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Mu opioid receptor in the human endometrium: dynamics of its expression and localization during the menstrual cycle

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Objective: To study the dynamics of the expression and localization of the mu opioid receptor (MOR) in human endometrium throughout the menstrual cycle.
Design: Analysis of human endometrial samples from different menstrual cycle phases (menstrual, early/midproliferative, late proliferative/early secretory, midsecretory, and late secretory) by reverse transcription–polymerase chain reaction, Western blot, and immunohistochemistry.
Setting: Academic research laboratory.
Patient(s): Women from the Human Reproduction Unit of the Cruces University Hospital, fulfilling the following criteria: normal uterine vaginal ultrasound; absence of endometriosis, polycystic ovary syndrome, implantation failure, or recurrent miscarriage; and no history of opioid drug use.
Intervention(s): Endometrial samples of 86 women categorized into groups for the menstrual cycle phases: 12 menstrual, 21 early/midproliferative, 16 late proliferative/early secretory, 17 midsecretory, and 20 late secretory.
Main Outcome Measure(s): MOR gene and protein expression and localization in the different compartments of the human endometrium at different stages of the menstrual cycle.
Result(s): The expression of MOR mRNA and protein changed throughout the cycle in human endometrium. MOR expression increased during the proliferative phase and decreased during the secretory one. Lower values were found at menstruation, and maximum values around the time of ovulation. Small variations for each endometrial compartment were found.
Conclusion(s): The presence of MOR in human endometrium and the dynamic changes during the menstrual cycle suggest a possible role for opioids in reproduction events related to the human endometrium or endometriosis. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Mu opioid receptor, MOR, endometrium, menstrual cycle

The endogenous opioid peptides (EOPs) are derived from proopiomelanocortin (POMC), proenkephalin (PENK), and prodynorphin (PDYN) precursors and exert their effects by binding to the G-protein-coupled receptors δ-opioid receptor (DOR), κ-opioid receptor (KOR), and μ-opioid receptor (MOR) (1). EOPs are known to participate in the regulation of reproductive physiology at multiple sites (2) since opioid peptides and their precursors and receptors have been described in many of the male and female reproductive tissues. One of the best-known effects of opioid peptides on the reproductive system is their inhibitory role in the secretion of GnRH at the hypothalamic level and the tonic inhibition of the release of LH (3–5). But there is a growing body of evidence that indicates a participation of opioid peptides in the regulation of reproductive function through a direct local action within reproductive tissues (6).

One of the tissues where it is believed that the opioids are acting is the endometrium. Human endometrium is a complex tissue that regenerates and regresses with each menstrual

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cycle under hormonal control but also due to regulation by other factors like opioid peptides (7). In fact, the three opioid peptide precursors' mRNA has been described in endometrium: POMC in rat endometrial cells (6) and Ishikawa human endometrial adenocarcinoma cell line (8, 9); PENK in endometrial cells of human (10), cow (11), mouse (12), rat (6), and primate (13); and PDYN in human endometrial cells (14) and Ishikawa human endometrial cells (9). Moreover, opioid peptides such as β-endorphin and met-enkephalin have been found in the uterine fluid of the human and cow (15), and dynorphyns have been described in human and Ishikawa human endometrial cells (14), where β-endorphin has also been detected (8). Finally, DOR and KOR have been described in Ishikawa human endometrial cells, while MOR is absent (14, 16); in fact, MOR has been localized only in the uterine luminal epithelium cells of the pregnant mouse (17) and in endometriosis stromal cells (18–20). It has been reported that specific agonists for KOR bind to epithelial and stromal primary endometrial cell cultures (14, 21).

The presence of all the compounds of the opioid system in the endometrium denotes a role of this system in any of the processes holding the endometrium. On the one hand, it has been described that dynorphin, via KOR, may participate in the apoptotic processes related to endometrial tissue remodeling during early pregnancy or menstruation (21). On the other hand, it is known that estrogens and glucocorticoids suppress the secretion of endometrial β-endorphin, while the secretion of dynorphin is induced by GnRH, and that type-specific regulation of endometrial opioids suggests that each type of opioid peptide possesses a quite distinct physiological role within the uterine cavity (9). Regarding the possible role of the opioid system in implantation, it has been hypothesized that some peptides coming from PENK could locally modulate the immune response since the mRNA of PENK was dramatically increased in the vicinity of the implantation site of the pregnant mouse uterus (12). Finally, the absence of β-endorphin and met-enkephalin in the uterine fluid of postmenopausal women but the presence of both peptides during the menstrual cycle (with higher concentrations in the secretory phase than in the proliferative phase due perhaps to the stimulation by gonadal steroids) suggests a role for opioids during the menstrual cycle (15, 22). Moreover, the transient expression of KOR and MOR and the presence of PENK in the mouse myometrium could regulate the myometrial contractibility (17).

