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REPRODUCTIVE PHYSIOLOGY AND DISEASE

Evidence for expression and functionality of FSH and LH/hCG receptors in human endometrium

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Abstract

Purpose Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) mediate intracellular functions by binding their specific protein G-coupled gonadotrophin receptor, respectively FSH receptor (FSHR) and LH/choriogonadotrophin receptor (LHCGR). Whereas the expression of FSHR and LHCGR in mammals was considered gonad-specific and cell-specific, studies identified gonadotrophin receptors in human female extragonadal reproductive tissues. This study aims to demonstrate that gonadotrophin receptors are expressed in endometrium and mediates intracellular functions.

Methods Collected endometria ($n = 12$) from healthy patients (mean age of 36 ± 6) were primary cultured for 24 h. The presence of gonadotrophin receptors was evaluated by RT-PCR followed by the sequencing of the resulted amplicons and by immunohistochemistry in original samples. Endometrial primary cultures were treated with increasing concentration (range 0–100 ng/ml) of either recombinant human LH (rhLH) or recombinant human FSH (rhFSH). Endometria controls had gonadotrophin replaced by the same volume of the culture medium. In gonadotrophin-treated samples, it was evaluated the intracellular cyclic adenosine monophosphate (cAMP) content by enzymatic immunoassay and the expression of steroidogenic genes by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR).

Results The sequencing of the RT-PCR amplicons confirmed the presence of both gonadotrophin receptors and immunohistochemistry localized them on the membrane of endometrial glands cells throughout the glandular epithelium. The gonadotrophin-receptor complex was able to increase the intracellular cAMP in a dose-response and time-course manner and to induce steroidogenic genes expression.

Conclusion This study demonstrates that both gonadotrophin receptors are expressed along the glandular epithelium of endometria and they mediate the effects of gonadotrophins on intracellular functions.

Keywords FSHR · LHCGR · Endometrium · Gonadotrophin · Steroidogenic genes

Introduction

Gonadotrophins are glycoprotein hormones composed of two non-covalently linked protein subunits, the α and the β subunits, to which carbohydrate moieties are attached. While the α subunit (92 amino acids) presents identical amino acid sequence for all gonadotrophins, the β subunits are different and confer the unique biological properties and the receptor specificity of each of these hormones [1].

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) act in the ovaries and regulate ovarian cycle in which they exert their biological functions by directly binding the extracellular domain of their specific receptors, e.g., FSH receptor (FSHR) and LH/choriogonadotrophin receptor (LHCGR). Gonadotrophin receptors belong to the superfamily of the G-protein-coupled receptors (GPCRs) and share a common base structure composed by an extracellular N-terminal segment, seven hydrophobic transmembrane helices, and a C-terminal intracellular segment [2].

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In mammals, the expression of FSHR and LHCGR was traditionally considered gonad-specific and cell-specific. While the FSHR expression was thought to be strictly limited to the Sertoli cells in male testis [3–5] and to the granulosa cells (GCs) in female ovaries [6–8], the LHCGR was considered exclusively expressed in Leydig cells in the testis and in differentiated human GCs (hGCs), theca cells, and luteal cells in the ovary [9]. Recent studies have demonstrated that both receptors are present in extragonadal non-reproductive tissue [10]. LHCGRs were identified in a variety of extragonadal non-reproductive tissues, including sperm, seminal vesicles, prostate, prostate carcinomas, skin, breast cell lines, adrenals, and even neural retina tissues [11]. For that regard, the female reproductive tract, the LHCGRs presence in human endometrium have been reported at gene level by microarrays [10], by reverse transcriptase-polymerase chain reaction (RT-PCR) [12], and finally confirmed at level protein using immunohistochemical technique [13]. However, whereas the LHCGR presence and functionality was extensively studied and demonstrated through the female reproductive tract, much less information are available for the characterization of FSHR.

Today, the presence of FSHR in human extragonadal tissue like endometrium has been supported by a few evidences limited to microarrays analysis [10, 14, 15] or RT-PCR [16], where it was shown the presence of FSHR during the luteal phase of secretive endometrium. Other studies found FSHR to be expressed in osteoclasts, where it mediates bone resorption [7, 17] and in endothelial cells of tumor vessels, where its function is not fully elucidated [18]. In a study performed to analyze the expression of FSHR in human extragonadal reproductive tissue and developing placenta, FSHR was localized in maternal decidua on both decidual cells and endothelial cells of the blood vessels by immunohistochemistry [19].

