Expression of cystic fibrosis transmembrane conductance regulator in human endometrium

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BACKGROUND: As a cAMP-regulated Cl⁻ channel, cystic fibrosis transmembrane conductance regulator (CFTR) plays a critical role in the active secretion of electrolytes and fluid in epithelial cells. Women with CFTR gene mutations are less fertile, generally assumed to be due to cervical factors. However, there is little known about CFTR protein expression in human endometrium and its possible roles in reproduction. METHODS AND RESULTS: CFTR protein and mRNA levels in human endometrium were analysed using immunohistochemical and in situ hybridization methods, respectively. Significant expression of CFTR protein was only seen in the glandular cells from late proliferative to all secretory phases, consistent with western blot analysis. High levels of CFTR mRNA were present only around the ovulatory period. In cultured glandular cells, the production of CFTR protein and mRNA was stimulated by estradiol and inhibited by progesterone. A forskolin-activated Cl⁻ current in endometrial epithelial cells with a linear I–V relationship was detected by the whole-cell patch–clamp technique. CONCLUSIONS: (i) CFTR mRNA and protein were localized in human endometrial epithelial cells and the amounts varied in a cyclic manner; (ii) CFTR expression in cultured glandular cells was up- and downregulated by estradiol and progesterone, respectively; and (iii) CFTR in human endometrium functions as a cAMP-activated Cl⁻ channel.

Key words: CFTR/chloride channel/human endometrium/menstrual cycle

Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR) is a 170 kDa glycosylated protein that functions as a cAMP-regulated Cl⁻ channel, encoded by the CFTR gene located on the long arm of chromosome 7 (Riordan et al., 1989; Bear et al., 1992). CFTR is known to present in a variety of epithelial linings, including the lungs, intestine, pancreas and salivary glands (Trezise and Buchwald, 1991; Kartner et al., 1992), as well as the female reproductive tract (Tizzano et al., 1994). Mutations within this gene lead to cystic fibrosis (CF), a severe autosomic recessive disease characterized by chronic duct obstruction in the respiratory airway and most of the exocrine glands, all due to abnormal transportation of electrolytes and fluid. CFTR gene mutations are also found to impact in human reproduction. A vast majority (97–98%) of male CF patients are infertile due to azoospermia associated with the absence of the vas deferens (Kaplan et al., 1968; Landing et al., 1969), whereas females with CF also have reduced fertility (Kopito et al., 1973), generally attributed to dense cervical mucus. It is well known that the uterine fluid has a variable ionic composition throughout the menstrual cycle (Casslén and Nilsson, 1984; Matthews et al., 1993) to provide an appropriate environment for a series of reproductive events, especially the initial phase of implantation. Endometrial epithelium, as a lining of the uterus, expresses the CFTR gene that was reported in several species including human (Trezise et al., 1993; Rochwerger and Buchwald, 1993; Mularoni et al., 1995; Sweezey et al., 1996; Chan et al., 2002). Wang’s (Wang et al., 2003) report indicated that CFTR-mediated bicarbonate secretion in mouse endometrium has a critical role in sperm capacitation, defects in which may result in reduced fertility. As a regulator of epithelial ion transport and extracellular fluid composition, the variation in CFTR expression and function is very likely to alter the volume and composition of uterine fluid to satisfy the different reproductive requirements. To date, no direct evidence of a cAMP-activated Cl⁻ channel in the human endometrium has been provided. The aim of the present study was to examine CFTR expression in human cyclic endometrial tissues and in cultured epithelial cells, as well as the functional response of CFTR as a cAMP-regulated Cl⁻ channel with the whole-cell patch–clamp technique.

Materials and methods

Immunohistochemistry and in situ hybridization methods were applied to detect the localization of CFTR protein and mRNA in human cyclic endometrial tissues and cultured endometrial epithelial
cells, respectively. The CFTR protein expression in human endometrium was analysed further by the western blot technique. The whole-cell patch–clamp technique was performed to record Cl\(^-\) current in cultured endometrial epithelial cells.

