Effect of mifepristone and levonorgestrel on expression of steroid receptors in the human Fallopian tube

A.Christow, X.Sun and K.Gemzell-Danielsson 1

Division of Obstetrics and Gynecology, Department of Woman and Child Health, Karolinska Hospital, S-171 76 Stockholm, Sweden

1To whom correspondence should be addressed. E-mail: kristina.gemzell@kbh.ki.se

It is likely that mifepristone or levonorgestrel in the future will find extended use for contraceptive purposes. It is therefore essential to characterize the modes of action of these compounds. To assess the effect on the human Fallopian tube, 24 women with regular menstrual cycles and proven fertility, admitted to the hospital for voluntary sterilization by laparoscopic technique, were randomly allocated to a control or one of two treatment groups. Treatments were given with either a single dose of 200 mg mifepristone or 0.75 mg levonorgestrel in two doses 12 h apart, on day LH 2. Surgery was performed on day LH 4 to LH 6. Steroid receptor expression was analysed by immunohistochemistry, Western blot and RT–PCR. In the controls, there was a higher concentration of progesterone receptors in the stromal cells in the isthmic region than in those in the ampullar region. Treatment with mifepristone increased the progesterone receptor concentration in epithelial and stromal cells and increased the estrogen receptor concentration in epithelial cells. No effect on steroid receptor concentration was found following levonorgestrel. The contraceptive effect of post-ovulatory mifepristone has previously been considered to be dependent on an effect on the endometrium. However an effect on the Fallopian tube could contribute to alter the peri-implantation milieu influencing fertilization and embryo development.

Key words: emergency contraception/Fallopian tube/levonorgestrel/mifepristone/steroid receptors

Introduction

Progesterone plays an essential role in a number of events related to human fertility including ovulation, development of uterine endometrium, implantation and pregnancy. The activity of the Fallopian tubes and the intraluminal environment, of importance for successful fertilization and transport of the fertilized ovum to the uterine cavity, also seems to be regulated by ovarian steroids.

The influence of ovarian steroids on the Fallopian tube is likely to be mediated through their respective receptors. Steroid receptors have been found both in the human endometrium and Fallopian tube (Amso et al., 1994). The number of receptors seems to vary according to the menstrual cycle. The cyclic changes in both hormone and receptor levels are critical in determining the functional state of the human endometrium and could also be expected to be critical for tubal functions, such as capacitation of spermatozoa and tubal transport (Kervancioglu et al., 2000).

Progesterone receptor (PR) antagonists or antiprogestins, such as mifepristone act as a fertility regulator by blocking the action of progesterone. The effect depends on the time of treatment and the doses given. We have previously shown that once-monthly administration of 200 mg mifepristone on day LH+2 is a highly effective contraceptive method (Gemzell Danielsson et al., 1993). It has also been demonstrated that post-coital administration of mifepristone is a very effective method for emergency contraception. Another alternative is treatment with high doses of the progesterone analogue, levonorgestrel. Both methods have been shown to be more effective and to have significantly fewer side-effects than the standard Yuzpe treatment (Cheng et al., 2000). It is likely that mifepristone or other antiprogestins or levonorgestrel in the future will find extended use for contraceptive purposes. It is therefore essential to characterize the possible modes of action of these compounds.

In this study, the variations of estrogen receptors (ER) and PR in the Fallopian tubes of women without any hormonal treatment or treated with either mifepristone or levonorgestrel are reported. The expression of steroid receptors in different segments of the Fallopian tube are compared at the time when transport of the fertilized ovum would be expected to occur.

