

Evaluation of steroid receptors, coregulators, and molecules associated with uterine receptivity in secretory endometria from untreated women with polycystic ovary syndrome

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Objective: To evaluate gene and protein expression of steroid receptors, nuclear receptor coregulators, and uterine receptivity markers in midsecretory phase endometria from untreated women with polycystic ovary syndrome (PCOS).

Design: Case-control study.

Setting: Hospital research unit.

Patient(s): Eight patients with PCOS and eight fertile women of similar age to those with PCOS.

Intervention(s): Endometrial samples were obtained from women with PCOS (PCOSE) and normal (NE) women during the midsecretory phase of the menstrual cycle.

Main Outcome Measure(s): Expression studies (immunohistochemistry, reverse transcription-polymerase chain reaction [RT-PCR] and Western blot).

Result(s): Endometria from PCOS exhibit higher levels of messenger RNA (mRNA) and protein for estrogen receptor α and coactivators than NE. Epithelial cells had a greater expression of progesterone receptor in PCOSE, whereas, no differences were observed in gene and protein expression of the nuclear corepressor (NcoR) and the antiadhesion molecule mucin type-1 (MUC-1) between PCOSE and NE. Immunodetection for the coactivator ARA70 was higher in PCOSE than in NE; in contrast, expression of β_3 -integrin in epithelia was lower in PCOSE than in control endometria.

Conclusion(s): The higher response to steroid hormones of endometria from untreated PCOS-women induces diminished expression of β_3 integrin, which partially explain implantation failure in PCOS patients.

Key Words: Polycystic ovary syndrome, endometrium, steroid receptors, coregulators, receptivity markers

The physiology of the human endometrium is highly dependent on the action of estrogens and progesterone of ovarian origin (1) and, thereby, on the spatial and temporally expression of their specific receptors (2, 3) and nuclear receptor coregulator proteins throughout the menstrual cycle (4). Nevertheless, few investigations have addressed the role of androgens and the androgen receptor (AR) in the endometrium of normal women (5, 6). These reports have indicated a cyclic expression of AR (5, 7–9) and a regulatory action of androgens on the expression of estrogen receptors (ER) (6).

Besides progesterone, which is crucial for normal implantation and pregnancy (10), other molecules have been implicated in the acquisition of endometrial receptivity, including

the adhesion $\alpha_v\beta_3$ integrin (11) and the antiadhesion mucin type-1 (MUC-1) molecules (12). As known, $\alpha_v\beta_3$ integrin is perhaps the best-characterized marker of uterine receptivity and the expression of MUC-1 is up-regulated throughout the period of receptivity (13).

Importantly, a very low expression of this antiadhesion molecule is found in the surface epithelium, as well as, in flushings of endometria from patients with recurrent miscarriage (14, 15).

Scarce knowledge is available regarding the expression and cell distribution of steroid receptors, nuclear receptor coregulators or marker molecules of uterine receptivity in women with ovulatory dysfunction and chronic hyperandrogenism, clinically evaluated as polycystic ovarian syndrome (PCOS).

As known, PCOS is the commonest endocrine-metabolic disorder that affects women in reproductive age (16, 17); a high number of them present infertility and recurrent miscarriage (18). Moreover, when ovulation is restored, these patients exhibit a high rate of spontaneous miscarriage (19–21).

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Recently, we and other investigators demonstrated an elevated expression of ER and its coactivators in endometria of women with PCOS (4, 22). In fact, the latter report indicates that PCOS women treated with clomiphene citrate exhibited elevated levels of p160 steroid receptor coactivators, which in addition to the overexpression of ER may increase the sensitivity of PCOS endometrium to the action of estrogens or androgens.

Likewise, studies assessed in a similar model have indicated that the poor reproductive performance of these patients may be, in part, due to the down-regulation of the endometrial expression of $\alpha_v\beta_3$ integrin (5).

Studies regarding uterine receptivity in PCOS-women in the absence of pharmacological treatment are lacking, the investigations conducted until now have been mostly assessed under clomiphene citrate treatment. Nevertheless, several reports have indicated that in endometria of regularly cycling women, clomiphene citrate might induce an asynchrony in endometrial development (23) and a reduction in glandular density with an increase in the number of vacuolated cells (24).

Recently, we have reported an aberrant endometrial β_3 -integrin expression in conjunction with a failure in the down-regulation of progesterone receptor (PR) during the window of implantation in women treated with clomiphene citrate (25). Therefore, it is of clinical importance to gain knowledge about the molecular behavior of the secretory endometrium of PCOS women in a spontaneous cycle. For this purpose, we explored whether gene and protein expression of steroid receptors, nuclear receptor coregulators and endometrial receptivity markers in midsecretory phase endometria from PCOS women are different from those of normal women.

MATERIALS AND METHODS

Hormone determinations were assayed as previously described elsewhere (22, 26, 27). The antibodies for estrogen receptor- α (ER α), estrogen receptor- β (ER β), androgen receptor (AR), nuclear corepressor (NCoR), ARA70, MUC-1 and β_3 subunit were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); for glycodeilin from R&D Systems, Inc. (Minneapolis, MN); for AIB1/SRC-3 from Pharmingen (San Diego, CA); and for progesterone receptor (PR) from DAKO Co. (Carpinteria, CA).

Anti-rabbit/anti-mouse/anti-goat polyclonal second antibody and labeled streptavidin biotin kit were purchased from DAKO. Protease-inhibitor cocktail obtained from Roche Mol Biochemicals (Mannheim, Germany), BSA Protein Assay kit from Pierce (Rockford, IL), peroxidase-conjugated anti-mouse monoclonal IgG, β -actin antibody and chemiluminescence substrate (ECL Western Blotting Analysis System from Amersham Biosciences, Piscataway, NJ). TRIzol reagent was obtained from Invitrogen (Carlsbad, CA), and

Revertaid H Minus M-Mulv and Taq DNA polymerase were obtained from Fermentas (Hannover, MD).