**Human endometrial tissue**

The endometrial specimens were all taken from patients, aged 23–45 years old, attending the in- and out-patient clinics at our Department, between January 1999 and March 2002. Patients were undergoing hysterectomy for uterine myoma or endometriosis, or endometrial biopsy before assisted reproductive techniques, with no sign of endometrial disease. The present study was approved by the Ethics Committee at Peking University Medical Center. All patients had regular menstrual cycles (28–35 days), and none were given hormonal therapy for at least 3 months before surgery. The specimens were fixed in 4% paraformaldehyde solution or immediately frozen in liquid nitrogen. Fresh samples in the proliferative phase of the menstrual history and histological examination according to the criteria of Noyes et al. (1975).

**Culture of human endometrial epithelial cells**

Human endometrial cell culture was performed according to a slightly modified method of Liu and Teng (1979). Briefly, the endometrial tissues were digested by collagenase (type II, 1 mg/ml, Gibco, New York, NY) in RPMI 1640 medium (Gibco) in a 37°C water bath for 1 h with shaking. After enzyme digestion, the suspension consisted of single stroma and fragments of epithelial glands. These two populations were separated by differential sedimentation at unit gravity. The collected epithelial cell clumps were cultured on coverglasses in Petri dishes (35 mm, Corning, NY). RPMI 1640 medium containing fetal bovine serum (FBS; 10%, Hyclone, UT), insulin (2 U/100 ml), penicillin and streptomycin (100 U/ml) was changed after 24 h. 17β-Estradiol (3.6 \times 10^{-8} \text{mol/l}; Sigma, St Louis, MO) and progesterone (10^{-8} \text{mol/l}; Sigma) were added to the medium (2% FBS) according to experimental requirement. The cultured cells were subjected to immunohistochemical and in situ hybridization analysis.

**Immunohistochemical detection of CFTR in human endometrium**

Paraformaldehyde-fixed and paraffin-embedded human endometria were cut into 5 µm sections, then deposited on glass slides coated with 3-aminopropyltriethoxy silane (APES; Sigma). The sections were then dewaxed with xylene, rehydrated through sequentially graded ethanol and rinsed with phosphate-buffered saline (PBS). They were subsequently incubated in methanol containing 3% H\(_2\)O\(_2\) for 10 min at room temperature to inhibit the endogenous peroxidase activity. Non-specific staining was blocked by a 30 min incubation for 10 min at room temperature. The sections were then incubated at 4°C overnight with polyclonal goat anti-human CFTR antibody (Santa Cruz Biotechnology, Santa Cruz, CA) which recognizes a CFTR epitope at the N-terminus, followed by the incubation with biotinylated secondary antibody (Maixin Bio, Fuzhou, China). The CFTR antigen was visualized by using streptavidin–peroxidase and 3,3′-diaminobenzidine (DAB; Sigma). The sections were counter-stained with Harris’ haematoxylin. Negative controls were performed by replacing the primary antibodies with non-immune serum.

The cultured endometrial glandular cells on coverglasses were also tested for CFTR expression by using the same immunohistochemical method after fixing in cold acetone for 10 min.

**Western blot analysis**

To substantiate further a cyclic dependence of CFTR protein expression in human endometria, western blot analysis was performed. The frozen human endometrial samples were lysed in a ice-cold buffer containing 50 mmol/l Tris–HCl, 150 mmol/l NaCl, 100 µmol/l phenylmethylsulphonyl fluoride (PMSF), 1% Triton X-100, 0.02% sodium azide (pH 7.4). Tissue lysates were centrifuged for 10 min at 1000 g to remove non-lysed cells; the supernatants were spun again at 12 000 g for 20 min at 4°C. Aliquots of the total proteins (100 µg) were electrophoresed in an 8% SDS–polyacrylamide gel for separation, then electroblotted to a polyvinylamide difluoride (PVDF) membrane. Following transfer, the membrane was saturated with 5% skimmed milk in Tris-buffered saline (TBS) containing 0.5% Tween-20 for 2 h, and then incubated with a 1:100 dilution of the goat anti-human CFTR antibody (Santa Cruz Biotechnology) at 4°C overnight, followed by an alkaline phosphatase-conjugated antibody (1:500 dilution) for further incubation for 1 h at 37°C. The CFTR protein was finally visualized using 4-nitroblue tetrazolium chloride (NBT) plus 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) solution.