Materials and methods

The study included 24 women (aged 26–45 years) with regular menstrual cycles (24–35 days interval) and proven fertility (median 3 gravida, 2.5 para, range 1–9 and 0–6 respectively), admitted to the hospital for voluntary sterilization by laparoscopic technique and using the ordinary plastic ring. All women gave written informed
consent prior to inclusion and the study was approved by the Karolinska Hospital Ethics Committee. None of the women had been treated with any hormonal contraceptives for a minimum of 3 months prior to the study. The participants were randomly allocated to a control or one of two treatment groups using sealed envelopes. Women allocated to the treatment groups were given either a single dose of 200 mg mifepristone \( (n = 8) \) or 0.75 mg levonorgestrel in two doses 12 h apart \( (n = 8) \), immediately after ovulation (day LH + 2). Surgery was performed on day LH + 4 to LH + 6 in both the control and treatment groups. At surgery, the piece of the Fallopian tube surrounded by the plastic ring on both sides was excised. The biopsy from the right side was taken from the isthmic part of the tube while on the left side the biopsy was taken from the distal, ampullar part of the tube. The day of the LH peak was estimated by Clear plan \( \text{Searle Unipath, Bedford, UK} \) measurement in urine twice daily from day 10 to the day of the LH peak, performed by the women themselves. In addition, all patients collected daily morning urine samples during the cycle for analyses of estrone- and pregnane-diol-glucuronide and LH. The expression of PR and ER in the tubal biopsies was analysed by immunohistochemistry and Western blot and PR mRNA was examined by RT–PCR.

**Hormone determination**

Daily morning urine samples were analysed for estrone- and pregnane-diol-glucuronide and LH using enzyme immunoassays \( \text{Cekan et al. 1986} \). The hormones were expressed in nmol per mmol creatinine for estrone- and pregnane-diol-glucuronide and per mmol creatinine for LH \( \text{McTaff and Hunt, 1976} \). For creatinine analysis, a commercial kit \( \text{Sigma Diagnostics, St Louis, MO, USA} \) was used.

**Immunohistochemistry**

Each biopsy was immediately frozen in liquid nitrogen. Thereafter biopsies were serially frozen and sectioned to 9 \( \mu \)m using a Reichert–Jung Cryocut 1800 \( \text{Cambridge Instruments GmbH, Nussloch, Germany} \). The sections were placed on glass slides and immersed in 100% methanol for 30 s, and acetone for 3 min to complete the fixation. Thereafter, the mounted sections were wrapped in parafilm and stored at \(-70^\circ C\).

Human PR was detected in the Fallopian tube sections by using the Abbott Pgr-ICA monoclonal assay system \( \text{Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA} \) according to the instructions from the manufacturer. The sections were first incubated with a blocking reagent for 15 min in a humidified chamber at room temperature. One sample from each specimen was incubated with the primary antibody, an IgG fraction of a monoclonal (rat) antibody to human PR, and the other was incubated with the control antibody, normal non-immune rat serum (serving as a negative control) for 30 min. The slides were washed in PBS twice for 5 min each and were then incubated with goat anti-rat immunoglobulin (bridging antibody) for 30 min, followed by two further washes with PBS. Horse-radish peroxidase \( (HRP) \)-rat anti-HRP \( (PAP \text{ complex}) \) in PBS was applied to the sections for 30 min, followed by two PBS washes. The slides were flooded with freshly prepared diaminobenzidine–hydrogen peroxide solution for 6 min and rinsed with distilled water before counterstaining with haematoxylin, dehydration in alcohol and xylene and mounting. Localization of ER was performed in a similar way with the monoclonal antibody \( \text{1D5} \) \( \text{Zymed Laboratories Inc., San Francisco, CA, USA} \) and the Vectastain Elite ABC immuno-peroxidase detection system \( \text{Vector Laboratories Inc., Burlingame, CA, USA} \), which detects both ER\( _{\alpha} \) and ER\( _{\beta} \), according to the instructions of the manufacturer. Endometrial tissues served as positive controls.

Immunohistochemical staining was evaluated blindly by two independent persons, using a Zeiss light microscope at \( \times 200 \) magnification. The staining intensity was graded on a scale of 0 = 0% stained cells, 1 = faint (<25% stained cells), 2 = moderate (25–50% stained cells), 3 = strong (50–75% stained cells) and 4 = very strong (>75% stained cells).

Two specimens, one treated with mifepristone and the other treated with levonorgestrel, could not be analysed with immunohistochemistry because material was too scarce. One patient in the control group was excluded since she was found to be pregnant prior to surgery.