As can be seen, there is evidence to suggest that the opioid system is involved in some endometrial functions, but from our point of view there is a gap in the field, since to date there is no detailed study about MOR in the mammalian endometrium during the menstrual cycle. Therefore, the aim of the present study was to analyze the dynamics of the expression of the MOR gene and protein, as well as the localization of MOR, in human endometrium throughout the menstrual cycle.

MATERIALS AND METHODS

Ethics Statement

Written informed consent was obtained from all participants at the time of tissue collection, and ethical approval was provided by the Clinic Research Ethics Committee of the Basque Health System in the Cruces University Hospital (ethics approval no. CEIC EI4/36, 02/2015).

Human Endometrial Tissues

Endometrial tissues for this study were obtained by endometrial biopsy from 86 women ages 22–39 years with regular menstrual cycles (25–35 days) who had not undergone hormone treatment in the previous 3 months. Samples were collected from patients using a Cornier pipelle (Laboratoires CCD). All the participants were patients from the Human Reproduction Unit of the Cruces University Hospital, fulfilling the following criteria: normal uterine vaginal ultrasound; absence of endometriosis, polycystic ovary syndrome, implantation failure, or recurrent miscarriage; and no history of opioid drug use. Prophylactic antibiotics were not used. There was no case of infection or other side effects. Endometrial dating was determined histologically by an experienced pathologist (L.A.) according to the criteria of Noyes et al. (23).

These samples were categorized in groups for the different menstrual cycle phases: phase I, menstrual (days 1–5, n = 4); phase II, early to midproliferative (days 6–10, n = 7); phase III, late proliferative to early secretory (days 11–19, n = 9); phase IV, midsecretory (days 20–24, n = 7); and phase V, late secretory (days 25–28+, n = 8). Collected tissues were either snap-frozen in liquid nitrogen and stored at −80°C for protein and mRNA extraction or fixed in buffered formalin (pH 7.4) and processed to paraffin wax blocks for immunohistochemistry (IHC).

Reverse Transcription

RNA from endometrial tissue (obtained from some samples of each stage) and cerebral cortex (positive control) were isolated with the RNasy mRNA Purification Kit (Ambion). The procedure for obtaining the cDNA was performed with ImProm-II Reverse Transcription System (Promega) according to the manufacturer's instructions. Briefly, about 150 ng of RNA and random primers was heated at 65°C for 10 minutes and chilled on ice for 5 minutes. Then, after adding the reverse transcription mix, the mixture was annealed at 25°C for 5 minutes. A first-strand synthesis reaction was carried out at 55°C for 60 minutes, and the reverse transcriptase was inactivated at 70°C for 15 minutes.

Real-Time Quantitative Polymerase Chain Reaction (PCR) Analysis

Real-time quantitative reverse transcriptase (RT)-PCR was performed on 29 endometrial samples throughout the menstrual cycle; phase I (n = 5), phase II (n = 9), phase III (n = 3), phase IV (n = 6), and phase V (n = 6). Quantitative PCR was performed in three replicates with the StepOne thermocycler using a TaqMan assay (Applied Biosystems), specifically designed for recognizing MOR (Hs01053957_m1). The thermal profile for this PCR consisted of a "holding stage" of 20 seconds at 95°C and 40 cycles with two steps: 1 second at 95°C and 20 seconds at 60°C. We used GAPDH

(Hs99999903_m1) as the endogenous control gene. The quantitative amount of mRNA in each sample was determined by the $2^{-\Delta\Delta CT}$ method using GAPDH as the reference gene. The average ΔCt of the cortex samples was used as the calibrator.

Sodium Dodecyl Sulfate (SDS)/PAGE and Immunoblotting

For the immunoblotting the following endometrial samples were used: from phase I, n = 3; phase II, n = 5; phase III, n = 4; phase IV, n = 4; and phase V, n = 6. The endometrial tissue and human cerebral cortex were processed as described elsewhere (24), and the membrane fraction was collected in the SDS sample buffer (50 mmol/L Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.1% bromophenol blue). The sample of human brain cortex was kindly donated by Dr. Leyre Urigüen (University of the Basque Country, Leioa, Basque Country).