Subjects

Human endometria were obtained with a Pipelle suction curette from the corpus of the uteri of women with PCOS, 29.5 ± 5.4 years old, body mass index (BMI) 29.9 ± 1.2 kg/m². The diagnosis of PCOS was made according to the presence of chronic anovulation or oligo-amenorrhea, hirsutism, plasma T concentration >0.6 ng/mL, or free androgen index (FAI) >5.0 and characteristic ovarian morphology on ultrasound based on the criteria described by Adams et al. (28).

The endometrial samples utilized in the present investigation were obtained from a total of 42 PCOS patients attending the Infertility Clinic, where 8 biopsies corresponded to endometria from the midsecretory phase (cycle days 19–23). In fact, the eight women studied had ovulated according to plasma P measurements >4 ng/mL. The mean values \pm SEM obtained for PCOS patients were: T = 0.69 ± 0.03 ng/mL; FAI = 11.3 ± 1.39 ; progesterone = 6.24 ± 1.14 ng/mL. Hyperprolactinemia, androgen secreting neoplasm, Cushing's syndrome, and 21-hydroxylase deficiency, as well as thyroid disease, were excluded by appropriate tests.

Control endometrium was obtained from eight healthy women during the secretory phase of the menstrual cycle according to plasma progesterone measurements, at the time of bilateral tubal ligation at the San Borja-Arriarán Clinical Hospital, National Health Service, Santiago, Chile. Age and BMI were similar to those of women with PCOS (33.9 ± 1.3 years and 30.6 ± 6.0 kg/m², respectively). None had taken oral contraceptives or other medications for at least 3 months before starting the study.

Plasma T concentration and FAI were significantly lower respect to women with PCOS (T = 0.47 ± 0.06 ng/mL; FAI = 3.14 ± 1.07 , $P < .05$).

The histological dating of tissue samples were classified as belonging to mid secretory phase (cycle days 19–23) according to established criteria (29) by an experienced histopathologist. Furthermore, the profile of glycodeilin expression was similar in the two types of endometria, NE and PCOSE, indicating the normal response of endometrial cells to progesterone. This investigation was approved by the Institutional Ethics Committee of the San Borja-Arriarán Clinical Hospital and an informed written consent was obtained from all subjects.

Tissue Preparation

Endometrial tissue samples were divided into three pieces. Two pieces of each sample were frozen in liquid N₂ and maintained at -70°C for reverse transcription-polymerase chain reaction (RT-PCR) and Western blot (WB) protocols. Another piece was fixed in 4% buffered formalde-

hyde for 24 hours, embedded in paraffin and cut into 5- to 6- μ m thick sections before histological and immunohistochemical studies.

Immunohistochemical Detection

Paraffin sections of human endometrial tissue were deparaffinized in xylene and hydrated gradually through graded alcohols. Incubating the samples in 3% hydrogen peroxide for 5 minutes prevented endogenous peroxidase activity. The sections were incubated in 10 mM of sodium citrate buffer (pH 6.0) at 95°C for 20 minutes, except for ER β where the sections were incubated with glycine 0.05 M, EDTA 0.01% buffer (pH 3.15).

Nonspecific antibody binding was prevented with 4% PBS-BSA for 1 hour. Primary antibody of ER α (monoclonal; 1/200), ER β (Polyclonal; 1/400), AR (monoclonal; 1/75), AIB1/SRC-3 (monoclonal; 1/100), NCoR (polyclonal; 1/100), ARA70 (polyclonal; 1/100) and MUC-1 (monoclonal; 1/100) was applied to the samples and incubated overnight at 4°C; the antibody for β_3 subunit (polyclonal; 1/100) and glycodeilin (polyclonal; 1/800) was incubated for 1 hour at 37°C and the antibody for PR was incubated 10 minutes without dilution, according to the instructions of manufacturer.

The second antibody used in all cases was a biotinylated anti-rabbit/anti-mouse/anti-goat immunoglobulin. The reaction was developed by the streptavidin-peroxidase system and 3,3' diaminobenzidine was used as chromogen; counterstaining was performed with hematoxylin. The slides were evaluated in a Nikon optical microscope (Nikon Inc., Melville, NY).

The immunochemical evaluation was determined as the percentage of positive stained cells. In all cases, at least 500 cells were evaluated for each sample by three independent observers, and blinded to patient category.

RNA Isolation and Semiquantitative RT-PCR

Total RNA was isolated using TRIzol Reagent from endometrial tissue according to the manufacturer's instructions. Total RNA was then reverse transcribed, and cDNA was subjected to PCR using specific primers for ER α and ER β (22), AR (5), ARA70 (30), NCoR (31), MUC-1 (32), PR (sense, 5'-GGT CTA CCC GCC CTA TCT CA-3', antisense, 5'-GGC TTG GCT TTC ATT TGG AA-3'), PR-B (sense, 5'-GAG GGG GCA GTG GAA CTC AG-3', antisense, 5'-AGG GGA ACT GTG GCT GTC GT-3'), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Semiquantitative RT-PCRs were achieved in the exponential linear zone amplification for each gene studied. Two μ g of total RNA were used for reverse transcription in a total volume of 20 μ L using the Revertaid H Minus M-Mulv Reverse.

The PCR condition for ER β , AR and MUC-1 was 3 mM of MgCl₂, 0.25 mM of dNTP, 0.625 U of Taq DNA poly-

merase, and 0.4 μ M of each primer; for ER α , it was 2 mM of MgCl₂, 0.15 mM of dNTP, 1.5 U of Taq DNA polymerase, and 0.4 μ M of each primer; for PR and PR-B, it was 1.75 mM of MgCl₂, 0.35 mM of dNTP, 1 U of Taq DNA polymerase, and 0.4 μ M of each primer; for NcoR, it was 1.5 mM of MgCl₂, 0.25 mM of dNTP, 0.625 U of Taq DNA polymerase, and 0.4 μ M of each primer; for ARA70, it was 0.8 mM of MgCl₂, 0.25 mM of dNTP, 0.625 U of Taq DNA polymerase, and 0.2 μ M of each primer. The PCR amplification was performed in the Thermocycler, model PTC-100 (MJ Research Inc., Watertown, MA).