**Western blot**

Frozen tissues were homogenized and cells were lysed in ice-cold sample buffer \( [50 \text{mmol/l Tris–HCl, PH 7.5, 150 mmol/l NaCl, 1% Nonidet} \ (P-40, 0.5\% \text{ sodium deoxycholate, 0.1}\% \text{ sodium dodecyl sulphate} (SDS)), \text{containing a protease inhibitor cocktail, and were centrifuged at} 1200 \text{g for 10 min at} 4^\circ \text{C to remove debris. Protein concentration was determined following standard protocols and using bovine serum albumin protein standards. For electrophoresis, equal amounts of protein (50 \mu\text{g}) of each sample were loaded per well. Equal loading was verified by immunoblotting with actin antibodies (data not shown). Electrophoresis was performed by a 8% SDS–polyacrylamide gel electrophoresis under reducing conditions. Proteins were transferred to nitrocellulose membranes (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The membranes were incubated for at least 1 h at room temperature in blocking solution (10 mmol/l Tris–HCl, PH 7.5, 150 mmol/l NaCl, 0.05 \text{ Tween 20 and 5% blocking reagent; Bio-Rad Laboratories, Hercules, CA, USA}). Thereafter the membranes were incubated overnight at 4°C with 1:1000 dilution of a monoclonal anti-PR antibody (Abbott Laboratories) or a 1:1000 dilution rabbit polyclonal anti-ER\( _{\beta} \) antibody (Santa Cruz Biotechnology, Inc., Scandinavian Diagnostic Services, Philadelphia, PA, USA). Blots were washed 3\times10 \text{ min in TBST (10 mmol/l Tris–HCl, pH 7.5, 150 mmol/l NaCl, 0.05 Tween 20) and incubated with the second antibody (peroxidase-conjugated anti- mouse IgG, 1:5000 in 10 mmol/l Tris–HCl, pH 7.5, 150 mmol/l NaCl, 0.05 Tween 20 and 5% blocking reagent or peroxidase-conjugated anti-rabbit IgG, 1:5000, both from SDS, Santa Cruz Biotechnology) for 1 h at room temperature. After extensive washing, detection was carried out with ECL Western blotting detection reagents (Amersham Pharmacia Biotech AB) and Hyperfilm.**

**RNA isolation and RT–PCR**

Total tissue RNA was isolated by using Ultraspec-RNA Isolation system \( \text{Biotech Laboratories, Inc., USA} \) according to protocols provided by the manufacturer. The quality of each total RNA sample was checked and controlled by using the following steps: (i) measurement of optical density; (ii) running of a denaturing RNA gel sample was checked and controlled by using the following steps: (i) measurement of optical density; (ii) running of a denaturing RNA gel. Electrophoresis was performed with levonorgestrel, could not be analysed with immunohistochemistry because material was too scarce. One patient in the control group was excluded since she was found to be pregnant prior to surgery. The primer sequences for \( \text{PR and GADPH are given in Table I. Amplification was carried out on a Mastercycler gradient (Eppendorf–Netheler–Hinz GmbH, Hamburg, Germany). For amplification, 1/10 of cDNA reaction mixture was used in the presence of 0.1 mmol/l each dNTP. The concentrations, in 25 \mu\text{ PCR buffer were: primers, 0.5}\text{ mmol/l, MgCl}_{2}, 2\text{ mmol/l, and 2.5 IU of Taq polymerase (Eppendorf). The amplification profile consisted of 34 cycles with denaturation at} 92°C \text{ for 1 min, annealing at} 58°C \text{ for 35 s, and extension at} 72°C \text{ for 1 min.**}
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Figure 1. The expression of progesterone receptors at different localizations of human Fallopian tube in controls and after treatment with mifepristone or levonorgestrel using immunohistochemical staining. (A) Isthmic region of control sample. (B) Ampullar region of control sample (same patient as in A). (C) Isthmic region after treatment with mifepristone. (D) Ampullar region after treatment with mifepristone (same patient as in C). (E) Isthmic region after treatment with levonorgestrel. (F) Ampullar region after treatment with levonorgestrel (same patient as in E). Original magnification ×200. Scale bar = 100 μm.