**In situ hybridization**

The presence of CFTR mRNA in human cyclic endometria and cultured epithelial cells was detected by the in situ hybridization method described previously (Engelhardt et al., 1992). The 862 bp antisense and 978 bp sense CFTR probes were prepared from human CFTR cDNA which was subcloned into pBluescript plasmids, linearized with XbaI or HindIII, then transcribed from either the T7 or T3 promoters to generate single-stranded cRNA probes. The cRNA probes were labelled with digoxigenin (DIG) RNA labelling mix (Roche Molecular Biochemicals, Mannheim, Germany). In brief, the labelling process was carried out at 37°C for 2 h. Then, DNPase I was added to remove template DNA. The riboprobes were precipitated with LiCl and ethanol, and stored in diethylypyrocarbonate (DEPC)-treated water at −80°C. To promote riboprobe entry, the tissue sections and cultured cells fixed on coverglasses were rinsed with 1 mol/l HCl for 10 min and then digested with proteinase K (Sigma) at 37°C for 30 min, and post-fixed in 4% paraformaldehyde. The hybridization was performed at 42°C overnight by using 500 ng/ml of DIG-labelled hCFTR RNA probe. After serially washed in 2 × SSC containing 50% formamide, 2 × SSC, 0.1 × SSC at 37°C for 30 min, the sections were incubated with horse serum (1:100 dilution) for 1 h, and then with the anti-DIG antibody (1:500 dilution) conjugated with alkaline phosphatase for 1 h at room temperature. The CFTR mRNA staining was visualized by the precipitate of dark purple colour developed via the presence of NBT/BCIP solution under a light microscope.

**Whole-cell patch–clamp measurement**

Cells were plated on coverglasses at low density. Current recordings were obtained using a patch–clamp amplifier (Inbio PCII-C patch clamp, Inbio Instruments, Wuhan, China). Patch pipettes were pulled from glass on a microelectrode puller (Narishige, Japan) to a resistance of 4–6 MΩ. In the whole-cell recordings, the pipette solution contained 140 mmol/l CsCl, 2 mmol/l MgCl\(_2\), 0.5 mmol/l EGTA, 10 mmol/l glucose, 2 mmol/l Mg-ATP and 5 mmol/l HEPES (pH 7.35, 298 mOsm/kg). The bath solution contained 170 mmol/l Tris–HCl, 1 mmol/l MgCl\(_2\), 2.5 mmol/l CaCl\(_2\), 5 mmol/l HEPES and 10 mmol/l glucose (pH 7.4, 325 mOsm/kg). The membrane was voltage-clamped to a holding potential of −70 mV and stepped to levels between −80 and +80 mV at 20 mV intervals. The whole-cell...
currents were filtered at 1 kHz, and digitized. All the patch–clamp recordings were made at room temperature.

**Statistical analysis**

Immunohistochemical and *in situ* hybridization stainings in human endometrial tissues were graded visually by using an arbitrary scale (0 = absent, 1 = light, 2 = moderate, 3 = intense) by two independent observers who were blinded to clinical information. While the computerized densitometric analyses were performed by the CAMIS system (Micheal-Audi Technology, Beijing, China) to measure the presence of CFTR mRNA and protein in cultured human endometrial glandular cells. The integrated optical density (IOD) was measured according to grey scale and represents the comparative concentration of CFTR protein or mRNA in the pictures. At least five images per slide were evaluated.