The PCR products were electrophoretically resolved on 1% agarose gel and stained with ethidium bromide. The bands were evaluated using an image analyzer (Kodak 1D Image Analysis software, Rochester, NY) and normalized relative to the GAPDH PCR product and expressed as arbitrary units (AU).

Western Blot Analysis

Endometrial tissues were homogenized in a lysis buffer (20 mM of HEPES, 2 mM, EGTA 2 mM of EDTA, and 15 μ M of PMSF) containing a protease-inhibitor cocktail. After centrifugation at 10,000 \times g for 20 minutes, protein concentrations were determined using the BSA Protein Assay Kit. Total proteins (50 μ g) were denatured and fractionated using 7.5% 1D-SDS-PAGE and transferred to nitrocellulose membrane. Membranes were blocked for 1 hour in TBST (20 mM of Tris, pH 7.5; 137 mM of NaCl; and 0.1% Tween-20) containing 10% nonfat dry milk.

The membranes were washed twice for 5 minutes each time with TBST and incubated with antibodies against AR (monoclonal; 1/100) and ER α (monoclonal; 1/300) overnight at 4°C, or β -actin (monoclonal; 1/15,000) for 2 hours at room temperature. The membranes were then washed twice for 30 minutes each time with TBST and incubated for 1 hour at room temperature with peroxidase-conjugated anti-mouse monoclonal IgG, followed by washed twice for 30 minutes each time with TBST.

The immunoreactive protein complexes were visualized by the chemiluminescence substrate. The bands were evaluated using Kodak 1D Image Analysis software, normalized relative to β -actin and expressed as AU.

Statistical Evaluation

The data was analyzed using Kolmogorov-Smirnov test normal distribution. Comparisons between groups were performed by Student's *t*-test. The significance level was set at 5%. Results are expressed as mean \pm SEM.

RESULTS

Messenger RNA Levels for Steroid Receptors and Coregulators

As known, steroid receptors are expressed in a cyclic manner in human NE throughout the menstrual cycle; there-

fore, our first interest was to examine whether messenger RNA (mRNA) levels for AR and the isoforms for ER and PR in secretory PCOSE were similar to secretory endometrial biopsy specimens from control women. The semi-quantitative RT-PCR analysis demonstrated that the mRNA of the α isoform of ER was significantly increased in PCOSE compared with NE ($P < .05$), whereas ER β mRNA abundance was similar in both types of endometria (Fig. 1).

A significant increase in AR mRNA was observed in the endometria of women with PCOS, which were obtained during the secretory phase ($P < .001$) (Fig. 1). No differences were observed in the amount of mRNA for total PR (Fig. 1) and its isoforms between both groups of

endometria, even though the PR-B message was higher than that for PR-A (PR-B: NE 1.23 ± 0.07 AU, PCOSE 1.30 ± 0.10 AU; PR-A: NE 0.27 ± 0.14 AU, PCOSE 0.16 ± 0.08 AU).

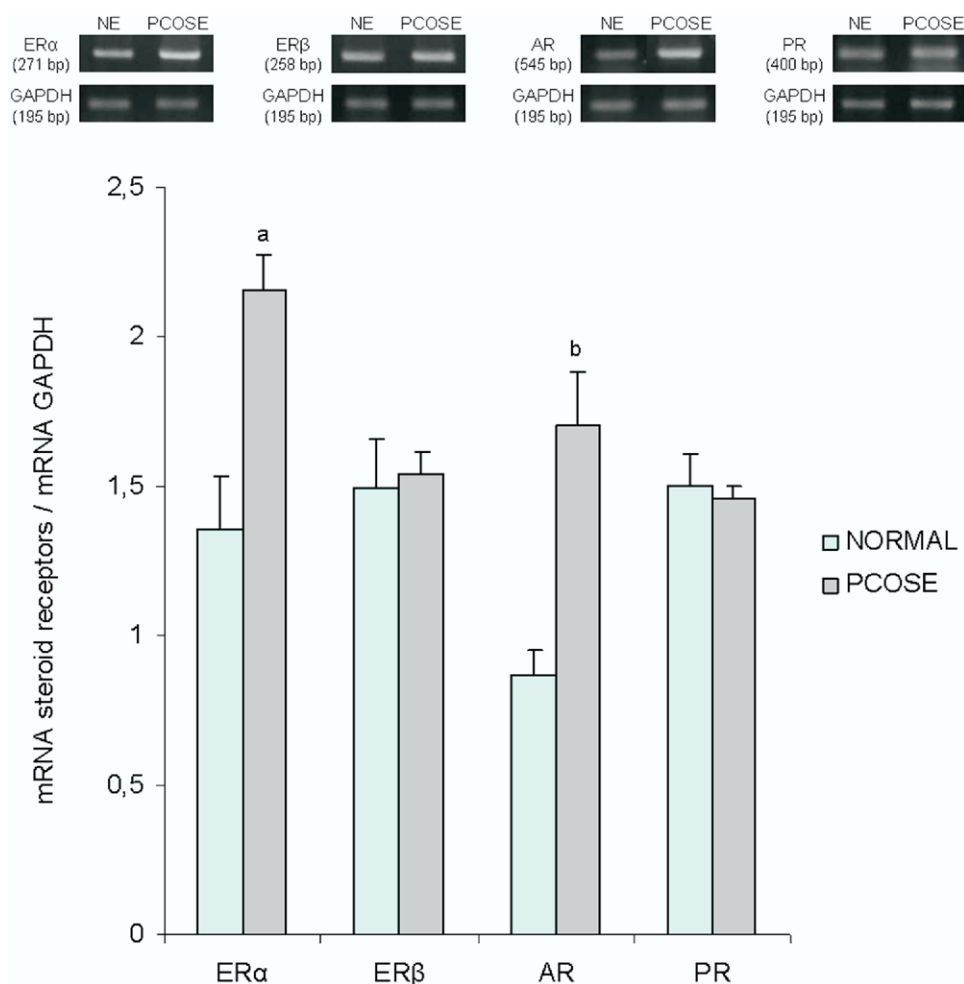
The mRNA expression of the steroid receptor coactivator ARA70 in endometrium of cycling fertile women during the secretory phase was significantly lower than in PCOSE ($P < .05$), whereas, the message for the corepressor NCoR was similar between both groups of endometria (Table 1).