Table I. Position and sequence of synthesized oligonucleotides used for PCR amplifications of PR and GADPH mRNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense primers</th>
<th>Sequence</th>
<th>Antisense primers</th>
<th>Sequence</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>1978–2001</td>
<td>GTGGCGGTTCCAAATGAAAGCCAAG</td>
<td>2690–2714</td>
<td>AATTCAACACTCAGGCCGACGGACT</td>
<td>737</td>
</tr>
<tr>
<td>GADPH</td>
<td>152–175</td>
<td>CCACCATGGGCAAATTCCATGGA</td>
<td>726–749</td>
<td>TCTAGACGCGGTCAGGTCCACC</td>
<td>598</td>
</tr>
</tbody>
</table>

1 min, and extension at 72°C for 1 min. GADPH cDNA was amplified for 28 cycles, and its level served as loading control. The PCR products were electrophoresed through a 1.5% agarose gel and visualized with ethidium bromide. A 100 bp ladder (Amersham Pharmacia Biotech) was used as a DNA standard for each gel. Fallopian tube cDNA were used in early experiments to establish the range of linearity between signal intensities and amounts of transcript in samples. The optimal PCR cycle number for each message was
chosen to yield product levels at the linear portion of the serial dilution curve. All PCR conditions were optimized for quantification of relative message contents with respect to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) product levels.

The films were scanned for measurements of integrated optical density, and the arbitrary densitometric level of respective signals was determined. The relative mRNA amounts of PR were expressed as a ratio of PR to G3DPH.

Statistics
Differences in urinary hormone concentrations between the control and treatment cycles were analysed using Student’s t-test. The unpaired two-group Mann–Whitney U-test was used for evaluating differences in PR and ER levels between the control and treatment groups. PR mRNA levels were presented as a mean ± SE. Data were analysed by Student’s t-test (paired or unpaired where relevant). P < 0.05 was considered as statistically significant.

Results
All women showed a normal, ovulatory cycle with a midcycle urinary LH peak and normal luteal phase estrone- and pregnanediol-glucuronide levels (data not shown). No significant changes occurred in cycle length, bleeding pattern or in hormonal profiles following treatment (data not shown). There was a good correlation (<1 day difference) between the preliminary dating of the LH peak based on Clear plan measurement and the hormonal levels in urine as determined by enzyme immunoassay.

Immunochemical localization of steroid receptors in the human Fallopian tube
Immunohistochemical staining for PR and ER was located to the cell nucleus in stromal and epithelial cells (Figures 1 and 2). The staining of the glandular cells was not evenly distributed but was present in some, but not all, cells. No staining could be observed in the perivascular cells. In the control group, the intensity of staining for PR in stromal cells was significantly less in the ampullar than in the isthmic parts of the Fallopian tube, while there was no difference in epithelial expression of PR (Table II).

Following treatment with mifepristone, a significant increase in PR concentration was found in both epithelial and stroma cells when compared with the control group (P < 0.05). The increase was most pronounced in the ampullar part of the Fallopian tube (P < 0.01) while the change in the isthmic part of the tube was not statistically significant. In contrast, treatment with levonorgestrel did not change PR levels significantly in any part of the tube compared with controls (Figure 1).

No significant differences in spatial expression of ER in the control group (untreated women) could be demonstrated. The ER concentration in epithelial and stromal cells did not differ significantly between the ampullar part and isthmic part of the Fallopian tube (Table III).
A significant increase in ER was found in the epithelial cells but not in the stromal cells after treatment with mifepristone (P < 0.01, total), especially in the isthmic region of the Fallopian tube (P < 0.05). Treatment with levonorgestrel did not result in any changes in ER expression.

Steroid receptor levels in the human Fallopian tube
The expression of PR at different locations of human Fallopian tube in controls and after treatment with mifepristone and levonorgestrel were also assessed by Western blot analysis. It was demonstrated that both PR-A and PR-B, but especially PR-B, were increased after treatment with mifepristone, consistent with that demonstrated by immunohistochemistry (Figure 3). In contrast, treatment with levonorgestrel did not change the PR levels significantly.

