P_{450}Arom induction in isolated control endometrial cells by peritoneal fluid from women with endometriosis

Jazmin Castro, B.Sc.,[a] *Marisa Torres, B.Sc.,*[a] *Hugo Sovino, M.D.,*[a,b] *Ariel Fuentes, M.D.,*[a,b] *M. Angélica Boric, B.Sc.,*[a] *and M. Cecilia Johnson, M.Sc.*[a]

[a] Institute of Maternal and Child Research, Faculty of Medicine, University of Chile; and [b] San Borja Arriarán Clinical Hospital, Santiago, Chile

Objective: To study the effect of peritoneal fluid from women with (PF-E) and without (PF-C) endometriosis on P_{450}Arom expression in endometrial cells.
Design: Experimental study.
Setting: University research unit.
Patient(s): Forty women of reproductive age with (n = 22) or without (control; n = 18) endometriosis.
Intervention(s): Peritoneal fluid and eutopic endometrial samples were obtained during surgery from women with (n = 13 and 9, respectively) and without (n = 4 and 14, respectively) endometriosis.
Main Outcome Measure(s): Expression study for P_{450}Arom, steroid factor 1 (SF-1), chicken ovalbumin upstream transcription factor I (COUP-TFI), and COUP-TFII messenger RNA (reverse transcriptase–polymerase chain reacion) and/or protein (immunoblot) in isolated endometrial epithelial cells transfected or not with expression vector containing SF-1, COUP-TFI, or COUP-TFII complementary DNAs.
Result(s): Basal messenger RNA and/or protein expression of P_{450}Arom and SF-1 were augmented in endometriosis, and that of COUP-TF was diminished. In control cells, $(Bu)_2$cAMP and PF-E increased P_{450}Arom and SF-1 expression (but not COUP-TF expression) in a dose-dependent way, an effect not observed with PF-C, adsorbed PF-E, or 10^{-5} M indomethacin. Transfected cells confirmed these results. Any treatments modified the studied molecules in endometriosis cells.
Conclusion(s): These data indicate that molecules contained in PF-E favor an estrogenic microenvironment, suggesting a role in the etiopathogenesis of endometriosis enabling the survival, maintenance, and growth of endometrial implants in the ectopic locations. (Fertil Steril® 2010;94:2521–7. ©2010 by American Society for Reproductive Medicine.)

Key Words: P_{450}Arom, SF-1, endometriosis, peritoneal fluid, cell culture, eutopic endometrium

Endometriosis is an estrogen-dependent pathology characterized by the presence of a functional endometrium outside the uterine cavity (1, 2). Because almost all women have retrograde menstruation (3), the coexistence of a menstrual efflux removal defect in the peritoneal cavity and an aberrant expression of some molecules in eutopic and ectopic endometria has been postulated (4). The peritoneal fluid is increased in endometriosis and contains high concentrations of macrophages and monocytes, which secrete many molecules that favor the angiogenesis, viability, and adhesion of the implant (5–7).

The enzyme P_{450}Arom, which catalyzes the conversion of androgen to estrogen, is coded by *Cyp19A1*, a gene regulated by 10 tissue-specific promoters (8–10). Its abnormal expression in eutopic and ectopic endometria of women with endometriosis (11–13) is regulated in a similar manner as the ovarian gene through promoter II by steroid factor 1 (SF-1), a 67-kDa monomer that positively regulates steroidogenic genes, and negatively by the 90-kDa homodimer chicken ovalbumin upstream transcription factor (COUP-TF) (14, 15). Steroid factor 1 competes with higher affinity for the same upstream site on promoter II than COUP-TF (14). Two isoforms of COUP-TF are described: COUP-TFI mediates process on organogenesis, cellular differentiation, and homeostasis, and COUP-TFII is highly expressed in uterine epithelial cells (16).

Chicken ovalbumin upstream transcription factor I is more expressed in normal endometrium, whereas SF-1 is mainly expressed in eutopic and ectopic endometria of women with endometriosis (11, 17). Therefore, the aberrant expression of P_{450}Arom described in ectopic and in eutopic endometria would depend on the transcription factors available in the tissue (4, 9, 12, 14, 17–23).

Prostaglandin E_2 (PGE_2) is also an inducer of P_{450}Arom expression in endometriotic cells. Estrogen increases PGE_2 formation by the stimulation of cyclooxygenase type 2 (COX-2), which catalyzes

Received November 11, 2009; revised and accepted March 11, 2010; published online April 28, 2010.
J.C. has nothing to disclose. M.T. has nothing to disclose. H.S. has nothing to disclose. A.F. has nothing to disclose. M.A.B. has nothing to disclose. M.C.J. has nothing to disclose.
Supported by Grants 1040412 and 1080229 from the Fondo Nacional de Ciencias y Tecnología, Santiago, Chile.
Reprint requests: M. Cecilia Johnson, M.Sc., P.O. Box 226-3, Santiago, Chile (FAX: 56-2-424-7042; E-mail: cjohnson@med.uchile.cl).

prostanoid synthesis in uterine endothelial cells (24). Thus, a positive feedback between prostaglandins and local estrogen production is established, favoring the cell proliferation and inflammation that characterizes this disease (11, 24, 25).

Here, we study the effect of peritoneal fluid from women with endometriosis on *Cyp19A1* expression through SF-1, COUP-TFI, and COUP-TFII regulation in epithelial cell cultures from eutopic endometrium of women with and without endometriosis.