Data were analysed using the Wilcoxon–Mann–Whitney U-test, analysis of variance (ANOVA) and Student’s *t*-test where appropriate. A *P*-value of < 0.05 was considered statistically significant.

**Results**

**Localization of CFTR protein and mRNA in human endometrium**

CFTR protein and mRNA were detected in the human endometrium throughout the menstrual cycle by immunohistochemistry and *in situ* hybridization, respectively. Using immunohistochemistry with a specific antibody directed against the N-terminus, CFTR was seen in the membrane and cytoplasm of luminal and glandular epithelial cells. No CFTR signal was detected in stroma cells. CFTR localized not only in the apical surface in the epithelial cells but also in the basolateral membrane. The expression pattern was cycle dependent, as illustrated in Figure 1. In the early proliferative phase, almost no staining was detected, but low or moderate staining in the mid-proliferative phase was found in most of the assayed samples. The middle and late proliferative phase showed significantly more staining than the early proliferative phase. While higher amounts of CFTR antigen were seen in glandular cells from late proliferative compared with all of the secretory phases, no statistically significant differences were found among those secretory subgroups (Table I).

Similar to the distribution of CFTR protein in human endometrium, CFTR mRNA also only located in epithelial glandular cells, but the cyclic change of CFTR mRNA was quite different from that of CFTR protein. The maximal level was only seen in the late proliferative phase, occasionally in the early secretory phase; negative or weak staining was found in the other phases, as shown in Figure 2 and Table I.

**Expression of CFTR protein and mRNA in cultured endometrial epithelial cells**

The regulation of CFTR expression by ovarian steroids was studied further *in vitro*. When cultured endometrial epithelial
cells were incubated with estradiol \(3.6 \times 10^{-8}\) mol/l) for 72h, CFTR protein and mRNA were both present at high levels. Neither progesterone (100 nM) alone nor progesterone plus estradiol \(3.6 \times 10^{-8}\) mol/l) induced the production of CFTR protein and mRNA in the cultured cells (Figures 3 and 4). The concentrations of CFTR protein and mRNA in cultured endometrial epithelial cells were analysed quantitatively by the IOD method and are summarized in Table II.

### Table I. Number of examined patients according to intensity of CFTR mRNA and protein staining in the endometrium throughout the menstrual cycle

<table>
<thead>
<tr>
<th>Period</th>
<th>Total cases</th>
<th>Intensity of CFTR mRNA staining</th>
<th>Total cases</th>
<th>Intensity of CFTR protein staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Proliferative phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Middle</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>Late</td>
<td>5</td>
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<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Secretory phase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Middle</td>
<td>5</td>
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<td>0</td>
</tr>
<tr>
<td>Late</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*aStatistically significantly lower compared with the late proliferative phase (P < 0.05; Wilcoxon–Mann–Whitney U-test).
bStatistically significantly higher compared with the early-proliferative phase (P < 0.05; Wilcoxon–Mann–Whitney U-test).

**Figure 2.** CFTR mRNA detection in human endometrium by *in situ* hybridization throughout the menstrual cycle. Signal was restricted to the glandular and luminal epithelium. No positive staining was seen in early-proliferative phase (A), but it appeared strongly in late-proliferative (B) and early-secretory endometria (C). Negative staining in the late-secretory (D) endometrium.

### CFTR protein immunoblot (western blot) analysis in human endometrium

To demonstrate the presence of CFTR protein, tissue lysates derived from cyclic human endometria were examined by western blot analysis with aspecific antibody raised against the N-terminus of CFTR. Figure 5 provided evidence for the existence of CFTR protein in endometrial tissue from late proliferative to all secretory phases, but there were no...
distinct immunoreactive bands in the tissue at early and mid proliferative phases. The specific immunoreactive bands had an estimated molecular weight of 170 kDa, the same as previous reports for CFTR (Denning et al., 1992).