The expression of ERβ at different locations of human Fallopian tube in controls and after treatment with mifepristone and levonorgestrel is illustrated in Figure 4. Expression of ERβ showed an increase after treatment with mifepristone. Treatment with levonorgestrel did not change ERβ levels significantly compared with the controls.

Expression of progesterone receptor mRNA in the human Fallopian tube
A significantly lower level of PR mRNA was found in the ampullar part of the Fallopian tube compared with the isthmic region in the control group (P < 0.05) (Figure 5, Table IV). Following treatment with mifepristone, a significant increase in the PR mRNA level was found in both isthmic and ampullar regions (P < 0.05, total compared with control; P < 0.01). Treatment with levonorgestrel did not affect the PR mRNA level compared with the control.

Discussion
In the present study, the distribution of ER and PR in the Fallopian tube was studied on cycle days LH+4 to +6, i.e. approximately the time for the embryo, when still in the Fallopian tube, to reach the morula to blastocyst transitional stage. The PR, but not ER, were found to be differently expressed along the Fallopian tube when assessed by immunohistochemistry and RT–PCR. The finding of a spatial difference in steroid receptor expression is in contrast to earlier findings (Press et al., 1986) using immunohistochemical methods, but in accordance with another study (Amso et al., 1994) which reported spatial, differently expressed steroid receptors during the menstrual cycle. It can be speculated that this may have implications for regulation of local factors which may affect the development of the embryo during its sojourn through the Fallopian tube. The preimplanting embryo is exposed to a constantly changing, hormonally orchestrated milieu during its time in the oviduct and uterine lumen. It is likely that in the Fallopian tube, as in the uterus, secretion of local factors such as cytokines is regulated by steroidal hormones. In addition, factors including seminal components, other cytokines (from the embryo, semen or uterine fluid) and signals released from the embryo itself could be influential locally.

An understanding of the mechanism of action of contraceptive methods is essential for the development of new methods as well as for optimal use of those already available. Emergency contraception is defined as any drug or device used after unprotected intercourse to prevent an unwanted pregnancy and is thus a method which is used after coitus but before pregnancy occurs. Treatment with either levonorgestrel only
Steroid receptors in the human Fallopian tube

Figure 2. The expression of estrogen receptors at different localizations of human Fallopian tube in controls and after treatment with mifepristone or levonorgestrel using immunohistochemical staining. (A) Isthmic region of control sample. (B) Ampullar region of control sample (same patient as in A). (C) Isthmic region after treatment with mifepristone. (D) Ampullar region after treatment with mifepristone (same patient as in C). (E) Isthmic region of treatment with levonorgestrel. (F) Ampullar region after treatment with levonorgestrel (same patient as in E). Original magnification ×200. Scale bar = 100 μm.

or mifepristone has emerged as the most effective hormonal methods for emergency contraception with very low side-effects (Cheng et al., 2000). Nevertheless, the mechanism of action of these regimens in humans when used for contraceptive purposes and especially as emergency contraceptives is not fully explained.

A large number of factors have been suggested to mirror endometrial receptivity. It could be assumed that probably one of the most important is the PR concentration since many, if not all, of the local factors in the endometrium are progesterone regulated. Early luteal phase treatment with mifepristone causes changes in endometrial secretory activity, and in expression of steroid receptors, integrins and leukamia inhibitory factor at the expected time of implantation (Gemzell Danielsson et al., 1994, 1997; Marions et al., 1998). Changes also occur in the composition and amount of uterine fluid (Gemzell Danielsson and Hamberg, 1994). Once-a-month treatment with a single dose of 200 mg of mifepristone on day LH+2 has been shown to be an effective contraceptive method (Gemzell Danielsson et al., 1993). Endometrial receptivity and develop-
nounced in the endometrial epithelial cells. The effect of early regulation of endometrial PR is inhibited, being most pro-

Following treatment with mifepristone, the normal down-

progesterone receptors (Gemzell-Danielsson et al., 1994).