MATERIALS AND METHODS

Subjects

Eutopic endometrium was obtained by laparoscopy with a Pippelle suction curette from 9 women age, 35.3 ± 6.4 years) for peritoneal endometriosis diagnosis (endometriosis group) and 14 women without endometriosis (age, 39.2 ± 5.1 years) for tubal ligation or hysterectomy for subserous or intramural myomas (control group); the women were without hormonal treatment in the last 3 months. A piece of tissue was fixed in formalin for dating (26), and the endometria were classified as proliferative (days 5–14; 3 control and 3 endometriosis samples) or secretory (days 15–28; 11 control and 6 endometriosis samples); the rest were used for cell preparation. Peritoneal fluid (PF) was obtained during surgery from 4 women without (PF-C, one pool) or 13 with (PF-E, three pools) peritoneal endometriosis, with each pool having 4 to 5 women in the proliferative phase of the menstrual cycle. Each PF pool was aliquoted and frozen at −80°C until use. To eliminate small molecules, some PF-E aliquots were adsorbed with activated charcoal and dextran (Sigma, St Louis, MO).

This study was approved by the institutional boards of University of Chile and the Metropolitan Central Health Service of Chile, and each patient provided written, informed consent before surgery.

Cell Culture

The endometrium was washed, minced, and digested as described by Pino et al. (27). The glands were obtained as indicated previously (28). Cells of second passages were cultured until subconfluence, incubated for 24 hours in defined medium (27), and treated (24 hours) with PF-E, PF-C, adsorbed PF-E, N6-2′-O-dibutyryl cyclic adenosine 3′,5′-monophosphate [$(Bu)_2$cAMP, 10^{-6} mol/L], or indomethacin (10^{-7}–10^{-5} mol/L, COX inhibitor) added 30 minutes before the PF-E addition.

Cell Transfection

Cells were transfected with the vectors pcDNA2, pCR3.1, and pcDNA3.0 containing SF-1 (pSF-1), COUP-TFI (pCOUP-TFI), and COUP-TFII (pCOUP-TFII) complementary DNAs (cDNAs), respectively (kindly donated by Drs. Ming-Jer Tsai and Keith L. Parker). The cells, cultured as above until 60% confluence, were transitorily transfected using 6 μL GeneJammer Reagent (Stratagene, La Jolla, CA) and 2 μg of plasmid DNA mixed to up 100 μL sterile Dulbecco's Modified Eagle Media (DMEM) for 10 minutes at room temperature. The volume was completed to 1 mL of culture medium and further incubated for 5 hours at 37°C. Later, another 1 mL was added, and the incubation continued for 24 hours. As transfection control, the cells were transfected with the vector cytomegalovirus containing β-galactosidase cDNA or with empty vector p-Cis (Promega, Madison, WI). After 48 hours the cells were cultured for 24 hours in defined medium and then treated for 24 hours with 10% PF-E, $(Bu)_2$cAMP, or 10% PF-E plus 10^{-5} mol/L indomethacin.

RNA isolation and RT-PCR

Total RNA and cDNA were obtained as indicated previously (27). The primers for COUP-TFI (NM005654; 380 bp) were upstream 5′-AAGCACTACGGCCAATTCAC-3′ and downstream 5′-GCGTTCATCCTCATCGAAGT-3′; for SF-1, P_{450}Arom, and COUP-TFII amplifications were previously reported (14, 21, 29).

One microliter of cDNA was added to 24 μL of a reaction mix that included 0.625 U of Taq DNA polymerase (Invitrogen Life Techonologies, Bethesda, MD), 0.4 mmol/L of each primer, 0.25 mmol/L 2′-deoxynucleoside 5′-triphosphate mix (0.20 mmol/L for SF-1), and 3 mmol/L $MgCl_2$ (1.5 mmol/L for P_{450}Arom). The amplification was at 94°C for 60 seconds, 50°C (P_{450}Arom), 51°C (COUP-TFII), 53.3°C (SF-1), or 55°C (COUP-TFI) for 60 seconds, and 72°C for 90 seconds and repeated for 30 (COUP-TFI and COUP-TFII), 33 (SF-1), or 35 (P_{450}Arom) cycles. As internal control, 18S ribosomal RNA (30) was amplified as previously described (31). Semiquantitation of PCR products was performed by image analysis (Kodak 1D Image Analysis; Rochester, NY), and their identity was confirmed by sequencing (Macrogen, Seoul, Republic of Korea).

Protein Homogenate Preparation and Immunoblot Analysis

Cytoplasmic protein homogenized in 50 μL lysis buffer A and nuclear proteins suspended in buffer B (1:1) were prepared as described previously (32). Thirty micrograms of cytoplasmic or nuclear proteins were resolved and electrotransferred into nitrocellulose membranes (BioRad, Hercules, CA). The immunoblot was done using antibodies against SF-1 (polyclonal, 1:800; ABR Affinity BioReagents, Golden, CO.), COUP-TFI (monoclonal; 1:1,000; R&D Systems, Minneapolis, MN), P_{450}Arom (monoclonal; 1:600; Serotec, Oxford, United Kingdom), TFIIB (monoclonal, 1:500; BD Biosciences Pharmingen, Chicago, IL), and β-actin (monoclonal, 1:15,000; Sigma). The bands were visualized, captured, and analyzed as indicated previously (27) and normalized with β-actin or TFIIB for cytosolic or nuclear extracts, respectively.