**Activation of Cl\(^{-}\) channels by forskolin in cultured endometrial epithelial cells**

Figure 6 shows an example of whole-cell Cl\(^{-}\) currents activated by forskolin (FSK, 10 \(\mu\)mol/l; Sigma), an adenylate cyclase activator, in cultured human endometrial epithelial cells. A small background leak conductance was observed under basal conditions (Figure 6A). Application of FSK to the bath solution elicited an elevated current in a time- and voltage-independent manner with a linear I–V relationship (Figure 6B and D). The stimulated channel activities were suppressed by diphenylamine-2-carboxylate (DPC, 100 \(\mu\)M; Fluka, Steinheim, Germany), a CFTR channel blocker (Figure 6C). These properties were consistent with those previously reported for CFTR in other epithelial cells (Schwiebert et al., 1994; Boockfor et al., 1998).

**Discussion**

Previous reports indicated that functional CFTR expression was detected in uterine endometrium and was influenced by ovarian hormones in some species, such as mouse, rat, guinea pig and rabbit (Trezise et al., 1992; Rochwerger and Buchwald, 1993; Mularoni et al., 1995; Sweezey et al., 1996; Chan et al., 2002). Tizzano et al. (1994) tested CFTR mRNA levels in female genitalia using in situ hybridization, and found that endometrial epithelial glands expressed CFTR at high levels only after puberty. No study exists on the patterns of CFTR expression in cyclic human endometrium, or cAMP-activated Cl\(^{-}\) channel function in endometrial epithelial cells. The present work is the first to demonstrate that the expression of CFTR protein and mRNA was dependent on the menstrual cycle phase in human endometrium.

In this paper, the immunohistochemical analysis of CFTR antigen used a specific antibody directed against the N-terminus of CFTR. The results showed that CFTR immunostaining was detected in a few cases (one in five) in the early proliferative endometrium, but was seen in epithelial and glandular cells at the mid proliferative phase. The strong staining was found in the late proliferative and all secretory phases. Interestingly, we not only observed a CFTR signal localized on the apical surface of epithelial cells, but it was also detected in the basolateral membrane. This is the same as reported by Cohn (1991), but the possible role of CFTR in the basolateral membrane needs further study. Our data provide evidence that in human endometrial epithelium, CFTR protein exists...
in the uterine inner mucosal lining. Furthermore, the expression may depend on estradiol levels. Rochwerger and Buchwald (1993) reported that estrogen stimulated CFTR mRNA and protein in rat uterine endometrial cells. Our results also found low CFTR protein expression at the late proliferative phase when estradiol rises. The western blot analysis also showed a similar pattern of phase dependency in CFTR protein expression with the expected molecular size from cyclic human endometrial tissue. To confirm this finding, we cultured human endometrial epithelial glandular cells in vitro, and found that estradiol significantly increased the contents of CFTR protein in the primary cultured cells, while progesterone alone, or together with estradiol, has no effect on CFTR production in those cultured cells. Sweezy et al. (1996) observed progesterone inhibition of CFTR expression using the cultured pancreatic epithelial cell model. In our work, the effect of progesterone on CFTR protein expression is different between in vivo and in vitro studies. We observed that the CFTR protein synthesis induced by estradiol could

### Table II. The integrated optical density of CFTR mRNA and protein in cultured endometrial epithelial cells

<table>
<thead>
<tr>
<th>Study group</th>
<th>Control</th>
<th>Estradiol (36 nM)</th>
<th>Progesterone (100 nM)</th>
<th>Estradiol (36 nM) + Progesterone (100 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR mRNA</td>
<td>0.066 ± 0.009</td>
<td>0.185 ± 0.061</td>
<td>0.054 ± 0.013</td>
<td>0.050 ± 0.017</td>
</tr>
<tr>
<td>CFTR proteins</td>
<td>0.064 ± 0.011</td>
<td>0.137 ± 0.030</td>
<td>0.071 ± 0.020</td>
<td>0.070 ± 0.035</td>
</tr>
</tbody>
</table>

*Versus estradiol group, P < 0.05 by analysis of variance.