Proteins were heated at 100°C for 5 minutes and were loaded onto 12% resolving gels and separated by one-dimensional SDS/PAGE (20–30 μg of endometrial tissue per lane; gray matter of the human prefrontal cerebral cortex, 30 μg). Proteins were then transferred to PVDF membranes (Amersham Biosciences), using the Mini Trans-Blot electrophoretic transfer system (Bio-Rad Laboratories). Blotted membranes were incubated with a primary polyclonal rabbit anti-MOR antiserum (1:2,500; Chemicon International) overnight at 4°C. The antibody was validated in a previous study by our research group (25, 26). The membrane was incubated for 2 hours at room temperature with a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Affinity BioReagents) diluted 1:2,500. Immunocomplexes were detected using enhanced chemiluminescence in a Chemi Doc system (Bio-Rad). To calculate the molecular weight of each band, the software Quantity One was used. To measure the changes along the menstrual cycle, results were normalized with the positive control ACTB (1:10,000; Sigma-Aldrich).

IHC

Samples (phase I, n = 4; phase II, n = 7; phase III, n = 9; phase IV, n = 7; and phase V, n = 8) were fixed in 4% neutral buffered formalin (pH 7.4) for 24–48 hours and stored in ethanol 70% until they were embedded in paraffin wax, and 4-μm sections were obtained with a Shandon AS 325 retraction microtome. For preliminary assessment of morphology, sections were stained with hematoxylin and eosin. On the basis on this assessment, samples were dated and grouped in one of the above described five phases.

Following deparaffination and rehydration, sections were microwave heated in citrate buffer (10 mM, pH 6) for 10 minutes for the antigen retrieval. Afterwards sections were incubated with 0.3% H_2O_2 in methanol (30 minutes) to block the endogenous peroxidase activity and with normal goat serum in PBS-0.1% Triton ×100 (10 minutes) to block nonspecific binding. Sections were then incubated in anti-MOR (Abcam; 1:1,200, overnight, 4°C) followed by the biotinylated anti-rabbit (1 hour) used as the secondary antibody in conjunction with the Vectastain H Elite ABC kit (Vector Laboratories). Immunostaining was visualized with Vector nova red (Vector Laboratories; ±1 minute). Slices were dehydrated and mounted with Vectamount (Vector Laboratories). Negative controls were performed by omitting the primary antibody before addition of the secondary antibody.

All images were examined and captured using Olympus BX50 optical microscopy (Olympus Optical Co.) connected to a digital color camera (Olympus XC50).

Evaluation of protein staining intensity and distribution was assessed using the semiquantitative histological score (HSCORE) system (27) and Digital-HSCORE, modified from Fuhrich et al. (28). Briefly, for the manual HSCORE, the whole sample was analyzed under 100× magnification and the following formula was used: $\text{HSCORE} = \sum \text{Pi} \times (i + 1)$, where i is the intensity of staining and Pi the percentage of stained cells with this intensity. The staining intensity was classified by three independent observers into four arbitrary categories: no labeling (0), weak (1), moderate (2), and strong (3). The D-HSCORE was performed using the image J software, as described elsewhere (28), but values of the arbitrary categories mentioned above in the range of color intensity (0–255) were established by three independent viewers (0: 255–235, 1: 234–174, 2:113–173, 3: 112–0).

Low intraobserver ($r = 0.983$; $P < .0001$) and interobserver ($r = 0.994$; $P < .0001$) differences for HSCORE in uterine tissues have been previously reported using this technique (27).

Data Analysis

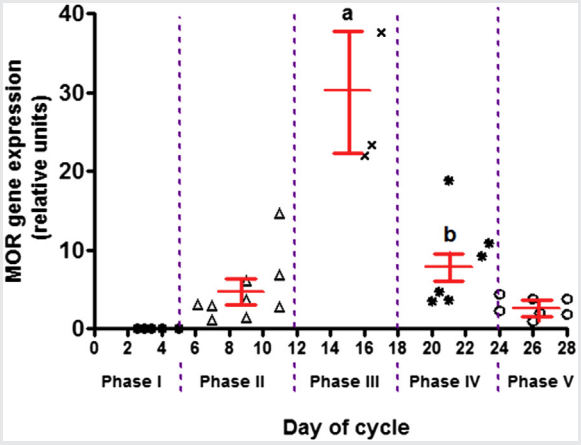
To study the changes of the MOR receptor throughout the menstrual cycle, Graph Pad Prism 5 program was used. All the results were pooled by above-mentioned menstrual cycle phases (phase I, phase II, phase III, phase IV, and phase V). The mRNA and protein expression was performed using the mean ± SEM for the one-way analysis of variance (ANOVA) tests. The post hoc test was Bonferroni. Immunohistochemical expression of MOR was analyzed with two-way ANOVA, considering the different compartments (luminal epithelium, glands, endothelium, and stroma) and different phases of the menstrual cycle. Bonferroni was used as a post hoc test. Significance was set at $P < .05$.