Statistical Analysis

The Mann-Whitney test for continuous variables and the Kruskal-Wallis test for discrete variables when comparing more than two categories were used. A P value of $<.05$ indicates statistical significance. Results are expressed as mean ± SEM and patient ages as mean ± SD.

RESULTS

Basal mRNA and Protein

Chicken ovalbumin upstream transcription factor II messenger RNA (mRNA) was lower in the endometriosis cells than in the control cells. In contrast, the SF-1 and P_{450}Arom mRNA levels, almost undetectable in control cells, were 93% and 305% higher in endometriosis cells (Table 1).

The nuclear COUP-TFI protein content was 50% higher in control compared with endometriosis cells; in contrast, the nuclear SF-1 and the cytoplasmic P_{450}Arom proteins were almost undetectable in control cells but strongly expressed in endometriosis cells ($P<.01$) (Fig. 1).

Effect of Treatments on mRNA and Protein Expression

Only in the control cells, SF-1 mRNA levels were increased 52% and 79% by PF-E (1% and 10%, respectively), 41% by $(Bu)_2$cAMP, and 152% by the combination with 10% PF-E compared with basal levels. Similarly, P_{450}Arom mRNA levels were increased 150% and 255% by PF-E (1% and 10%), and 246% and 582% by $(Bu)_2$cAMP alone or with 10% PF-E compared with basal levels, respectively. Chicken ovalbumin upstream transcription factor I and COUP-TFII were not affected (Table 1). Control PF or adsorbed PF-E did not modify any molecule studied in either cell culture group (Table 1).

The PF-E stimulatory effect on the mRNA of SF-1 and P_{450}Arom was reduced by indomethacin in a dose-dependent way only in control cells (10^{-7} mol/L: 48% and 11%; 10^{-6} mol/L: 67% and 36%; 10^{-5} mol/L: 99% and 90%, respectively) without affecting COUP-TFI and COUP-TFII, shown only for 10^{-5} mol/L dose in Table 1.

TABLE 1

COUP-TF I, COUP-TF II, SF-1, and P_{450}Arom mRNA levels in cultures of epithelial cell of endometrium from women without and with endometriosis.

Variable	Control cells				Endometriosis cells			
	COUP-TF I	COUP-TF II	SF-1	P_{450}Arom	COUP-TF I	COUP-TF II	SF-1	P_{450}Arom
Basal	0.84 ± 0.04	0.70 ± 0.03	0.29 ± 0.04	0.22 ± 0.04	0.71 ± 0.04	0.56 ± 0.04[a]	0.56 ± 0.04[a]	0.88 ± 0.05[a]
PF-E (1%)	0.84 ± 0.05	0.71 ± 0.06	0.44 ± 0.04[b]	0.55 ± 0.05[b]	0.71 ± 0.05	0.57 ± 0.04	0.56 ± 0.46	0.91 ± 0.05
PF-E (10%)	0.84 ± 0.05	0.70 ± 0.05	0.52 ± 0.04[b]	0.78 ± 0.04[b]	0.71 ± 0.04	0.51 ± 0.05	0.56 ± 0.04	0.89 ± 0.04
$(Bu)_2$cAMP (10^{-6} M)	0.84 ± 0.09	0.70 ± 0.06	0.41 ± 0.02[b]	0.76 ± 0.03[b]	0.64 ± 0.15	0.55 ± 0.09	0.57 ± 0.05	0.82 ± 0.06
PF-E (10%) + $(Bu)_2$cAMP	0.84 ± 0.03	0.70 ± 0.13	0.73 ± 0.08[b]	1.50 ± 0.07[b]	0.60 ± 0.06	0.56 ± 0.06	0.55 ± 0.05	0.82 ± 0.04
Adsorbed PF-E (10%)	0.84 ± 0.20	0.70 ± 0.11	0.31 ± 0.10	0.25 ± 0.12	0.70 ± 0.25	0.56 ± 0.12	0.55 ± 0.12	0.87 ± 0.37
PF-C (10%)	ND	ND	0.24 ± 0.07	0.23 ± 0.08	ND	ND	ND	ND
PF-E + indomethacin (10^{-5} M)	0.81 ± 0.21	0.70 ± 0.21	0.29 ± 0.05[c]	0.27 ± 0.06[c]	0.62 ± 0.25	0.52 ± 0.17	0.55 ± 0.11	0.85 ± 0.3

Note: Reverse transcriptase–polymerase chain reaction was performed in epithelial cell cultures of endometrium from women without (control cells) and with endometriosis (endometriosis cells). The amplified cDNA was normalized with 18S ribosomal RNA levels (AU). Results are given as mean ± SEM from 12 different control and 9 endometriosis cell cultures. ND = not determined.

[a] $P<.05$ vs. control cells.
[b] $P<.05$ vs. basal.
[c] $P<.05$ vs. PF-E.

Castro. Peritoneal fluid on P_{450}Arom expression. Fertil Steril 2010.

Only in control cells, the protein content of SF-1 and P_{450}Arom, but not COUP-TFI, increased by 1% PF-E (486% and 539%), 10% PF-E (971% and 914%), $(Bu)_2$cAMP (886% and 900%), and its combination with PF-E (1,114% and 2,053%) compared with basal levels, respectively. Indomethacin almost completely prevented the PF-E stimulatory effect on protein synthesis (Fig. 2).