Immunodetection of Steroid Receptors and Coregulators

The immunohistochemical localization and cell distribution of ER α was preferentially in the nucleus of epithelial and

FIGURE 1

The PCR amplification from cDNA of the endometria of normal women (NE) and women with PCOS (PCOSE) using primers for ER α (271 bp), ER β (258 bp), AR (545 bp), PR (400 bp), and GAPDH (195 bp). The normalized yield for ER α , ER β , PR, and AR PCR fragments relative to GAPDH PCR products from different endometria is presented. Values are mean \pm SEM of independent experiments for NE (n = 8) and PCOSE (n = 8). ^a $P < .05$ between NE and PCOSE; ^b $P < .001$ between NE and PCOSE.



Quezada. Characterization of endometria of PCOS-women. Fertil Steril 2006.

TABLE 1**Messenger RNA levels of coregulators in NE and PCOSE midsecretory endometria.**

	NE (AU)	PCOSE (AU)
ARA70	0.75 ± 0.11	1.41 ± 0.05 ^a
NCoR	2.89 ± 0.49	3.62 ± 0.41

Note: Values are mean ± SEM. NE = normal women; PCOSE = women with polycystic ovary syndrome. ^a $P < .05$ between NE and PCOSE.

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stromal cells (Fig. 2), with a greater percentage of epithelial cells exhibiting positive staining in the PCOSE samples than in NE ($P < .05$) (Table 2). In contrast, the β isoform of ER was mostly detected in the cytoplasm of cells from both endometrial cell compartments (Fig. 2), and no differences were observed in the percentage of positively stained cells between the two groups of samples. Furthermore, a significantly higher ratio of the isoforms α/β for ER was obtained in PCOSE compared with NE ($P < .05$).

For PR, nuclear and cytoplasmic immunostaining was found in both cell compartments from NE and PCOSE (Fig. 2). As presented in Table 2, the endometrium from cycling fertile women exhibit a higher percentage of positive cells for PR in the stromal compartment than in glands ($P < .001$). Importantly, the overall profile of PR expression was higher in PCOSE than in NE, particularly in the epithelial compartment where the immunodetection of PR was significantly increased ($P < .001$).

The localization of AR was preferentially at the cytoplasmic level of stromal and epithelial cells in both groups of endometria (Fig. 2), and no important differences in the percentage of positive cells for AR were obtained for the two groups of samples (Table 2).

The immunohistochemistry for the steroid receptor coactivators AIB1/SRC-3 and ARA70 and the corepressor NCoR is presented in Figure 3. The staining for AIB1/SRC-3 was observed at the nuclear and cytoplasmic level, being more intense in the epithelial compartment of endometria from fertile cycling women, although the percentage of positive cells was similar between both cell types (EEC $25.7 \pm 7.1\%$; ESC $25.8 \pm 7.3\%$).

For PCOSE, an overall increase in the percentage of positive cells was determined for AIB1/SRC-3 compared with NE (NE $25.8 \pm 7.2\%$; PCOSE $39.4 \pm 5.9\%$; $P < .05$).

The coactivator ARA70 was immunodetected at the cytoplasmic level of epithelial and stromal cells (Fig. 3). Importantly, a significantly greater percentage of positive cells with the highest intensity was detected in epithelia of PCOSE (NE $31.1 \pm 6.6\%$; PCOSE $50.1 \pm 10.2\%$, $P < .05$).

No important differences were obtained for the corepressor NCoR between NE and PCOSE.

Protein Expression of Steroid Receptors

Because ER α and AR messages were significantly different between endometria from fertile cycling women and patients bearing PCOS, protein expression for these receptors were performed by Western blot in endometrial samples obtained during the secretory phase of the menstrual cycle from both groups of women. As presented in Table 3, no statistical differences were observed for AR protein expression between NE and PCOSE.

In contrast, protein levels for ER α augmented in PCOSE compared with NE ($P < .05$; 30%), coincident with the findings for gene expression and immunohistochemical studies of the present investigation.

Levels of Uterine Receptivity-Related Molecules

The expression of MUC-1 was assessed by RT-PCR and immunohistochemistry in endometria obtained from normally cycling women and from women with PCOS. The abundance of MUC-1 mRNA was similar for both groups of endometria (NE: 2.7 ± 0.3 AU; PCOSE: 2.5 ± 0.1 AU), which is coincident with the results obtained from the immunohistochemical study, where an intense cytoplasmic staining for MUC-1 was found in the epithelial compartment of the two groups of endometria (Fig. 3).

On the other hand, the immunohistochemical evaluation of $\alpha_v\beta_3$ indicated that this integrin is found extensively in the cytoplasm of epithelial cells from both groups of endometria. Furthermore, our data revealed a significant decrease in the percentage of $\alpha_v\beta_3$ -positive cells in the epithelial compartment of PCOSE compared with NE ($P < .05$) (Table 2 and Fig. 3).