![Figure 4](https://example.com/f4.png)

**Figure 4.** The expression of CFTR mRNA was examined in cultured endometrial glandular cells by in situ hybridization, received estradiol (A), progesterone alone (B) or plus estrogen (C), (D) is control group.

![Figure 5](https://example.com/f5.png)

**Figure 5.** Western blot analysis of CFTR protein immunoprecipitated from human endometrium. lane 1. early-proliferative phase. lane 2: mid-proliferative phase; lane 3 and 4: late-proliferative phase; lane 5 and 6: early-secretory phase; lane 7: late-secretory phase; lane 8: negative control.
be abolished by adding progesterone to the culture medium simultaneously in vitro, whereas immunohistochemistry revealed a high level of CFTR protein in the whole secretory phase endometrium, coincident with the progesterone-dominated condition. This phenomenon may account for the serial action of estradiol and progesterone during the normal menstrual cycle. Once the CFTR protein is induced by estradiol stimulation during the proliferative phase, it will be resident for a long time in the cell plasma membrane. Lukacs et al. (1993) reported that the functional half-life of CFTR is >24 h; even after blocking protein synthesis for 72 h, the magnitude of the cAMP-activated depolarization remains at >40% of control conditions.

CFTR mRNA expression was also detected in cyclic endometrial tissues and cultured epithelial glandular cells using in situ hybridization. Significant levels of CFTR mRNA were seen in most of the late proliferative and a few of the early secretory endometrial tissues just around the ovulating period dominated by the estradiol peak, while CFTR mRNA was absent in late secretory phases under the effect of progesterone. This appearance conformed with what was seen in conditioned cell culture. The high level of CFTR mRNA was only detected in cultured epithelial glandular cells supplemented with estradiol, but not with progesterone, regardless of whether estradiol was present or not. The estradiol-upregulated and progesterone-downregulated CFTR mRNA in human endometrial epithelial cells is the same as reported in animal experiments (Rochwerger and Buchwald, 1993; Trezise et al., 1993; Rochwerger et al., 1994; Mularoni et al., 1995, 1996; Lee et al., 2001; Chan et al., 2002).

A disagreement is evident between the CFTR mRNA and protein expression in late secretory endometrium. The mRNA signal was negative in all four late secretory endometrial samples while continuous expression of CFTR proteins has been detected in epithelial cells. The discrepancy could be attributed to the comparatively long half-lives of CFTR protein. On the other hand, it is also possible that a lower sensitivity of our in situ hybridization methods limits our detection. Mularoni et al. (1996) detected the continuous CFTR mRNA expression in human endometrium using

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**Figure 6.** Characteristics of Forskolin (FSK)-stimulated whole cell currents in a cultured human endometrial epithelial cell. Whole-cell current recordings were obtained before (A) and after (B) FSK (10 μM) stimulation or after FSK stimulation followed by DPC (100 μM) (C). Currents were elicited by voltage pulse from –80 to 80 mV, in steps of 20 mV, with a holding potential of –70 mV. (D) Corresponding I-V relationship measured at peak current, 100 ms after voltage pulse, obtained from the cell at rests (black squares), FSK stimulation alone (triangle), or FSK stimulation followed by DPC addition (white squares).
competitive RT-PCR and indicated that there was a lower level of CFTR mRNA during the secretory phase, consistent with our result.

The characteristics of CFTR function on cultured endometrial glandular cells in estradiol-supplemented medium were studied by using the whole-cell patch-clamp technique monitoring ion channel activities. Our results first revealed the elevated Cl\(^{-}\) current in human endometrium induced by FSK, an adenylate cyclase activator, and exhibited a linear I–V relationship in a time- and voltage-independent manner. We further confirmed that the cAMP-activated Cl\(^{-}\) current in cultured endometrial epithelial cells is sensitive to DPC, a chloride channel blocker. These data agreed well with CFTR features reported in other epithelial cells (Schwiebert et al., 1994; Boockfor et al., 1998).