Results for mRNA and protein were similar for the three PF-E pools. No significant difference was observed between proliferative and secretory cells from control and endometriosis cultures (mRNA control cell data only: COUP-TFI: 0.70 ± 0.09 AU and 0.82 ± 0.08 AU; COUP-TFII: 0.77 ± 0.07 AU and 0.66 ± 0.04 AU; SF-1: 0.26 ± 0.04 AU and 0.30 ± 0.09 AU; P450Arom: 0.28 ± 0.0.8 AU and 0.221 ± 0.05 AU).

Cell Transfection

In pCOUP-TFI or pCOUP-TFII transfected cells, the COUP-TFI and COUP-TFII mRNA levels increased 51% and 64% in control cells and 20% and 112% in endometriosis cells compared with empty vector transfected cells, respectively. Similar to untransfected cells, the addition of PF-E, $(Bu)_2$cAMP, or indomethacin did not affect the COUP-TF mRNA expression (Fig. 3A).

In control cells, pSF-1 transfection increased SF-1 mRNA almost 50% and P_{450}Arom mRNA 305% compared with empty vector transfected cells. The addition of PF-E or $(Bu)_2$cAMP increased SF-1 (94% and 86%) and P_{450}Arom (82% and 46%) mRNA levels compared with basal pSF-1 transfected cells. Indomethacin almost completely blocked the PF-E stimulation on SF-1 mRNA and the pSF-1 transfection effect on P_{450}Arom mRNA levels (Fig. 3B and C).

In pCOUP-TFI transfected control cells, the presence of PF-E or $(Bu)_2$cAMP increased the P_{450}Arom mRNA level approximately 285% compared with empty vector or pCOUP-TFI transfected cells, an increase affected by indomethacin (Fig. 3C). Similar results were obtained with pCOUP-TFII transfection (data not shown).

In endometriosis cells, the pSF-1 transfection increased SF-1 and P_{450}Arom mRNA levels 33% and 31%, respectively, compared with empty vector transfected cells. However, the pCOUP-TFI transfection and the PF-E, $(Bu)_2$cAMP, or indomethacin treatments did not modify the enzyme mRNA level (data not shown).

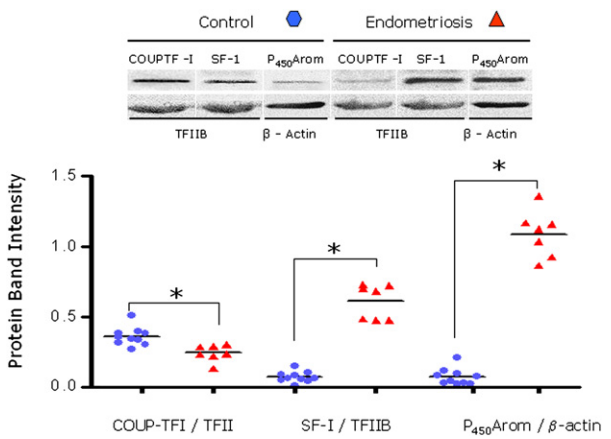
DISCUSSION

The mRNA, protein, and/or activity of P_{450}Arom have been shown in eutopic and/or ectopic endometria of women with endometriosis by several investigators using diverse techniques (4, 17, 20, 21, 23, 33); recently, Colette et al. (34) detected only low levels in ectopic endometrium. The cellular location of P_{450}Arom is controversial because some investigators found it mainly in the glandular and superficial epithelia (4, 35, 36), others in the stromal cell compartment (4, 13, 14), or in both (22, 37). In the present work, in epithelial cells isolated from the eutopic endometrium of women with and without endometriosis, we detected P_{450}Arom mRNA and protein, and moreover the negative and positive regulators COUP-TFI, COUP-TFII, and SF-1. Additionally, we analyzed the effect of peritoneal fluid obtained from these women on endometrial cells in vitro. The cells transfected with pSF-1 enabled us to gain insight into the mechanisms involved.

In control and endometriosis cell cultures, the expression patterns of P_{450}Arom, SF-1, COUP-TFI, and COUP-TFII were different according to endometrial origins but not by the stage of the menstrual cycle, confirming previous results (27, 38). The high SF-1

FIGURE 1

Protein content of COUP-TFI, COUP-TFII, SF-1, and P_{450}Arom in epithelial cell cultures of eutopic endometrium from women with and without endometriosis. Cytoplasmic and nuclear cell homogenates were obtained as described in Materials and Methods; all data were normalized with TFIIB (nuclear homogenates) or β-actin (cytoplasmic homogenates). Results are the mean ± SEM of endometrial cell cultures from women without (control; n = 10) and with (n = 7) endometriosis. *$P<.01$ vs. control.



Castro. Peritoneal fluid on P_{450}Arom expression. Fertil Steril 2010.

expression observed in endometriosis cells was concordant with a high P_{450}Arom expression that may favor local estrogen production, thus facilitating endometrial growth in ectopic locations. Although the enzyme activity was not analyzed, we previously reported a higher in vitro conversion of androgens to estrogens in eutopic endometrial explants from women with endometriosis than in those from control women (20). The high expression of both COUP-TFI and COUP-TFII found in the present study was enough to repress the *Cyp19A1* gene in control cells but apparently not enough to compete with the strong SF-1 content in the endometriosis cells; this agrees with the predominance of DNA–COUP-TF complex vs. DNA–SF-1 complex reported in isolated cells from control endometrium compared with ectopic endometrium (14).

