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Effects of variations in serum estradiol concentrations on secretory endometrial development and function in experimentally induced cycles in normal women

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Abstract

Eighteen normal women underwent pituitary down-regulation with leuprolide, followed by a 10-day treatment with 0.2 mg/d transdermal estradiol (E_2) with subsequent allocation to one of two 10-day estradiol regimens plus 40 mg daily intramuscular P: supraphysiologic (0.2 mg/d transdermal E_2 mg/d vaginal micronized E_2) or subphysiologic (no exogenous E_2 treatment). Average E_2 and P in the supraphysiologic, physiologic, and subphysiologic groups were 1,175.9 pg/mL and 17.5 ng/mL, 136.9 pg/mL and 21.2 pg/mL, and 23.8 ng/mL and 22.0 ng/mL, respectively, and there were no differences between groups in endometrial histology or expression of biomarkers of receptivity.

Keywords

Endometrium; receptivity; estrogen; luteal phase; beta-3 integrin; osteopontin

Although the corpus luteum secretes both estradiol (E_2) and progesterone (P), the effects of luteal E_2 on endometrial function remain unclear (1–3). Data from experimentally designed studies as well as IVF studies have been conflicting. Some support the concept of estrogen-enhancing implantation, whereas others suggest no impact or even harm (1–11). In an effort to clarify the relevance of E_2 levels during the luteal phase, we studied the effects of variations in serum E_2 concentrations on secretory endometrial function in modeled cycles in normal women. We evaluated the functional state by assessing histologic progression as well as expression of several biomarkers suggested to have key roles in the implantation process including the β3 integrin subunit, osteopontin (OPN), and estrogen and P receptors

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(12–20). We hypothesized that wide variations in luteal phase E_2 levels would have significant impact on these measures.

Approval for this study was obtained from the institutional review board of UNC Hospitals. Subjects were assigned to treatment groups using a blinded, block-randomized design. Subjects were healthy women, aged 18 to 34 years, having normal menstrual cycles and proven midsecretory phase endometrial β3 integrin subunit expression and histologic development. Power calculation determined that nine women were required in each treatment group for comparison with previously sampled control subjects to detect a significant difference in β3 integrin expression. All subjects were down-regulated with leuprolide acetate (Lupron, TAP Pharmaceuticals, Lake Forrest, IL) and then received two 0.1-mg E_2 patches changed on alternate days for 10 days (18). This was followed by one of two investigational treatment regimens for an additional 10 days. One group received treatment with micronized E_2 (2 mg twice daily, per vagina) and P in oil (40 mg daily, intramuscularly [IM]), in addition to continuing transdermal E_2. The second group received the same exogenous P treatment regimen, but no further E_2 treatment in any form. Subjects returned for serum E_2 and P concentrations on alternate days during P treatment. On the tenth day, endometrial biopsy was performed using an endometrial Pipelle (Milex Products Inc., Chicago, IL). A portion of each specimen was fixed in formalin before paraffin embedding and staining for histologic dating; the remainder was flash frozen at −90 °C for analysis by immunohistochemistry and immunoblot.

Results obtained from study subjects were compared with those obtained in two other groups of tissue specimens obtained previously. One group of control tissue specimens derived from normally cycling women sampled on randomly assigned days after LH surge in natural cycles (representing early, mid, and late luteal phase). The second was from a group of women first suppressed by GnRH agonist and then treated with a physiologic E_2 replacement regimen of two 0.1-mg patches changed on alternate days for 10 days followed by transdermal E_2 and exogenous P in oil (40 mg daily, IM) for an additional 10 days before biopsy (18).

Sections of each tissue specimen were examined by a gynecologic pathologist (blinded to the day of sampling) and assigned a histologic date (19). Immunohistochemical staining was performed using monoclonal antibodies specific for each of the following molecular markers of endometrial function or receptivity: estrogen receptor (ER)-α, PR-A/B, PR-B, β3 integrin, and OPN. The staining intensity was assessed using the following equation: H-score = $\Sigma\ Pi\ (i + 1)$, where I = intensity (1, 2, or 3, corresponding to weak, moderate, or strong, respectively) and Pi is the percentage of stained epithelial cells for each intensity (varying from 0–100%), as previously described (20).

Immunohistochemical stains were validated by immunoblot studies using antibodies specific for β3 integrin, ER-α, and OPN. Endometrial tissue specimens from all study and control groups were homogenized and pooled by each study condition (21). One hundred micrograms of each protein sample were denatured and fractionated using one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to a polyvinylidene fluoride membrane using an electroblotter (Bio-Rad, Hercules, CA). Immunoblots were repeated using antibodies against β-actin (constitutive protein) for validation of findings.

Eighteen women were recruited and enrolled in the study and 100% of enrolled subjects completed the protocol. There were no differences between groups in age, body mass index, or race. As expected, spontaneous cycle control subjects had mean E_2 and P concentrations that were low in the early luteal phase, peaked in midluteal, and fell in the late luteal phase.

Endometrial histologic dating also was uniformly “in phase” in natural cycles. The mean serum E_2 concentration in subjects receiving physiologic E_2 treatment (136.9 pg/mL) was not different from those observed during the midluteal phase (128.3 pg/mL) in normally cycling controls. However, mean serum E_2 level was significantly higher in subjects receiving supraphysiologic E_2 treatment (1175.9 pg/mL; $P<.0001$) and lower in subjects receiving only P treatment (21.2 pg/mL; $P<.0001$). P concentrations were similar in all study groups, generally higher than midluteal phase levels in normally cycling controls, but uniformly within the range observed during the normal luteal phase. Thus, the characteristics of modeled cycles achieved targeted hormonal levels.