RESULTS

Dynamics of MOR mRNA Expression in Human Endometrium during the Menstrual Cycle

There was no expression of MOR mRNA during the first days of the menstrual cycle (phase I), but, during the proliferative phase (phase II), the expression of MOR mRNA increased until the maximal MOR mRNA expression was observed during the late-proliferative phase (phase III). When the cycle continued to the secretory phase (phase IV), the expression of MOR mRNA decreased until almost the minimum in the last days of the cycle (phase V). The expression of MOR in phase III was significantly different in comparison with all other phases; moreover, the expression of MOR in phase I was significantly different ($P < .01$) in comparison with the expression in phase IV (Fig. 1).

FIGURE 1



Expression values of MOR in human endometrium throughout the menstrual cycle by quantitative RT-PCR. Phase I: menstrual phase (days 1–5); phase II: early- and midproliferative phase (days 6–11); phase III: late-proliferative and early-secretory phase (days 12–17); phase IV: midsecretory phase (days 18–23); phase V: late-secretory phase (days 24–28). The quantitative amount of mRNA in each sample was determined by the $2^{-\Delta\Delta CT}$ method using GAPDH as the reference gene. The average ΔCt of the cortex samples was used as the calibrator. Each data point in the graph belongs to one sample, and the mean ± SEM for each phase is shown in *red*. Significant differences between phases are indicated with different *letters*. $^{a}P < .05$ vs. all other phases. $^{b}P < .05$ vs. phase I.

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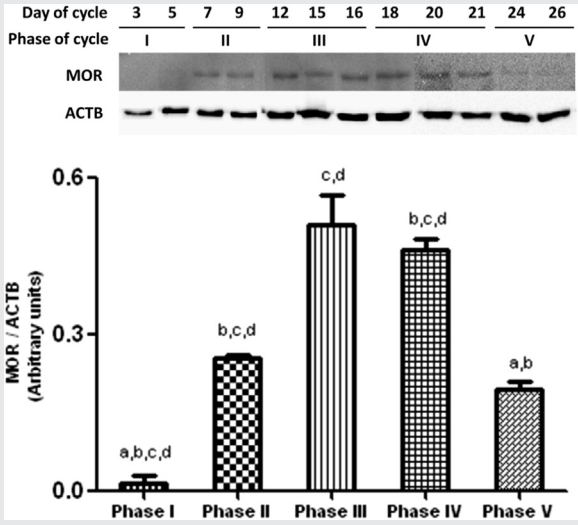
FIGURE 2



Expression of MOR in human endometrium throughout the menstrual cycle by Western blot. Phase I: menstrual phase (days 1–5); phase II: early- and mid-proliferative phase (days 6–11); phase III: late-proliferative and early-secretory phase (days 12–17); phase IV: mid-secretory phase (days 18–23); phase V: late-secretory phase (days 24–28). To determine MOR protein abundance, the same extracts were reblotted with anti-ACTB. The histogram shows the MOR expression mean ± SEM of three different experiments corrected by ACTB content. Significant differences between phases are indicated with different *letters*; $P<.01$ in all cases. Representative Western blot is shown.

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Dynamics of MOR Protein Expression in Human Endometrium during the Menstrual Cycle