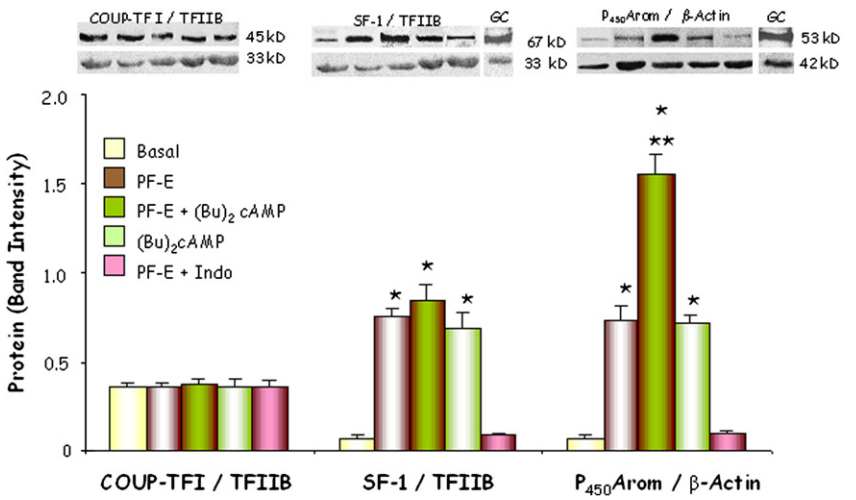
To date, few studies about peritoneal fluid have been reported, and none has evaluated its effects on P_{450}Arom, SF-1, and COUP-TF expression. The positive effect of PF-E on P_{450}Arom and SF-1 mRNA expression agrees with a report of a dose-dependent stimulatory effect of peritoneal fluid from women with mild endometriosis on eutopic endometrial cell proliferation (39); these data and the lack of PF-C effect support that some molecular components of PF-E might act in a mitogenic way to increase ectopic cell proliferation and viability. Besides, other reports did not find a proliferative effect in control cells treated with autologous PF (39, 40).

The lack of response of epithelial cells from endometriosis to PF-E in our cultures could probably be caused by the aberrant gene expression in the eutopic endometrium from these patients, as reported in several studies (1, 4, 23, 29). The baboon model for endometriosis suggests intercommunication and transference of substances between the peritoneal and uterine cavities (41). The peritoneal fluid can pass through the fallopian tubes until it reaches the endometrium by unclear mechanisms, although uterine waves present anterograde and retrograde movements (42, 43) that may contribute to the translocation of menstrual and peritoneal fluids.

Our data suggest that the molecular composition of PF-E could participate in the induction of SF-1 and P_{450}Arom expression. Recently the role of androstenedione on the up-regulation of

FIGURE 2

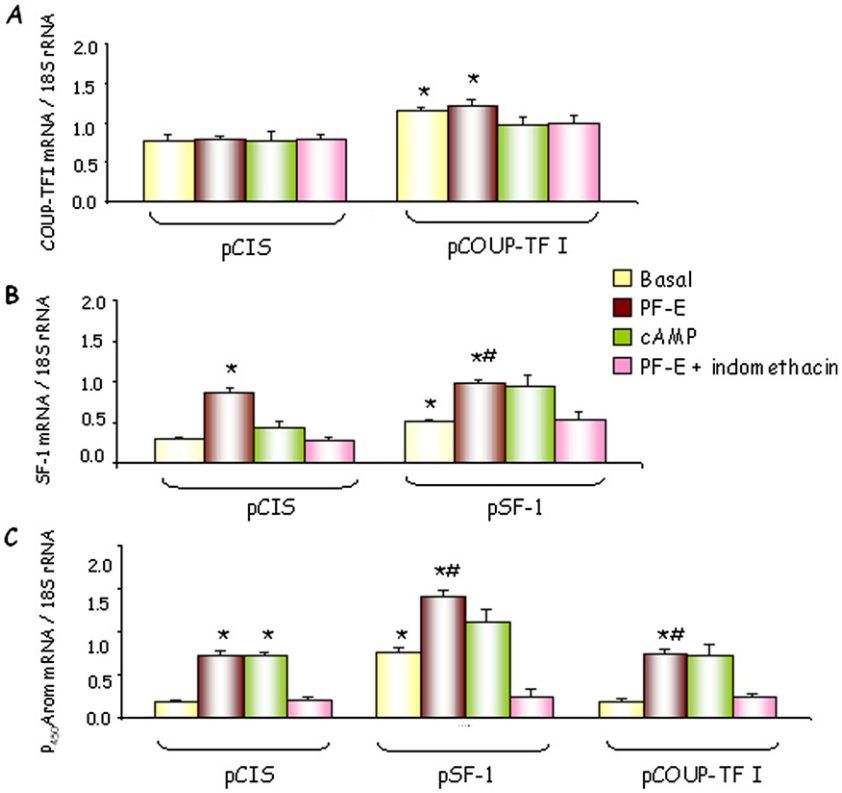
Protein content of COUP-TFI, SF-1, and P_{450}Arom in cultures of epithelial cells of endometrium from control women. Endometrial cells were incubated in the absence (basal) or presence of 10% PF-E with or without 10^{-6} mol/L $(Bu)_2$cAMP or 10^{-5} mol/L indomethacin for 24 hours. Positive control: human ovarian granulose cells (GC). All data were normalized with TFIIB (nuclear homogenates) or β-actin (cytoplasmic homogenates). Results are the mean ± SEM of endometrial cell cultures obtained from seven control women. *$P<.01$ vs. basal; **$P<.01$ vs. 10% PF-E or $(Bu)_2$cAMP alone.