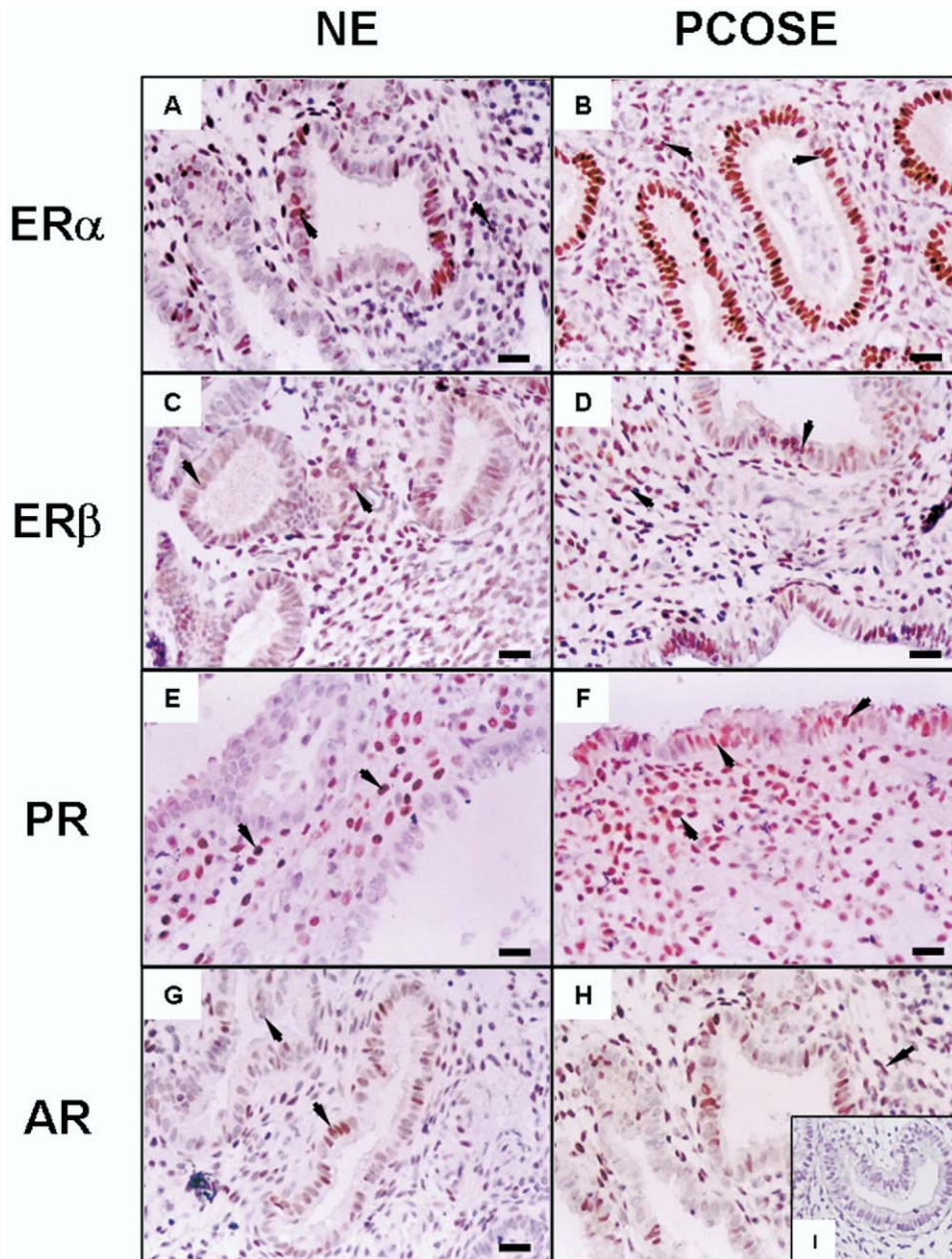
DISCUSSION

Several reports indicate that women with PCOS are often infertile, particularly due to ovarian failure and recurrent miscarriage. Even more, the restoration of the ovarian function in these patients does not lead in most cases to an improvement of their reproductive potential. Studies related to uterine receptivity in PCOS-women have been conducted under clomiphene citrate treatment, which reportedly elicits abnormal endometrial maturation. These findings suggest that long-lasting ER occupancy by clomiphene citrate may induce aberrant expression of proteins related to uterine receptivity when administered to normo-ovulatory women (25).

Therefore, to add to the understanding of the poor reproductive potential of PCOS patients, we consider of clinical importance to evaluate the expression of known proteins in the secretory endometria of PCOS women without pharmacological treatment.

FIGURE 2

Immunohistochemical detection of estrogen receptor α (ER α), estrogen receptor β (ER β), progesterone receptor (PR), and androgen receptor (AR) in paraffin wax sections of endometria obtained from normal women (NE) (*left panel*) and women with PCOS (PCOSE) (*right panel*). Positive nuclear staining was detected in epithelial cells for ER α (**A, B**) and for ER β (**C, D**) in the nuclei and cytoplasm of epithelial and stromal cells. Nuclear and cytoplasmic staining for PR (**E, F**) was detected in stroma and epithelia of both groups of endometria, with a higher number of positive cells in the stroma than in epithelia of NE. Positive nuclear and cytoplasmic staining was detected in stroma and epithelia for AR (**G, H**). Negative control (nonimmune rabbit serum) is used in place of the respective antibody (**I**). Arrowheads indicate positive staining of the respective proteins. Magnification in all panels is $\times 400$. Scale bars represent 30 μm .



Quezada. Characterization of endometria of PCOS-women. *Fertil Steril* 2006.

TABLE 2

Immunohistochemical detection of ER α , ER β , PR, AR, and β_3 -integrin subunit in epithelial and stromal compartments of midsecretory endometria from NE and PCOS.

	Epithelium compartment		Stromal compartment	
	NE	PCOSE	NE	PCOSE
ER α	40.4 \pm 8.0	67.9 \pm 9.1 ^a	46.7 \pm 10.5	50.1 \pm 8.4
ER β	11.7 \pm 2.9	7.9 \pm 1.2	17.6 \pm 11.7	13.3 \pm 3.8
PR	14.0 \pm 9.4	82.7 \pm 11.2 ^a	49.4 \pm 11.0 ^b	59.0 \pm 12.8
AR	67.0 \pm 5.3	58.0 \pm 8.5	68.7 \pm 7.1	70.3 \pm 6.0
β_3	83.1 \pm 3.6	53.5 \pm 11.0 ^c	7.9 \pm 3.6	10.3 \pm 1.2

Note: Values are mean \pm SEM. ER = estrogen receptor; PR = progesterone receptor; AR = androgen receptor; NE = normal women; PCOSE = women with polycystic ovary syndrome.