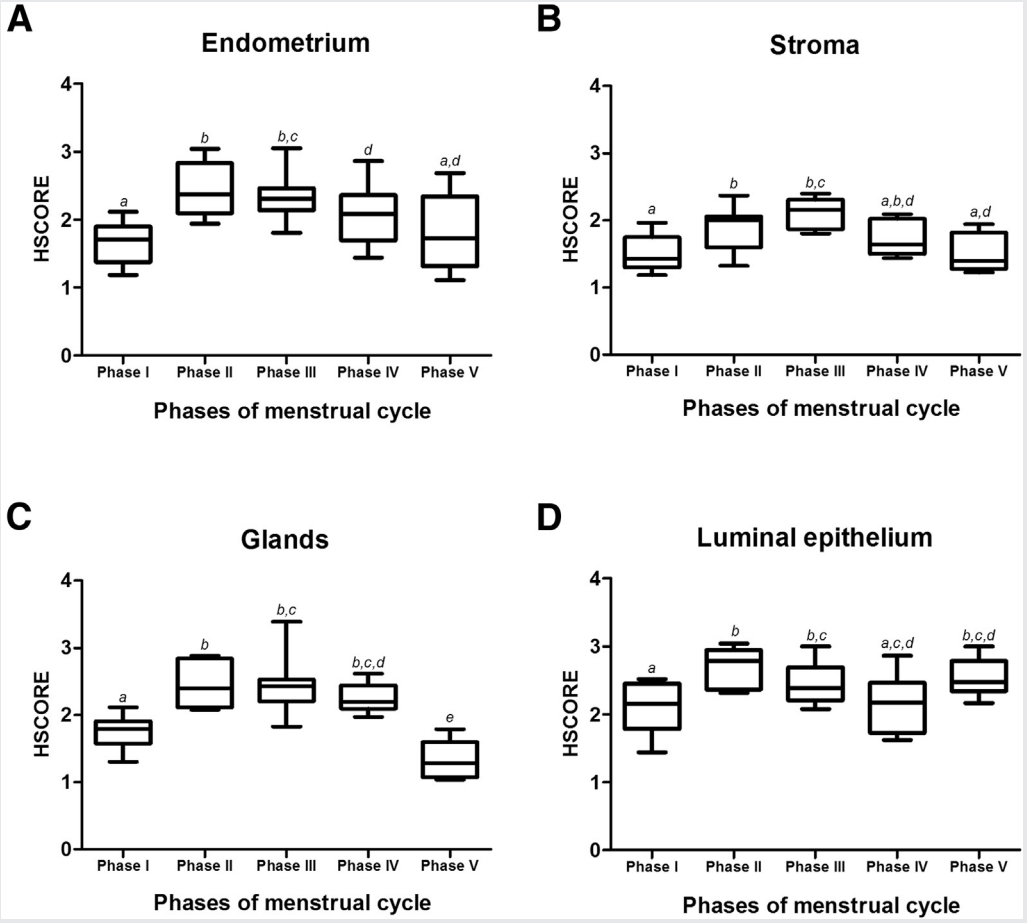
Figure 2 shows representative Western blot using human endometrium at different days of the cycle. The anti-MOR polyclonal antiserum labeled a band at about 70 kDa in endometrial tissue protein extracts, and we detected a band at about 42 kDa using anti-ACTB antiserum. The expression of MOR protein was almost zero during the first days of the menstrual cycle (phase I), but, during the proliferative phase (phase II), the immunodetection of MOR was significantly increased, reaching a peak around the late-proliferative and early-secretory phase (phase III). After that phase, in the midsecretory phase (phase IV), the immunodetection of MOR decreased significantly ($P<.01$), until it reached, in phase V, almost the value of the first phase.

MOR Immunoreactivity in Human Endometrium: Histomorphometric Analyses

Specific endometrial MOR staining was observed in all phases of the menstrual cycle (Fig. 3A). The lowest immunolabeling was detected both in the menstrual phase (phase I) and in the days before this phase, that is, in the late secretory phase (phase V). After the menstrual period, the expression of the protein increased significantly ($P<.001$, phase I vs. phase II), and MOR protein immunoreaction showed high intensity during the proliferative phase. The most intense staining was present in the days near the ovulation (late proliferative to early secretory phases or phase III). Afterwards, in the secretory period, the immunolabeling intensity decreases until it reaches the values observed in the menstrual phase. In fact, the menstrual phase immunolabeling values show significance ($P<.01$) between all the phases except the late secretory one.

All the studied endometrial regions (stroma, glands, and luminal epithelium; Fig. 3B, 3C, and 3D) were stained during the menstrual cycle, and the immunoreactive pattern that was shown for the whole endometrial tissue was maintained for all the studied areas, with some variations among them. In the stromal compartment (Fig. 3B), the staining increased significantly after menstrual period, during the proliferative phase, reaching a nadir in the late-proliferative to early-secretory period ($P<.01$ between phase I and phase II and $P<.001$ between phase I and phase III). From that moment, it showed a significant decline ($P<.01$ from phase III to IV and $P<.001$ from phase III to V). In the glands the maximum levels were reached already in the early proliferative (phase II) and maintained until the midsecretory phase (phase IV; Fig. 3C). From this moment, the staining was reduced significantly ($P<.001$), reaching the lowest observed values in the late secretory phase (phase V). It is interesting to remark how in the late secretory phase the expression of the MOR protein decrease is also significant with respect to the values found in the menstrual phase ($P<.01$). Analyzing the luminal epithelium

FIGURE 3



HSCORE values representing the immunoreactivity intensity of MOR protein staining in the whole endometrium (**A**), the stromal compartment (**B**), the epithelial glands (**C**), and the luminal surface of the endometrium (**D**). Box and whisker plots representing median, interquartile range, and minima/maxima for phase I: menstrual phase (days 1–5; n = 4); phase II: early- and mid-proliferative phase (days 6–11; n = 7); phase III: late-proliferative and early-secretory phase (days 12–17; n = 9); phase IV: midsecretory phase (days 18–23; n = 7); and phase V: late-secretory phase (days 24–28; n = 8). The different combinations of *letters* indicate significant differences between phases; $P<.01$ in all cases.