Castro. Peritoneal fluid on P_{450}Arom expression. Fertil Steril 2010.

FIGURE 3

(**A**) COUP-TFI, (**B**) SF-1, and (**C**) P_{450}Arom mRNA levels in control transfected cells. Epithelial cells from control endometrium transfected with empty plasmid (pCIS), pCOUP-TFI, or pSF-1 were cultured under basal condition or with 10% PF-E, 10^{-6} mol/L $(Bu)_2$cAMP, or 10^{-5} mol/L indomethacin for 24 hours. Endometrial cDNA was amplified as described in Materials and Methods; all data were normalized with 18S ribosomal RNA levels. Results performed in duplicate are the mean ± SEM of endometrial cell cultures obtained from six control women. *$P<.01$ vs. cells transfected with empty vector (pCIS), basal; #$P<.01$ vs. each basal group.



Castro. Peritoneal fluid on P_{450}Arom expression. Fertil Steril 2010.

P_{450}Arom expression with the recruitment of SF-1 has been reported (44). We could not exclude the participation of this steroid on the PF-E induction of P_{450}Arom and SF-1 expression; androstenedione is the major aromatase substrate present in the PF-E and is also removed together with other small molecules by active charcoal–dextran treatment.

The increase of P_{450}Arom expression by pSF-1 transfection confirms its role on *Cyp19A1* gene regulation in endometrial cells from women with endometriosis. This positive effect was augmented by PF-E and $(Bu)_2$cAMP, suggesting that other regulatory factors could also be regulating this promoter, probably through the cAMP pathway by protein kinase A (PKA) activation and cAMP response element–binding protein phosphorylation, as in the ovary and endometrial cells from women with endometriosis (4, 14). Additionally, the phosphorylation of SF-1 by PKA has been reported in the regulation of *Cyp17* (45), *CYP1B1* genes (46), and in normal endometrial cells cotransfected with pSF-1 and P_{450}Arom promoter II luciferase gene reporter (14).

The fact that the stimulatory effect of PF-E on P_{450}Arom and SF-1 mRNA was blocked by indomethacin in a dose-dependent way and was almost abolished with adsorbed PF-E suggests that prostaglandins may be responsible for the PF-E stimulatory actions. An association between P_{450}Arom, prostaglandins, and COX-2 has been described in breast cancer and in eutopic and ectopic endometria (4, 25, 47, 48). Recently a positive effect of PGE_2 on steroidogenic enzymes via SF-1 in endometriotic stromal cells has been reported (49, 50). Indomethacin inhibits both COX-1 and COX-2, reducing prostaglandin synthesis, which partially explains the negative effect on SF-1 and P_{450}Arom molecules but may not explain the lack of action of the prostaglandins present at high concentrations in peritoneal fluid from these patients, indicating that other mechanisms may be involved. In this respect, inhibitory effects of indomethacin on the PKA, Akt, and mitogen-activated protein kinase phosphorylation in mouse skin and adipocyte differentiation have been reported (51, 52).

In summary, the present study showed that PF-E induces aberrant expression of P_{450}Arom and SF-1 only in control endometrial epithelial cells. Similarly, $(Bu)_2$cAMP also stimulated their expression alone and P_{450}Arom expression in an additive manner with PF-E. It is unknown whether the altered composition of PF-E is a consequence or a cause of endometriosis (5, 53). The finding that control cells are the only ones affected by PF-E supports the hypothesis that molecular factors in this fluid could be participating in the etiopathogenesis of endometriosis, favoring the survival, maintenance, and growth of endometrial implants in the ectopic locations.

Acknowledgments: The authors thank Drs. Ming-Jer Tsai (Baylor College of Medicine, Houston, Texas), Keith Parker (Texas University Southwestern Medical Center, Dallas, Texas), Sedar Bulun (Feinberg School of Medicine, Northwestern University, Chicago, Illinois), and Franco de Mayo (Baylor College of Medicine, Houston, Texas) for providing the expression vectors that contain SF-1, COUP-TFI, and COUP-TFII cDNAs; Dr. Fernando Gabler (Faculty of Medicine, University of Chile) for his role in patient endometrial dating; and all the women who generously donated tissue, without whom this study would not have been possible.