Immunostaining for β3 integrin subunit and OPN throughout the luteal phase in spontaneous cycles and in modeled cycles are shown in Figure 1A. Strong staining for β3 integrin and OPN, typical during the midsecretory phase of natural cycles, was observed in all study groups, and H-scores were not different between groups (Bartlett’s test for equal variance; Fig. 1B). We also observed no difference in H-scores for ER-α, PR-A, and PR-B during the midsecretory phase in natural cycles versus modeled cycles (Bartlett’s test for equal variance >0.05, data not shown). In samples derived from natural cycles, staining for the β3 integrin subunit and OPN by Western immunoblot was strong during the midsecretory phase; staining for ER-α was strong in the early secretory phase and decreased during the mid- and late secretory phase (Fig. 1C). Results from modeled cycles were similar to those for tissues from midsecretory phase of natural cycles. Expression of β-actin was similar in all tissues, demonstrating equivalent levels of total protein expression (data not shown).

It has been assumed that morphologic and functional endometrial maturation relate directly to the levels of circulating sex steroids and that abnormally low or high E_2 and P concentrations or E_2/P ratios during the luteal phase are likely to have important clinical consequences. However, data from this and our previous study of modeled cycles in normal women question that notion seriously. Previously, we demonstrated that secretory histologic endometrial development is not sensitive to variations in circulating P concentrations (18). Our current study extends those observations, revealing that histologic endometrial maturation also appears insensitive to widely varying E_2 levels spanning the range between castrate and grossly supraphysiologic concentrations. These collected observations are striking, and suggest that the endometrium can tolerate a wide range in circulating E_2 and P levels during the luteal phase without significant consequence. Although histologic endometrial dating cannot reliably define a specific luteal day, we expected the extremes in E_2 concentrations imposed in our modeled cycles to result in discernible differences in endometrial histology (22, 23). That they did not supports the notion that histologic dating is not sufficiently sensitive to be an effective analytical tool for evaluating endometrial development and function.

To further investigate the effects of varying luteal phase E_2 concentrations on the endometrium, we examined the expression of a number of putative biomarkers of endometrial function, using immunohistochemistry and immunoblotting. Interestingly, expression of the β3 integrin subunit and OPN were similar under all experimental conditions, with both methods of analysis. The expressions of β3 integrin subunit and OPN have been viewed as measures of endometrial receptivity. To the extent they are, these observations suggest that widely varying serum E_2 levels also have no significant impact on endometrial receptivity and are consistent with studies finding no benefit from luteal phase E_2 supplementation on IVF outcomes (9).

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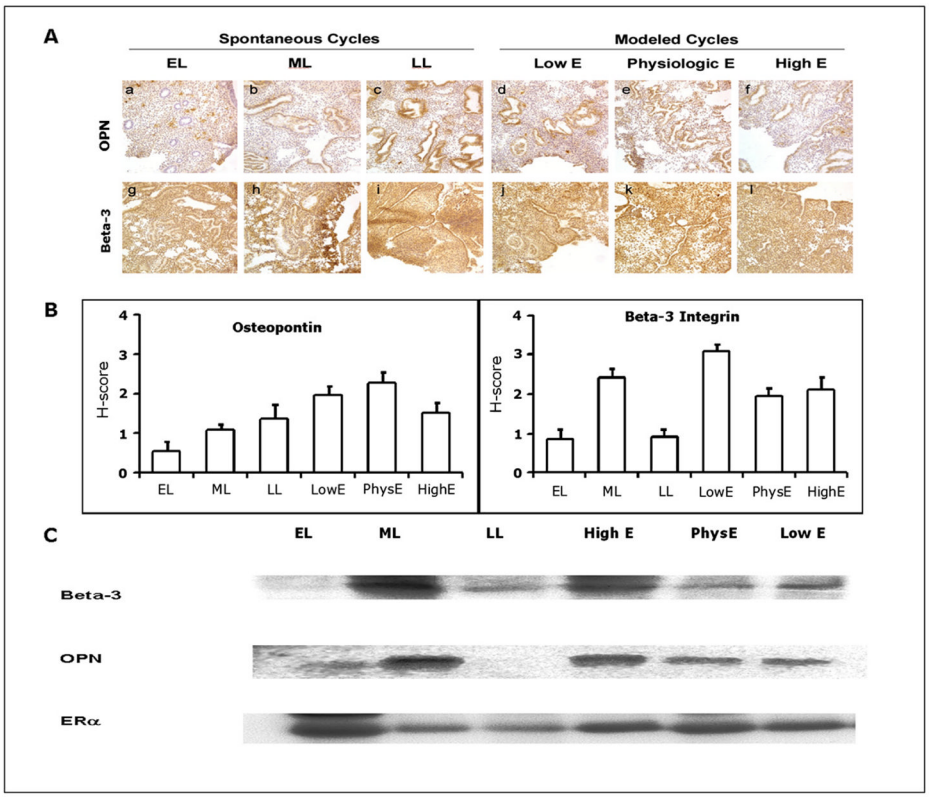

FIGURE 1.

(**A**) Photomicrographs showing immunohistochemical localization of two biomarkers of receptivity in endometrium from normal and study women. β3 integrin subunit (β3) and osteopontin (OPN) are strongly expressed in the receptive midluteal phase as well as in all study conditions. Original magnification, 20×. (**B**) H-scores of immunohistochemcial stains. ML not different than study samples for β3 and OPN; (**C**) Immunoblots for β3, OPN, and ER-α. Note strong expression of β3 and OPN in the normal midluteal samples as well as in the altered hormonal conditions. EL: early luteal, ML: midluteal, LL: late luteal, LowE: subphysiologic estrogen, PhysE: physiologic estrogen, HighE: supraphysiologic estrogen. Groll. No endometrial effect of luteal estrogen. Fertil Steril 2009.