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(Fig. 3D), it can be observed that protein concentration was enhanced after menstruation, reaching maximum values at the early-proliferative phase ($P<.001$). Afterward, the decease started ($P<.01$ between phases II and IV), but from midsecretory to late secretory a new increase was observed, showing a different trend compared with the other compartments.

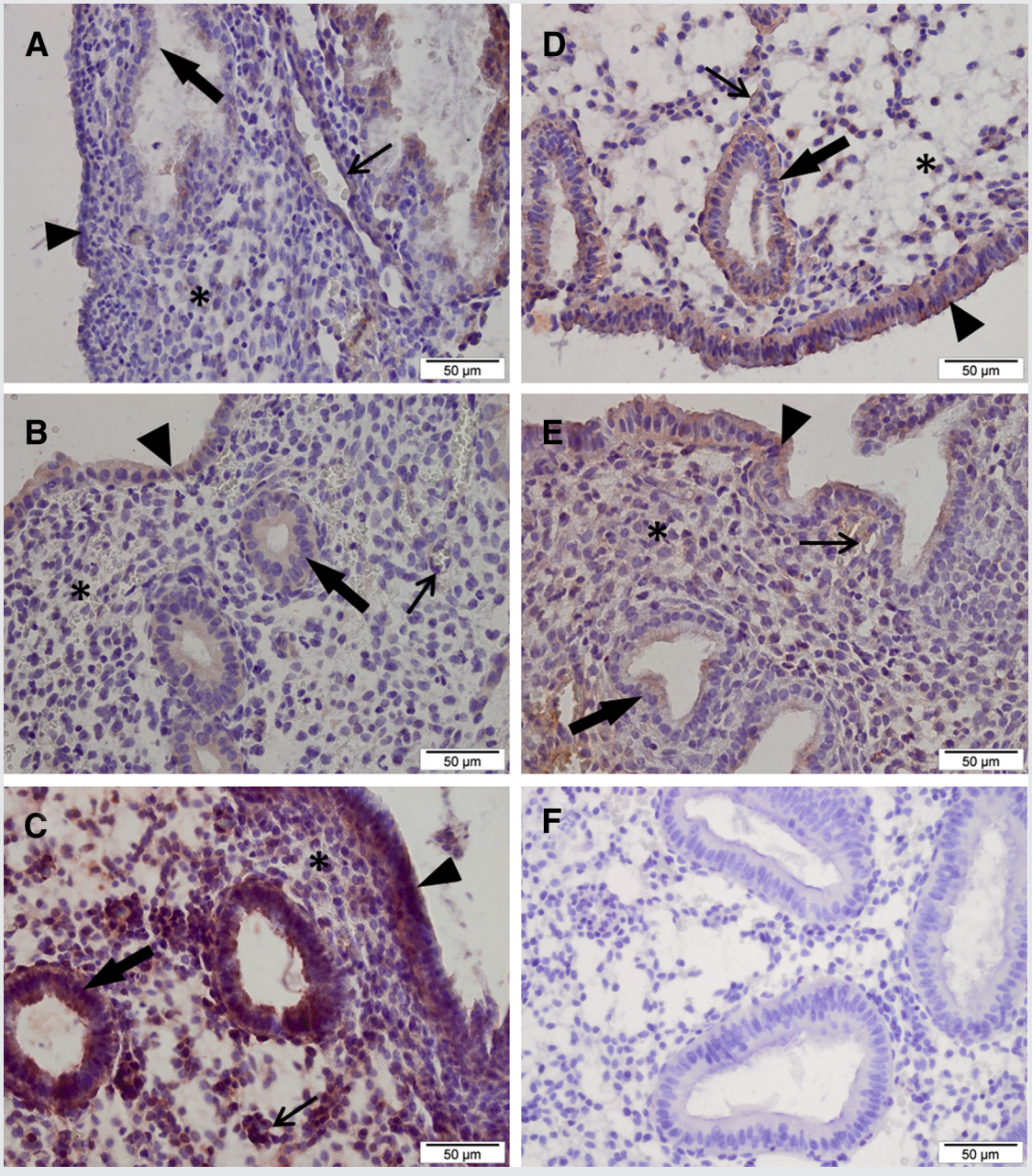
Immunohistochemical Localization of MOR in Human Endometrium

Figure 4 shows MOR distribution in the different menstrual cycle phases of the human endometrium.

Phase I. MOR expression in the menstrual endometrium (Fig. 4A): glands (arrows) and stroma (asterisk) near the basalis undergo collapse as the superficial tissue sloughs. Glands have tortuous shapes. MOR expression is weak, but it is a little bit more intense in the epithelial cells of the luminal surface of the endometrium (arrowheads) and gland epithelium (arrows). Epithelial expression is stronger in the apical region of the cells. In the glandular epithelium a light staining of some of the cells can also be observed. Most of the cells of the stromal compartment have no MOR protein expression, but those in which the staining appeared show higher intensity than the glandular ones.