REFERENCES

1. Giudice LC, Kao L. Endometriosis. Lancet 2004;364:1789–99.
2. Barlow DH, Kennedy S. Endometriosis: new genetic approaches and therapy. Ann Rev Med 2005;56:345–56.
3. D'Hooghe TM, Debrock S. Endometriosis, retrograde menstruation and peritoneal inflammation in women and in baboons. Hum Reprod Update 2002;8:84–8.
4. Bukulmez O, Hardy DB, Carr B, Word A, Mendelson CR. Inflammatory status influences aromatase and steroid receptor expression in endometriosis. Endocrinology 2008;149:1190–204.
5. Koninckx P, Kennedy S, Barlow D. Endometriotic disease: the role of peritoneal fluid. Hum Reprod Update 1998;4:741–51.
6. Mulayim N, Arici A. The relevance of the peritoneal fluid in endometriosis-associated infertility. Hum Reprod 1999;14:67–76.
7. Kyama CM, Overbergh L, Debrock S, Valckx D, Vander Perre S, Meuleman C, et al. Increased peritoneal and endometrial gene expression of biologically relevant cytokines and growth factors during the menstrual phase in women with endometriosis. Fertil Steril 2006;85:1667–75.
8. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Corbin C, Mendelson CR. Tissue-specific promoters regulate aromatase cytochrome P450 expression. Clin Chem 1993;39:317–24.
9. Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. Pharmacol Rev 2005;57:359–83.
10. Simpson ER. Aromatase: biologic relevance of tissue-specific expression. Semin Reprod Med 2004;22:11–23.
11. Bulun SE. Mechanisms of disease. Endometriosis. N Engl J Med 2009;360:268–79.
12. Acien P, Velasco I, Gutiérrez M, Martínez-Beltrán M. Aromatase expression in endometriotic tissues and its relationship to clinical and analytical findings. Fertil Steril 2007;88:32–8.
13. Noble LS, Simpson ER, Johns A, Bulun SE. Aromatase expression in endometriosis. J Clin Endocrinol Metab 1996;81:174–9.
14. Zeitoun K, Takayama K, Michael MD, Bulun SE. Stimulation of aromatase P450 promoter (II) activity in endometriosis and its inhibition in endometrium are regulated by competitive binding of steroidogenic factor-1 and chicken ovalbumin upstream promoter transcription factor to the same cis-acting element. Mol Endocrinol 1999;13:239–53.
15. Val P, Lefrançois-Martinez AM, Veyssière G, Martinez A. SF-1 a key player in the development and differentiation of steroidogenic tissues. Nucl Recept 2003;1:8–30.
16. Kurihara I, Lee DK, Petit FG, Jeong J, Lee K, Lydon JP, et al. COUP-TFII mediates progesterone regulation of uterine implantation by controlling ER activity. PLoS Genet 2007;3:e102.
17. Yang HJ, Shozu M, Murakami K, Sumitani H, Segawa T, Kasai T, Inoue M. Spatially heterogeneous expression of aromatase P450 through promoter II is closely correlated with the level of steroidogenic factor-1 transcript in endometrioma tissues. J Clin Endocrinol Metab 2002;87:3745–53.
18. Kitawaki J, Noguchi T, Amatsu T, Maeda K, Tsukamoto K, Yamamoto T, et al. Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium. Biol Reprod 1997;57:514–9.
19. Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Honjo H. Expression of aromatase cytochrome P450 in eutopic endometrium and its application as a diagnostic test for endometriosis. Gynecol Obstet Invest 1999;48:21–8.
20. Johnson MC, Pinto C, Alves A, Palomino A, Fuentes A, Boric MA, et al. P450Arom and estrogenic microenvironment of eutopic endometria in endometriosis. Rev Med Chile 2004;132:1475–82.
21. Dheenadayalu K, Mak I, Gordts S, Campo R, Higham J, Puttemans P, et al. Aromatase P450 messenger RNA expression in eutopic endometrium is not a specific marker for pelvic endometriosis. Fertil Steril 2002;78:825–9.
22. Wölfler MM, Nagele F, Kolbus A, Seidl S, Schneider B, Huber JC, et al. A predictive model for endometriosis. Hum Reprod 2005;20:1702–8.
23. Aghajanova L, Hamilton A, Kwintkiewicz J, Vo KC, Giudice LC. Steroidogenic enzyme and key decidualization marker dysregulation in endometrial stromal cells from women with versus without endometriosis. Biol Reprod 2009;80:105–14.
24. Tamura M, Deb S, Sebastian S, Kunihiro O, Bulun SE. Estrogen up-regulates cyclooxygenase-2 via estrogen receptor in human uterine microvascular endothelial cells. Fertil Steril 2004;81:1351–6.
25. Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshelwood MM, et al. Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. J Clin Endocrinol Metab 1997;82:600–6.
26. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. Fertil Steril 1950;1:3–25.
27. Pino M, Galleguillos C, Torres M, Sovino H, Fuentes A, Boric MA, et al. Association between MMP1 and MMP9 activities and ICAM1 cleavage induced by TNF in stromal cell cultures from eutopic endometria of women with endometriosis. Reproduction 2009;138:1–12.
28. Johnson MC, Maliqueo M, Boric MA, Villavicencio A, Vantman D, Vega M. Differential in vitro actions of nitric oxide on human endometrial cell survival. Fertil Steril 2004;81:176–84.
29. Matsuzaki S, Canis M, Vaurs-Barriere C, Pouly JL, Boespflug-Tanguy O, Penault-Llorca F, et al. DNA microarray analysis of gene expression profiles in deep endometriosis using laser capture microdiddection. Mol Hum Reprod 2004;10:719–28.
30. Schmittgen TD, Zakrajsek BA. Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR. J Biochem Biophys Methods 2000;46:69–81.
31. Johnson MC, Torres M, Alves A, Bacallao K, Fuentes A, Vega M, et al. Augmented cell survival in eutopic endometrium from women with endometriosis: expression of c-myc, TGF-beta1 and bax genes. Reprod Biol Endocrinol 2005;8:45–52.
32. Ponce C, Torres M, Galleguillos C, Sovino H, Boric MA, Fuentes A, et al. Nuclear factor kappaB pathway and interleukin-6 are affected in eutopic endometrium of women with endometriosis. Reproduction 2009;137:727–37.
33. Kyama CM, Overbergh L, Mihalyi A, Meuleman C, Mwenda JM, Mathieu C, et al. Endometrial and peritoneal expression of aromatase, cytokines, and adhesion factors in women with endometriosis. Fertil Steril 2008;89:301–10.
34. Colette S, Loussse JC, Defrère S, Curaba M, Heilier JF, Van Langendonckt A, et al. Absence of aromatase protein and mRNA expression in endometriosis. Hum Reprod 2009;24:2133–41.
35. Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Fushiki S, Honjo H. Deteccion of aromatase cytochrome P-450 in endometrial biopsy specimens as a diagnostic test endometriosis. Fertil Steril 1999;72:1100–6.
36. Torres M, Lucero Y, Galleguillos C, Sovino H, Fuentes A, Gabler F, et al. Estudio de moléculas involucradas en la desregulación de la síntesis y función de estrógenos en endometrio eutópico de mujeres con endometriosis. Medicina 2007;67:80.
37. Matsuzaki S, Canis M, Pouly JL, Déchelotte PJ, Mage G. Analysis of aromatase and 17 B-hydroxysteroid dehydrogenase type 2 messenger ribonucleic acid expression in deep endometriosis and eutopic endometrium using laser capture microdissection. Fertil Steril 2006;85:308–13.
38. Utsunomiya H, Cheng YH, Lin Z, Reierstad S, Yin P, Attar E, et al. Upstream stimulatory factor-2 regulates steroidogenic factor-1 expression in endometriosis. Mol Endocrinol 2008;22:904–1.
39. Meresman GF, Barañao RI, Tenenbaum A, Singla JJ, Neuspiller NR, Rumi LS. Effect of peritoneal fluid from patients with mild and severe endometriosis on endometrial stromal cell proliferation. Arch Gynecol Obstet 1997;259:109–15.
40. Braun DP, Ding J, Dmowski WP. Peritoneal fluid-mediated enhancement of eutopic and ectopic endometrial cell proliferation is dependent on tumor necrosis factor-α in women with endometriosis. Fertil Steril 2002;78:727–32.
41. Hastings JM, Fazleabas AT. A baboon model for endometriosis: implications for fertility. Reprod Biol Endocrinol 2006;4(Suppl 1):S7–20.
42. Bulleti C, Deziegler V, Polli V. Uterine contractility during the menstrual cycle. Hum Reprod 2000;15(Suppl 1):81–9.
43. Bulleti C, De Ziegler D, Levi Setti P, Cicinelli E, Polli V, Flamigni C. The patterns of uterine contractility in normal menstruating women. From physiology to pathology. Ann N Y Acad Sci 2004;1034:64–83.
44. Bulkumez O, Hardy DB, Carr BR, Auchus RJ, Toloubeydokhti T, Word RA, et al. Androstenedione up-regulation of endometrial aromatase expression via local conversion to estrogen: potential relevance to the pathogenesis of endometriosis. J Clin Endocrinol Metab 2008;93:3471–7.
45. Lund J, Bakke M, Mellgren G, Morohashi K, Döskeland S. Transcriptional regulation of the bovine CYP17 gene by cAMP. Steroids 1997;62:43–5.
46. Zheng W, Jefcoate C. Steroidogenic factor-1 interacts with cAMP response element-binding protein to