ET

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3) 1.0 (0–3) 1.0 (0–3) 1.0 (0–3) 1.0 (0–3)

1.0 (0–3) 1.0 (0–3) 1.0 (0–3) 1.0 (0–3)

1.0 (0–3) 1.0 (0–3) 1.0 (0–3) 1.0 (0–3)

Values are median and (range).

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Table II. The expression of progesterone receptor in Fallopian tubes after treatment with mifepristone or levonorgestrel

Table III. The expression of estrogen receptor in Fallopian tubes after treatment with mifepristone or levonorgestrel

Values are median and (range).

a,b,P < 0.05; d,e,P < 0.01.

Figure 3. Analysis of progesterone receptor (PR) expression at different locations of human Fallopian tube in controls and after treatment with mifepristone or levonorgestrel using Western blotting. (1) Isthmic region of control sample; (2) ampullar region of control sample; (3) isthmic region after treatment with mifepristone; (4) ampullar region after treatment with mifepristone; (5) isthmic region after treatment with levonorgestrel; (6) ampullar region after treatment with levonorgestrel. PR-A = PR subtype A; PR-B = PR subtype B.

Figure 4. Analysis of estrogen receptor β (ERβ) expression at different locations of human Fallopian tube in controls and after treatment with mifepristone and levonorgestrel using Western blotting. (1) Isthmic region of control sample; (2) ampullar region of control sample; (3) isthmic region after treatment with mifepristone; (4) ampullar region after treatment with mifepristone; (5) isthmic region after treatment with levonorgestrel; (6) ampullar region after treatment with levonorgestrel.

The ERβ subtype has been suggested to be responsible for down-regulation of PR in the endometrial luminal epithelium (Weihua et al., 2000). In the endometrium, ERα expression

luteal phase treatment of mifepristone in the endometrium is most pronounced at approximately cycle days LH +6 to LH +8, i.e. at the expected time of implantation (Gemzell-Danielsson, 1994; Cameron et al., 1997). On day LH +4, these changes are not yet fully apparent. Similar to the effect observed in the endometrium, this study has shown that PR increases in both the ampullar and isthmic region of the Fallopian tube following treatment with mifepristone. This increase in PR levels was significant already on days LH +4 to LH +6. Immunohistochemical staining localized the increased expression of PR to both stromal and epithelial cells. It seems likely that changes in progesterone-related events similar to those observed in the endometrium will also take place in the Fallopian tube following treatment with mifepristone, a possibility that requires further evaluation.

Exposure of monkey and rat embryos to antiprogesterone

in vitro does not cause any developmental abnormalities (Juneja and Dodson, 1990; Wolf et al., 1990; Hardy and New, 1991). Furthermore fertilization rates of human oocytes in vitro are not affected by mifepristone to monkeys (Messinis and Templeton, 1988). However, administration of mifepristone to monkeys at the post-ovulatory stage delays embryo development in the morula-to-blastocyst transition stage (Gosh et al., 1997). In rats, post-coital mifepristone causes accelerated oviductal embryonal transport, delays development beyond the morula stage and postpones endometrial receptivity (Psychoyos and Prapas, 1987). Thus the available data indicate that mifepristone has no direct effect on embryo development and that the effects seen in vivo are likely to be secondary to changes in the oviduct or uterine milieu. It is possible that these effects are mediated through the demonstrated changes in steroid receptor concentrations.

The ERβ subtype has been suggested to be responsible for down-regulation of PR in the endometrial luminal epithelium (Weihua et al., 2000). In the endometrium, ERα expression
Steroid receptors in the human Fallopian tube

Figure 5. RT–PCR products showing progesterone receptor (PR) mRNA expression. Optimization of the PCR cycle number for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (A) and PR (B). For amplification in the exponential phase of PCR, different numbers of cycles were tested for each message. Quantitative analysis of cycle dependency for the generated PCR signals revealed a strong linear relationship between cycles 24 and 30 for GAPDH \((r^2 = 0.9939)\) and between cycles 30 and 36 for PR \((r^2 = 0.9910)\). Expression of PR mRNA analysed by RT–PCR in Fallopian tube after treatment with mifepristone or levonorgestrel (C). (M) 100 bp ladder DNA marker; (1) control sample (isthmic region); (2) control sample (same patient, ampullar region); (3) treatment with mifepristone (isthmic region); (4) treatment with mifepristone (same patient, ampullar region); (5) treatment with levonorgestrel (isthmic region); (6) treatment with levonorgestrel (same patient, ampullar region); (7) negative control; (8) positive control, sample from pregnant patient’s Fallopian tube.