Phase II. MOR expression in the early to midproliferative endometrium (Fig. 4B): glands are small and tubular, both epithelium and stroma are regenerating, and it is usual to see mitotic activity. MOR expression is weak, but it is stronger in the epithelium: in the luminal surface of the endometrium, gland epithelium (arrows), and endothelium (thin arrows). MOR expression in the gland epithelium appears in the apical and basal region of the cells. The stromal compartment labeling reaches higher values than in the previous phase, but the increase is still higher in epithelia.

FIGURE 4



MOR localization in human endometrium during the menstrual cycle. Representative pictures of immunostaining with MOR antibody at menstrual phase, phase I (day 1, **A**); early- to midproliferative phase, phase II (day 6, **B**); late-proliferative to early-secretory phase, phase III (day 14, **C**); midsecretory phase, phase IV (day 20, **D**), and late-secretory phase, phase V (day 28, **E**). MOR immunoreactivity varies during the cycle in all endometrium compartments: epithelial gland cells (*arrows*), luminal epithelium (*arrowheads*), stroma (*asterisk*), and endothelium (*thin arrows*). (**F**) Day 14. Negative control by omitting the primary antibody before addition of the secondary antibody. Scale bar, 50 μm.

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Phase III. MOR expression in the late-proliferative to early-secretory endometrium (Fig. 4C): glands are tortuous with pseudostratified nuclei and sometimes subnuclear cytoplasmic vacuoles. Stroma is rather dense, fundamentally around the vessels, and shows moderate growth; small spiral arteries and thin-walled venules are present. MOR expression is very intense in the epithelial cells both in the luminal surface and glands. We can observe how some cells were deeply stained near the apical membrane, while others show stronger staining in the basal layer (we did not find immunostaining in the nucleus of the cells; Supplemental Fig. 1). Nevertheless, in this phase, the staining in the epithelia does not seem to be higher, and in the luminal epithelium we can also appreciate a decrease compared with the previous phase.

The endothelium is still highly stained. The stroma region around vessels has also strong staining, and it reaches its maximum MOR protein expression levels during the late-proliferative phase, near the ovulation days.

Phase IV. MOR expression in the midsecretory phase (Fig. 4D): glands now are even more tortuous and show intraluminal secretion. Basal vacuoles move to the apical region, and now nuclei are basally oriented. Stroma shows edema, which will be maximal at days 21–22. MOR immunoreactivity is quite intense at the epithelial cells and shows staining at the apical and basal region of the epithelial gland cells (arrows). Endothelium (thin arrows) and stroma also show MOR immunoreactivity.

Phase V. MOR expression in the late secretory phase (Fig. 4E): after the 20th day of the cycle, the stroma changes are more evident; nonetheless, the glands become more prominent. Predecidual stromal change is now evident around spiral arteries, with many interspersed leukocytes. MOR immunoreactivity is less intense at the gland epithelial cells and shows staining at the apical region fundamentally (arrows). The luminal epithelium maintains intense staining. In the stroma, immunoreactivity is less intense, but endothelium staining is maintained (thin arrows).

DISCUSSION

The presence of the endogenous opioid system (opioid peptides, their precursors and receptors) in the mammalian endometrium was described a few years ago, and since then, a number of physiological roles have been suggested (7). With our present work, we have elucidated that MOR is expressed in human endometrium, not only at the mRNA level but also at the protein level, and, moreover, we have checked how the dynamics of expression and localization of MOR changes throughout the menstrual cycle in a coordinated way in each region of the endometrium (luminal epithelium, glands, and stroma).

To date, the unique evidence about the presence of MOR in the human endometrium has been produced in two works focused on endometriosis where the authors speak about the expression of MOR mRNA on eutopic endometrial stromal cells (18, 19). Our data confirm that the mRNA of MOR is present in human endometrium, but we have also seen how that expression follows a cycle-dependent pattern where mRNA is absent in the first days of the menstrual cycle, increases its expression during the early proliferative phase to reach a maximum at the late-proliferative phase and after, and decreases again until almost its minimum levels throughout the secretory phase. This fact could explain why in previous works performed with eutopic endometrium some patients did not show expression of MOR (18, 19), since the investigators only differentiated the patients' endometrium between the proliferative and secretory phases without taking into account the day of the cycle, while important changes are detected within these two phases. In the present work, we also have validated the expression pattern of mRNA by the immunodetection of the MOR protein, which showed a very similar pattern to that of mRNA using Western blot and IHC.