^a $P < .001$ between epithelia of NE and PCOSE.

^b $P < .001$ between epithelia and stromal compartment of NE.

^c $P < .05$ between epithelia of NE and PCOSE.

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In the present investigation, we studied gene and protein expression and localization of steroid hormone receptors and its coregulators and proteins related to uterine receptivity in spontaneous secretory endometria from PCOS patients, and compared with that of fertile cycling women. Besides the histological evaluation of PCOS endometria indicating that ovulation had occurred, the profile of glycodeilin expression was similar to that of the NE. Both observations assure that the endometrial cells responded to the action of progesterone.

As known, glycodeilin is a major secretory product of epithelial cells induced directly by progesterone and has been suggested to have utility in detecting hormonally inadequate cycles (33, 34).

The diminution on the expression of steroid receptors in the endometrium during the secretory phase of a normal menstrual cycle, have been largely documented. In the present study, our results indicate an overexpression of the α isoform of ER in the secretory endometrium of PCOS women, at the level of both gene and protein expression, in agreement with our previous report (22), whereas, no changes were observed for the β isoform of ER.

The long lasting progestin-unopposed exposure to estrogens could partially explain the up-regulation of ER α in the endometria of these women (35), even when the ovarian function is temporally restored. In several tissues, both isoforms of ER present antagonistic biological effects (35); thus, the estrogenic action is better determined by the relative expression of the two isoforms of ER. In our model, the ratio α/β of ER was higher in the endometria of PCOS-women than in NE, suggesting a predominance of the ER α -mediated actions in those tissues.

We also observed overexpression of AR mRNA in secretory PCOSE, coincident with the data previously reported in

clomiphene-citrate-treated PCOS-women (5). It has been suggested that the high plasma levels of androgens observed in these patients could induce AR expression, although it cannot be ruled out the effect of estrogens. In fact, in an in vitro system using Ishikawa cell line it has been described that dihydrotestosterone, E $_2$, and diethylstilbestrol up-regulate AR protein expression (5).

Nevertheless, no increase in AR protein level in endometria from PCOS-women was detected in the present study, which suggests that cell line cultures may not accurately represent the in vivo situation.

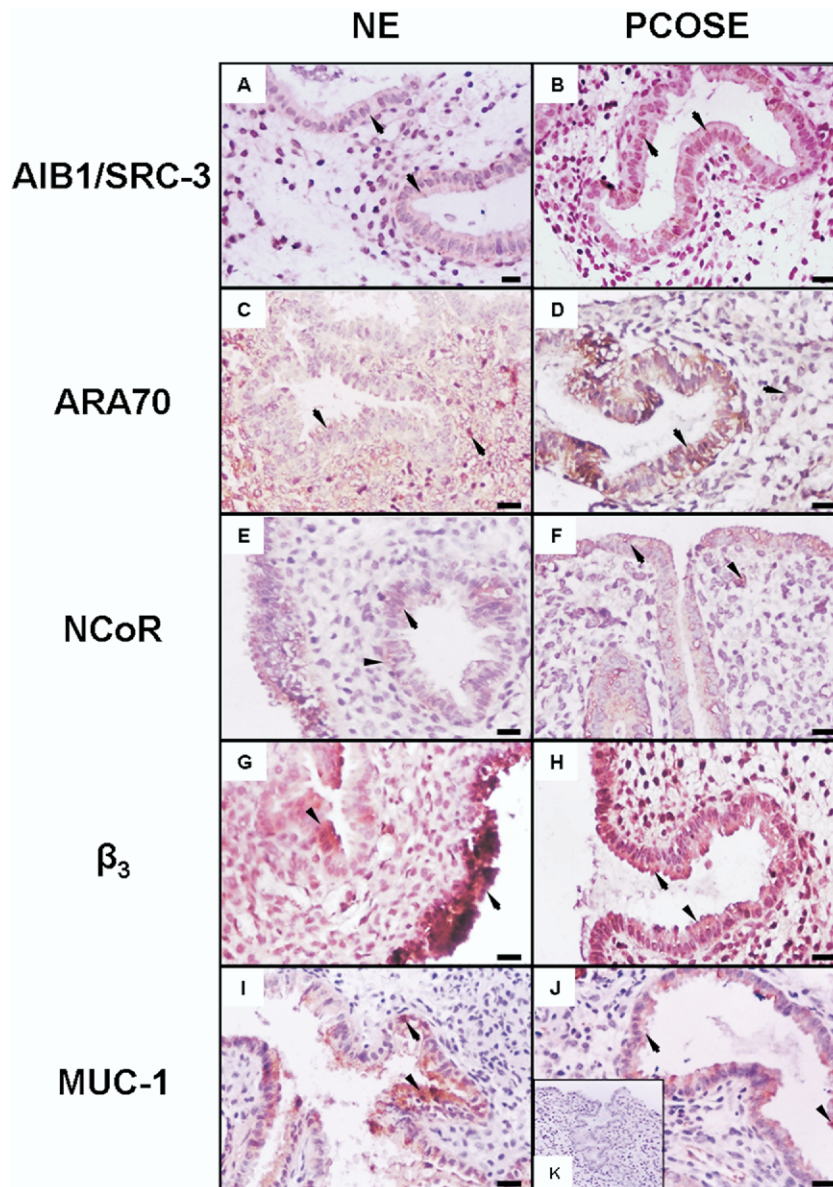
Besides the higher expression of estrogen receptors in PCOSE, the present study demonstrates that the coactivators of steroid receptors are overexpressed in those endometria, indicating an increased sensitivity to the action of steroid hormones. It is important to note that the present study describes for the first time gene and protein expression for ARA70 in normal and pathological human endometria.