mediate cAMP stimulation of CYP1B1 via a far upstream enhancer. Mol Pharmacol 2005;67:499–512.
47. Brueggemeier R, Quinn A, Parrett M, Joarder F, Harris R, Robertson F. Correlation of aromatase and cyclooxygenase gene expression in human breast cancer specimens. Cancer Lett 1999;140:27–35.
48. Davies G, Martin L, Sacks N, Dowsett M. Cyclooxygenase-2 (COX-2), aromatase and breast cancer: a possible role for COX-2 inhibitors in breast cancer chemoprevention. Ann Oncol 2002;13:669–78.
49. Bulun SE, Utsunomiya H, Lin Z, Yin P, Cheng YH, Pavone ME, et al. Steroidogenic factor-1 and endometriosis. Mol Cell Endocrinol 2009;300:104–8.
50. Attar E, Tokunaga H, Imir G, Yilmaz MB, Redwine D, Putman M, et al. Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. J Clin Endocrinol Metab 2009;94:623–31.
51. Petersen RK, Jorgensen C, Rustan AC, Froyland L, Muller-Decker K, Furstenberger G, et al. Arachidonic acid-dependent inhibition of adipocyte differentiation requires PKA activity and is associated with sustained expression of cyclooxygenases. J Lipid Res 2003;44:2320–30.
52. Chun KS, Akunda JK, Langenbach R. Cyclooxygenase-2 inhibits UVB-induced apoptosis in mouse skin by activating the prostaglandin E2 receptors, EP2 and EP4. Cancer Res 2007;67:2015–21.
53. Oral E, Olive D, Arici A. The peritoneal environment in endometriosis. Hum Reprod Update 1996;2:385–98.