Our findings strongly contradict the widely accepted idea that MOR is not present in human endometrium, based on a study on Ishikawa human endometrial adenocarcinoma cells (16). However, the aforementioned work did not include any experiment with normal endometrial cells. So, from our point of view, in comparing our data with those of that article, two hypotheses could be made: Ishikawa cells have the phenotype of endometrial cells at the beginning or the end of menstrual cycle or MOR certainly disappears in endometrial adenocarcinoma. In any case, it seems that the use of the Ishikawa cells would not be the best option to extrapolate the results obtained from a healthy endometrial tissue model for MOR.

The cyclic upregulation and downregulation observed on MOR lead us to hypothesize that MOR has a defined role throughout the menstrual cycle but especially during the period from the midproliferative to the midsecretory phase, where the expression of MOR is the largest of the entire cycle. That idea is reinforced with the data of the works that measured the presence of EOP in endometrium: in one work, β-endorphin was not detected in the human endometrium in the proliferative phase, while it appeared in secretory phase (22). In another work, the investigators did not find many differences during the cycle in β-endorphin levels, but instead they detected significantly higher concentrations of met-enkephalin in human uterine fluid during the secretory phase in comparison with the proliferative phase, and, additionally, the concentration of met-enkephalin in the uterine fluid in superovulated cows was higher than that in control cows (15). Moreover, as would be expected, it has been described that the transcript of the precursor of met-enkephalin (PENK) increased in the proliferative phase and decreased in the secretory phase of primate and human endometrium (10, 13). Thus, it seems that in the postovulatory period, the endometrium may secrete high levels of met-enkephalin and maybe β-endorphin and that the existence of MOR could be necessary for the function of those peptides. In addition, it has been hypothesized that those peptide-level changes in the cycle could be regulated by steroid hormones. In this sense the influence of gonadal steroids on the synthesis and secretion of opioid peptides in the endometrium (15) has been suggested, and it has been also proposed that P could induce the synthesis of opioid peptides in endometrial glandular cells (22). Otherwise, the absence of β-endorphin and met-enkephalin in the uterine fluid of postmenopausal women and the recovering of EOP levels after a steroid replacement (15) make clear the important role of the opioid system during the menstrual cycle and the necessity of the hormonal regulation of this system for it to work. Even so, a reduction in endogenous opioid tone as a trigger for initiating the LH surge in proestrus rats has been also proposed (5, 29).

Although the expression pattern of mRNA and the immunodetection of the MOR protein using Western blot and IHC for the whole endometrium showed a very similar pattern, there are some differences regarding the localization of the receptor in different compartments of the endometrial biopsies. The stromal compartment is the most representative area, and the expression of MOR follows the same pattern as that of the whole endometrium, increasing during the proliferative phase and decreasing in the mid-late secretory phases, but, for example, the expression pattern of MOR

in luminal epithelium shows a new rise in the late-secretory phase. It will be interesting to check where exactly the expression of MOR increases during the proliferative phase because it seems that this event is essential to inhibiting the inflammatory process occurring during menstruation, via inhibition of cAMP (30). This kind of regulation has been described in other communication systems of endometrium, some of them very related to the opioid system as, for example, the cannabinoid system (31).

In conclusion, our data indicate that MOR is expressed in human endometrium and that its expression pattern changes during the menstrual cycle in a different manner in all the studied endometrial compartments. These findings suggest that MOR could have several functions in the complex remodeling process that endometrium undergoes every month and, therefore, in early events of reproduction. Even so, as the MOR knockout mice are able to have offspring (32), it will be interesting to elucidate what is the real role of MOR in reproduction events since this issue has been not studied in depth to date.

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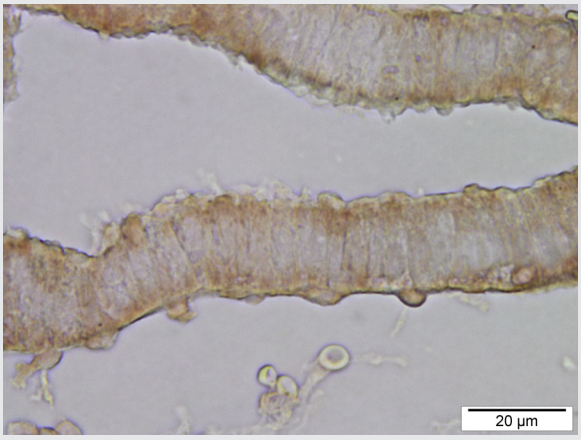
SUPPLEMENTAL FIGURE 1



High magnification picture of MOR immunolocalization in human endometrium. Scale bar, 20 μm.

Totorikaguena. MOR in human endometrium. Fertil Steril 2017.