Progesterone receptor is another molecule with an important regulatory action in endometrial function, particularly during the implantation window. In endometria from fertile cycling women obtained during the midsecretory phase, stromal cells but not epithelial cells, express PR (36, 37), which could indicate an important role of progesterone in stroma predecidualization. The abnormal abundance of PR in the epithelial compartment of midsecretory endometria may indicate an alteration in uterine receptivity as reported for women with luteal phase defect (37).

Importantly, the findings of the present study indicate a persistent PR at the epithelial level of PCOSE, which suggest an alteration on the expression of uterine receptivity-related molecules. In fact, we found that β_3 -integrin is diminished in the epithelial compartment of PCOSE, which could explain in part the failure in implantation of this group of women.

FIGURE 3

Immunohistochemical localization of steroid receptor coactivators AIB1/SRC-3 and ARA70 and the corepressor NCoR, and the mucin MUC-1 and β_3 -integrin subunit in paraffin wax sections of normal women (NE) (*left panel*) and women with PCOS (PCOSE) (*right panel*). Nuclear and cytoplasmic positive staining for AIB1/SRC-3 (**A, B**) was detected in NE and PCOSE, with an intense staining in the epithelia of both groups of endometria. The steroid receptor coactivator ARA70 (**C, D**) was detected predominantly in the cytoplasmic of epithelial and stromal cells of both NE and PCOSE. The positive staining was visibly increased in epithelia of PCOSE. The immunostaining for the corepressor NCoR (**E, F**) was detected preferentially in the cytoplasm of epithelia and stroma of both types of endometria, and no differences were observed in the intensity of the staining between NE and PCOSE. Immunohistochemical staining for β_3 -integrin subunit (**G, H**) was detected in the cytoplasm of epithelial cells of NE and PCOSE, being markedly lower in PCOSE epithelia. The antiadhesion molecule MUC-1 (**I, J**) was immunolocalized in the epithelial compartment of both groups of endometria, with an intense positive staining in the cytoplasm of epithelial cells. Negative control (nonimmune rabbit serum) is used in place of the respective antibody (**K**). Arrowheads indicate positive staining of the respective proteins. Magnification in all panels is $\times 400$. Scale bars represent $30 \mu\text{m}$.



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TABLE 3**Western blot analysis of AR and ER α in midsecretory endometria from NE and PCOSE.**

	NE (AU)	PCOSE (AU)
AR	1.02 \pm 0.10	1.17 \pm 0.04
ER α	1.32 \pm 0.07	1.74 \pm 0.08 ^a

Note: Values are mean \pm SEM. AR = androgen receptor; ER = estrogen receptor; NE = normal women; PCOSE = women with polycystic ovary syndrome; AU = arbitrary units.

^a $P < .05$ between NE and PCOSE.

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These data partially agree with the information reported earlier (5), whereas in a cell culture system, androgens and estrogens exerted a negative effect on the expression of β_3 -integrin.

Furthermore, our results indicate that the persistence of PR in addition to increase ER α in the epithelial compartment of PCOSE may induce an inhibition of β_3 -integrin expression. As reported, the withdrawal of steroid action at the epithelial level of endometria, represented by down-regulation of epithelial PR and ER α , plays a crucial role in the expression of molecules involved in normal uterine receptivity, including β_3 -integrin subunit (38). Therefore, our data strongly suggest that the increase in both PR and ER α in the epithelial compartment of PCOSE may induce the diminution of β_3 -subunit expression.

Interestingly, the similar expression of MUC-1 in both groups of endometria represents the action of progesterone on the endometrial tissue. As known, in addition to glycodeclin, MUC-1 is another molecule where production is directly stimulated by progesterone (39).

In conclusion, the data of the present study indicate that the higher expression of steroid receptors and coactivators in PCOSE increase the sensitivity to steroid action in the endometria of those patients. In addition, the persistence of epithelial PR induces an aberrant expression of β_3 -integrin, adhesion molecule, which is important for the implantation process. Moreover, our results suggest that not only AR is related to β_3 -integrin as previously reported (5), but also the levels and cell localization of PR and ER α in epithelia of PCOSE, which are more likely to regulate the expression of this adhesion molecule.

Therefore, the results obtained in this study may suggest important mechanisms related to implantation failure exhibited by PCOS-women.

