Distribution of cyclooxygenase-1, cyclooxygenase-2, and cytosolic phospholipase A_2 in the luteal phase human endometrium and ovary

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Objective: To identify the distribution of the enzymes cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and cytosolic phospholipase A_2 ($cPLA_2$) in the human ovary and endometrium.
Design: Prospective clinical study.
Setting: Hospital-based unit for reproductive health and research laboratories.
Patient(s): Twenty-nine healthy fertile women with normal menstrual cycles.
Intervention(s): Endometrial and ovarian biopsy samples were obtained from healthy, fertile women in the luteal phase of the menstrual cycle or during caesarean section.
Main Outcome Measure(s): Pinopode formation and immunohistochemical staining of $cPLA_2$, COX-1, and COX-2.
Result(s): In the endometrium, the immunostaining of $cPLA_2$ was most intense in the luminal epithelium when pinopodes were present. The staining of both COX-1 and COX-2 was most intense in the epithelial cells, with the stroma staining positive only for COX-2. The endometrial vessels expressed COX-2 but not COX-1. The staining of COX-1 and COX-2 was intense on the surface epithelial cells on the outer lining of the ovary.
Conclusion(s): This study details the distribution of these prostaglandin synthase enzymes and emphasizes their importance for the functions of both the endometrium and the ovary. (Fertil Steril® 2005;83:156–62. ©2005 by American Society for Reproductive Medicine.)

Key Words: COX, cPLA2, endometrium, pinopodes

The cyclooxygenase (COX) enzymes exist in three isoforms; COX-1, COX-2, and the recently identified COX-3. The different COX genes are regulated by two independent systems, although the enzymatic reactions they catalyze are identical (1). These isoforms may be involved in the same processes or may co-coordinately function in other processes. There are also situations where the enzymes are redundant, with one isoform replacing the function of another isoform if one is absent (2).

Data from studies with knockout mice clearly show that loss of COX-2 has more dramatic effects than loss of COX-1 on the reproductive functions. Knockout mice lacking COX-2 are infertile (3, 4), and show multiple reproductive failures, including failures in ovulation, fertilization, implantation, and decidualization (5, 6). Studies in mice show that PGE_2 derived from COX-2 is essential for ovulation (7), and prostacyclin (PGI_2) derived from COX-2 is involved in implantation (6). However, some of the data concerning implantation recently have been questioned (8), with these investigators suggesting that implantation may only be delayed in the COX-2 knockout mice.

The eicosanoids, products of arachidonic acid metabolism, are suggested to play a crucial role during fertilization, ovulation, and implantation. Phospholipase A_2 (PLA_2) is a key regulator of eicosanoid biosynthesis, liberating arachidonic acid from lipid membranes for subsequent metabolism by COX-1 and COX-2. This forms the crucial intermediates of prostaglandin endoperoxides (PGG_2 and PGH_2) in the prostaglandin and thromboxane synthesis pathways. Cytosolic phospholipase A_2 ($cPLA_2$) is essential for implantation in the rodent; females with the null mutation in $cPLA_2$ produce small litters due to a deferred implantation window and aberrant uterine spacing of the embryos (9). However, the exact functions of prostaglandins in human ovulation, fertilization, and implantation are not completely understood.

The endometrium is only receptive during a limited period of time, often called the implantation window. This occurs approximately 7 days after ovulation and is marked by both biochemical and structural changes (10). Ultrastructural changes of the endometrium during implantation include the occurrence of pinopodes on the apical surface of the luminal

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TABLE 1

Timing and grouping of biopsy samples by morphologic evaluation of the endometrium using scanning electron microscopy.

Group	Developmental stage	Day after the LH surge	Number of biopsy samples
A	Before pinopodes	LH+0 to LH+5	6
B	With pinopodes	LH+6 to LH+9	12
C	After pinopodes	LH+10 to LH+14	7

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epithelial cells in the endometrium. Pinopodes were first identified in rodents where they were shown to absorb uterine secretions (11, 12). However, the function of pinopodes in the human endometrium is not clear.

The distribution of cPLA$_2$ in the human endometrium during the luteal phase has previously not been examined, but the presence of COX-1 and COX-2 has been shown (13, 14). The relationship of the expression of these enzymes to the presence of pinopodes, and thus to the implantation window, has not been studied.

Although the importance of prostaglandins for normal ovarian function is acknowledged, the distribution of cPLA$_2$, COX-1, and COX-2 in the human ovary had yet to be studied. We examined the localization of these three enzymes in both the endometrium and the ovary.

MATERIAL AND METHODS

Clinical Material

Endometrial biopsy samples were obtained from 25 healthy, fertile female volunteers who had a history of regular menstrual cycles (25 to 35 days). The mean age of the women was 39 years (range: 28 to 44 years).

Ovarian biopsy samples were obtained from 5 of these 25 women who were undergoing a sterilization operation during the luteal phase. A further four ovarian biopsy samples were obtained from women during caesarean sections. The mean age of these women was 33 years (range: 25 to 39 years). None of the women had used steroidal contraceptives or an intrauterine device for at least 3 months before the study, had had no miscarriages or deliveries within 1 year before the study, or had any history of pelvic inflammatory disease.

TABLE 2

Timing of biopsies of the ovary.

Group	Day	Number of biopsy samples
D	Caesarean section	4
E	LH+2	1
F	LH+6, LH+7	2
G	LH+10	1
H	LH+14	1

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The ethics committees of the Karolinska Institutet at the Karolinska Hospital and Huddinge University Hospital approved the study. Informed consent was obtained from all participating women.

Biopsies

Endometrial biopsy samples were obtained in the luteal phase from the anterior wall of the uterine cavity using a Randall curette (Stille Werner AB, Stockholm, Sweden) without dilatation of the cervix. The number and dating of endometrial biopsy samples are shown in Table 1. The day of sampling was randomly selected between days LH+1 to LH+14. Participants identified the day of the LH surge by testing their morning urine using a dipstick test (Clearplan; Unipath Ltd, Bedford, United Kingdom). The endometrial specimen was divided into two pieces. One of the pieces was processed for scanning electron microscopy and the other for immunohistochemical examination.

Samples for scanning electron microscopy were immediately fixed in a solution containing 2.5% (wt/vol) glutaraldehyde, 0.5% paraformaldehyde, 0.1 mol/L sucrose, 0.1 mol/L sodium cacodylate, and 3 mmol/L calcium chloride (pH 7.4). The endometrial biopsy samples processed for immunohistochemistry were fixed in 4% phosphate buffered formaldehyde for a maximum of 24 hours and then stored in 70% ethanol until processing. The ovarian biopsy samples were fixed in Bovin's solution for a maximum of 24 hours before dehydration and storage in 70% ethanol. The number and dating of ovarian biopsy samples are shown in Table 2.

Scanning Electron Microscopy

Samples were washed twice in a buffer containing sodium cacodylate, (0.15 mol/L), and calcium chloride (3 mmol/L, pH 7.4), and once in distilled water. The specimens were