Table IV. The relative levels of mRNA expression for PR in Fallopian tubes and treatment with mifepristone or levonorgestrel

<table>
<thead>
<tr>
<th>Location</th>
<th>Control ((n = 5))</th>
<th>Mifepristone ((n = 5))</th>
<th>Levonorgestrel ((n = 5))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isthmic region</td>
<td>1.09 ± 0.31</td>
<td>1.67 ± 0.42(^a)</td>
<td>1.10 ± 0.43</td>
</tr>
<tr>
<td>Ampullar region</td>
<td>0.75 ± 0.37(^a)</td>
<td>1.34 ± 0.21(^a)</td>
<td>1.02 ± 0.31</td>
</tr>
<tr>
<td>Total</td>
<td>0.92 ± 0.37</td>
<td>1.50 ± 0.36(^b)</td>
<td>1.06 ± 0.35</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

\(^aP < 0.05\); \(^bP < 0.01\).

decrees in glands and stroma during the secretory phase while ER\(^\beta\) declines in glandular but not stromal cells (Critchley et al., 2001). Only ER\(^\beta\) was present in the vascular endothelial cells of the endometrium. In the present study, immunostaining for the ER was present mainly in glandular, but also in stromal, cell nuclei. The effect of mifepristone on ER was found, in contrast with the effect on PR, to be more pronounced in the isthmic region and significant only in the epithelial cells.

In an early study (Kesserti et al., 1974), it was shown that a single dose of norgestrel 0.4 mg 3–10 h post-coitus changed the composition of the uterine fluid, reduced the number of spermatozoa recovered from the uterine cavity from 3 h after treatment, caused immobilization of spermatozoa and increased viscosity of cervical mucus beginning at 9 h. Levonorgestrel pre-ovulation has also been shown to have an inhibitory effect on follicular development and ovulation (Landgren et al., 1989; Marions et al., 2001). Treatment with levonorgestrel immediately after ovulation or during the luteal phase does not seem to have an effect on endometrial development and function (Marions et al., 2001). No effect has been found on endometrial PR concentrations. The same was true for the Fallopian tube as demonstrated in the present study.

Recently it has been shown that the efficacy of levonorgestrel for emergency contraception is dependent on how soon post-coitus the treatment is given. The pregnancy rate was shown to increase from 0.5% when treatment was given 12 h after intercourse to 4.1% when given at 61–72 h post-coitus (Piaggio et al., 1999). This is also in agreement with a greater contraceptive effect of levonorgestrel on ovarian and sperm function rather than on the function of the Fallopian tube or the endometrium.

In conclusion, this study has shown that there is a spatial...
expression of PR within the human Fallopian tube with higher levels of receptors being expressed in the isthmic part than the ampullar part of the tube. This may reflect the importance of a tight regulation of the tubal microenvironment during early embryonic development and at the morula-to-blastocyst transition stage, i.e. days LH+4 to LH+6.

Following treatment with 200 mg mifepristone on cycle day LH+2, PR mRNA concentrations were significantly increased in the ampullar and isthmic region while the effect on the protein levels reached significance only in the ampullar region of the tube. Following mifepristone, there was also a significant increase in ER concentrations in epithelial cells and this was especially pronounced in the isthmic region.

No effect on PR or ER could be found following treatment with 0.75 mg levonorgestrel twice 12 h apart on LH+2 compared with control. This is consistent with previous findings of the lack of effect of levonorgestrel on endometrial PR expression. The various effects of mifepristone on reproductive functions is consistent with the high efficacy of this compound for preventing pregnancy when used as an emergency contraceptive or as a once-a-month post-ovulatory contraceptive method.

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