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REFERENCES

- Brenner RM, West NB. Hormonal regulation of the reproductive tract in female mammals. *Annu Rev Physiol* 1975;37:273–302.
- Katzenellenbogen, BS. Dynamics of steroid hormone receptor action. *Annu Rev Physiol* 1980;42:17–35.
- Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS Jr. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metab* 1988;67:334–40.
- Gregory CW, Wilson EM, Apparao KB, Lininger RA, Meyer WR, Kowalik A, et al. Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. *J Clin Endocrinol Metab* 2002;87:2960–6.
- 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.
- Maliqueo MA, Quezada S, Clementi M, Bacallao K, Anido M, Johnson C, et al. Potential action of androstenedione on the proliferation and apoptosis of stromal endometrial cells. *Reprod Biol Endocrinol* 2004; 10:81.
- Slayden OD, Nayak NR, Burton KA, Chwalisz K, Cameron ST, Critchley HO, et al. Progesterone antagonists increase androgen receptor expression in the rhesus macaque and human endometrium. *J Clin Endocrinol Metab* 2001;86:2668–79.
- Fujimoto J, Nishigaki M, Hori M, Ichigo S, Itoh T, Tamaya T. The effect of estrogen and androgen on androgen receptors and mRNA levels in uterine leiomyoma, myometrium and endometrium of human subjects. *J Steroid Biochem Mol Biol* 1994;50:137–43.
- Lovely LP, Appa Rao KB, Gui Y, Lessey BA. Characterization of the androgen receptor in a well-differentiated endometrial adenocarcinoma cell line (Ishikawa). *J Steroid Biochem Mol Biol* 2000;74: 235–41.
- Baulieu EE. Contraception and other clinical applications of RU-486, an antiprogestosterone at the receptor. *Science* 1989;245:1351–7.
- 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* 1992;90:188–95.
- Carson DD, DeSouza MM, Kardon R, Zhou X, Lagow E, Julian J. Mucin expression and function in the female reproductive tract. *Hum Reprod Update* 1998;4:459–64.
- Hey NA, Graham RA, Seif MW, Aplin JD. The polymorphic epithelial mucin MUC1 in human endometrium is regulated with maximal expression in the implantation phase. *J Clin Endocrinol Metab* 1994;78: 337–42.
- Hey NA, Li TC, Devine PL, Graham RA, Aplin JD. MUC-1 in secretory phase endometrium: expression in precisely dated biopsies and flushings from normal and recurrent miscarriage patients. *Hum Reprod* 1995;10:2655–62.
- Serle E, Aplin JD, Li TC, Warren MA, Graham RA, Seif MW, et al. A morphological and immunohistochemical study of endometrial development in the peri-implantation phase of women with recurrent miscarriage. *Fertil Steril* 1994;62:989–96.
- Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol* 1935;29:181–91.
- Homburg R. Polycystic ovary syndrome from gynecological curiosity to multisystem endocrinopathy. *Hum Reprod* 1996;11:29–39.
- Franks S. Polycystic ovary syndrome. *N Engl J Med* 1995;333:853–61.
- Dor J, Itzkowicz DJ, Mashiach S, Lunenfeld B, Serr DM. Cumulative conception rates following gonadotropin therapy. *Am J Obstet Gynecol* 1980;136:102–5.

20. Okon MA, Laird SM, Tuckerman EM, Li TC. Serum androgen levels in women who have recurrent miscarriages and their correlation with markers of endometrial function. *Fertil Steril* 1998;69:682–90.
21. Tulppala M, Stenman UH, Cacciatori B, Ylikorkala O. Polycystic ovaries and levels of gonadotrophins and androgens in recurrent miscarriage: prospective study in 50 women. *Br J Obstet Gynaecol* 1993;100:348–52.
22. Maliqueo M, Clementi M, Gabler F, Johnson MC, Palomino A, Sir-Petermann T, et al. Expression of steroid receptors and proteins related to apoptosis in endometria of women with polycystic ovary syndrome. *Fertil Steril* 2003;80:812–9.
23. Bonhoff A, Naether O, Johannisson E. Effects of clomiphene citrate stimulation on endometrial structure in infertile women. *Hum Reprod* 1996;11:844–9.
24. Sereepapong W, Suwajanakorn S, Triratanachat S, Sampatanukul P, Pruksananonda K, Boonkasemsanti W, et al. Effects of clomiphene citrate on the endometrium of regularly cycling women. *Fertil Steril* 2000;73:287–91.
25. Palomino WA, Fuentes A, Gonzalez RR, Gabler F, Boric MA, Vega M, et al. Differential expression of endometrial integrins and progesterone receptor during the window of implantation in normo-ovulatory women treated with clomiphene citrate. *Fertil Steril* 2005;83:587–93.
26. Bazaes RA, Salazar TE, Pittaluga E, Pena V, Alegria A, Iniguez G, et al. Glucose and lipid metabolism in small for gestational age infants at 48 hours of age. *Pediatrics* 2003;111:804–9.
27. Codner E, Okuma C, Iniguez G, Boric MA, Avila A, Johnson MC, et al. Molecular study of the 3 beta-hydroxysteroid dehydrogenase gene type II in patients with hypospadias. *J Clin Endocrinol Metab* 2004;89:957–64.
28. Adams J, Polson DW, Franks S. Prevalence of polycystic ovaries in women with anovulation and idiopathic hirsutism. *BMJ* 1986;293:355–9.
29. Noyes RN, Hertig AT, Rock J. Dating the endometrial biopsy. *Fertil Steril* 1950;1:3–25.
30. Nessler-Menardi C, Jotova I, Culig Z, Eder IE, Putz T, Bartsch G, et al. Expression of androgen receptor coregulatory proteins in prostate cancer and stromal-cell culture models. *Prostate* 2000;45:124–31.
31. Kurebayashi J, Otsuki T, Kunisue H, Tanaka K, Yamamoto S, Sonoo H. Expression levels of estrogen receptor α , estrogen receptor β , coactivators and corepressors in breast cancer. *Clin Cancer Res* 2000;6:512–8.
32. Hey NA, Meseguer M, Simon C, Smorodinsky NI, Wreschner DH, Ortiz ME, et al. Transmembrane and truncated (SEC) isoforms of MUC1 in the human endometrium and Fallopian tube. *Reprod Biol Endocrinol* 2003;1:2.
33. Tulppala M, Julkunen M, Tiitinen A, Stenman UH, Seppala M. Habitual abortion is accompanied by low serum levels of placental protein 14 in the luteal phase of the fertile cycle. *Fertil Steril* 1995;63:792–5.
34. Klentzeris LD, Bulmer JN, Seppala M, Li TC, Warren MA, Cooke ID. Placental protein 14 in cycles with normal and retarded endometrial differentiation. *Hum Reprod* 1994;9:394–8.
35. Matthews J, Gustafsson JA. Estrogen signaling: a subtle balance between ER alpha and ER beta. *Mol Interv* 2003;3:281–92.
36. Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty KS Jr. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. *J Clin Endocrinol Metab* 1988;67:334–40.
37. Lessey BA, Yeh I, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P, et al. Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. *Fertil Steril* 1996;65:477–83.
38. Lessey BA. Two pathways of progesterone action in the human endometrium: implications for implantation and contraception. *Steroids* 2003;68:809–15.
39. Meseguer M, Aplin JD, Caballero-Campo P, O'Connor JE, Martin JC, Remohi J, et al. Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod* 2001;64:590–601.