means of reverse transcription polymerase chain reaction or other molecular technique.

Conclusions

Our findings show that the use of micronized progesterone in a dose of 200 mg/d for 10 days by women with PCOS did not promote the same modifications of control group during the secretory phase regarding the immunoexpression of epithelial cells or histomorphological changes. The obesity may be an additional factor, which might interfere with adequate endometrial transformation during the implantation window.

Declaration of Conflicting Interests

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