CFTR, as a cAMP-activated Cl\(^{-}\) channel, plays a critical role in electrolyte and fluid secretion. When it was stimulated by intracellular cAMP, the channel opened and allowed Cl\(^{-}\)-efflux. The expression of functional CFTR in human endometrial epithelium in a cycle-dependent manner was thought to be associated with the composition of human uterine fluid. Twenty years ago, Casslén and Nilsson (1984) first compared the concentrations of inorganic ions between human uterine fluid and serum, and found that the concentration of potassium and calcium, but not chloride, varied cyclically, both having lower values at mid cycle than that in the proliferative and luteal phases. The permeability for cations and anions is considered an active process. The present study provided significant evidence showing the abundant CFTR mRNA and protein expressed in endometrial epithelial cells around the ovulatory period. It may facilitate sperm migration because active Cl\(^{-}\) secretion drives Na\(^{+}\) and fluid from plasma into the uterine lumen to produce the optimal electrolyte composition and sufficient fluid volume. This work is also the first to reveal that the CFTR protein, but not mRNA, exists in endometrial epithelium throughout the menstrual cycle except the early proliferative phase. The uterine electrolyte environment may vary with the cycle, but the regulation and mechanism of fluid movement across the epithelium remain poorly understood. Previous reports indicated that prostaglandin E\(_2\) may induce Cl\(^{-}\) secretion involved by CFTR to contribute to the higher Cl\(^{-}\) concentration during blastocyst implantation in the rat (Fong and Chan, 1998; Deachapunya and O’Grady, 1998). Another study of human tubal electrophysiology showed that platelet-activating factor (PAF) released by gametes or embryos could stimulate chloride ion movement across the tubal epithelium, thereby increasing the rate of production of human oviductal fluid (Downing et al., 2002). Though little is known about the electrolyte composition of human uterine fluid during the implantation window because of the difficulty in collecting samples accurately, thick and dense uterine fluid is considered to benefit implantation (Chien et al., 2002). However, as some cytokines, including interleukin-1β (IL-1β), can stimulate CFTR mRNA expression (Nakamura et al., 1992; Besancon et al., 1994; Cafferata et al., 2000), high levels of IL-1β have been observed in women with recurrent failed embryo transfer (Inagaki et al., 2003) presumed to produce excessive uterine fluid.

In addition, CFTR may act as a regulator of other channels and transporters including the amiloride-sensitive Na\(^{+}\) channel and Cl\(^{-}\)/HCO\(_3\)\(^{-}\) exchangers (Lee et al., 1999). In mouse endometrium, activation of CFTR can inhibit Na\(^{+}\) absorption (Chan et al., 2001). A study by Wang et al. (2003) demonstrated that CFTR in mouse endometrium can mediate bicarbonate secretion directly, defects in which may result in impaired sperm capacitation and decreased fertilizing capacity. Variable CFTR expression was detected in human endometrium; the absence of CFTR-mediated bicarbonate secretion, as well as the thick cervical mucus, might also account for the lower female fertility in CF. The effect of CFTR on implantation, however, is less clear.

In conclusion, in the present study, we found that CFTR mRNA and protein were localized in human endometrial epithelial cells and the amounts varied in a cyclic manner, CFTR expression in cultured glandular cells was up- and down-regulated by estradiol and progesterone, respectively, and that CFTR in human endometrium functions as a cAMP-activated Cl\(^{-}\) channel. Further research into CFTR regulation may improve our knowledge about human reproductive physiology and pathophysiology, which may provide better treatments in some cases of infertility, such as defective uterine bicarbonate secretion.

Acknowledgements

The authors wish to thank Professor Bo Zhang for technical assistance and Dr Bonnie Olsen of North Carolina University for providing pBluescript plasmids containing human CFTR cDNA.

References


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Submitted on March 19, 2004; accepted on August 11, 2004