The objective of this in vitro study is to confirm the presence of both gonadotrophin receptors at human endometrial level and to verify their functionality by analyzing the effects exerted by gonadotrophins.

Materials and methods

Endometrium tissue primary culture

Endometria biopsies ($n = 12$ total) were collected during the proliferative phase of menstrual cycle (from day 5 to day 14 of the ovulatory cycle) of healthy regular menstruating women (mean age of 36 ± 6) that underwent to diagnostic hysteroscopy before in vitro fertilization (IVF) procedure for male infertility factor. Institutional review board (IRB) approval was obtained for this study. All patients were required to give written informed consent to allow recording and using of their laboratory and clinical data related to medical history for clinical research purpose.

Collected endometrial samples were singularly transferred in 1X Dulbecco's phosphate-buffered saline (DPBS, Sigma-Aldrich, St. Louis, MO, USA) and immediately processed under sterile conditions at room temperature. Single endometrium biopsy (5–7 mm^3) was washed twice with 1X DPBS in a Petri dish, then cut in smaller pieces of approximately of 0.5–1 mm^3 using sterile scalpel. Pieces obtained were singularly deposed in a 12 multiwell plates or treated for RNA isolation and immunohistochemical analysis. Deposed pieces were cultured for 24 h with RPMI 1640 (Life technologies, UK) supplemented with 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, USA) and 10% fetal bovine serum (FBS) South America (EU Approved, Carlo Erba, Italy), without antibiotics before the gonadotrophin treatments to avoid side effects due to previous surgery. Endometria primary cultures were maintained at 37 °C under a controlled atmosphere of 5% CO_2.

Cell culture

hGCs were purified from the ovarian medium aspirated of women that underwent IVF protocol, as previously described [20]. hGCs were plated (10^5 cells/well) in 12 multiwell plates, then maintained at 37 °C under a controlled atmosphere of 5% CO_2 for 6 days to avoid side effect due to previous IVF hormone stimulatory treatment. Medium was daily changed with fresh culture medium (McCoy 5A medium (Carlo Erba, Italy) supplemented with 5% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B (Sigma-Aldrich, St. Louis, MO, USA)). Primary hGC cultures were FSH-treated as previously described [21].

The IPLB-LdFB cell line derived from the fat body of the caterpillar of the gypsy moth, *Lymantria dispar* (Lepidoptera), were cultured in Ex-Cell 400 medium (JRH Biosciences, KS, USA) at 26 °C as previously described [22].

Treatments

Gonadotrophin treatments on cultured endometria were performed by dissolving increasing concentration (range 0–100 ng/ml) of either recombinant human LH (rhLH, Luveris ®, Merck Serono, Italy) or recombinant human FSH (rhFSH, Gonal-F ®, Merck Serono, Italy) in incubation buffer (RPMI 1640; 2 mM L-glutamine; 0,5% FBS). Samples were evaluated after 24 h of incubation with gonadotrophins. For cultured endometria controls, only incubation buffer was used.

Polymerase chain reaction sample preparation and reverse transcription (RT)

Original pieces and gonadotrophin-stimulated endometrium samples were directly lysed in 1 ml of commercial product Tri-Reagent® (Sigma-Aldrich, St Louis, MO, USA) and

immediately processed for total RNA extraction following the product's datasheet, then suspended in 20 μl of RNase-free water. Total extracted RNA was digested with DNase I (Promega, Madison, WI, USA) for 30 min at 37 °C then evaluated by spectrophotometry using Nanodrop ND-1000 (Thermo Fisher Scientific, Waltham, MA, USA) to determine quality and concentration. Two micrograms of total RNA from each sample were reverse transcribed to cDNA using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA), following manufacturer's instructions. The thermal profile applied was 5 min at 25 °C, 30 min at 42 °C, and final 5 min at 85 °C using 2720 Thermal Cycler (Applied Biosystem, Waltham, MA, USA).

RT-PCR and sequencing

The cDNAs obtained from original pieces were employed as single template in RT-PCR reactions using Taq DNA polymerase (Sigma-Aldrich, St Louis, MO, USA) optimized for subsequent sequencing of the resulted amplicons. Briefly, reactions were assembled in 25 μl final volume by mixing 2 μl of cDNA, 0,1 μM of each gonadotrophin receptor pair of primer listed in Table 1, 200 μM deoxynucleotide (dNTP) mix, 1X polymerase chain reaction (PCR) buffer, and molecular biology grade water (Sigma-Aldrich, St Louis, MO, USA). Reactions were performed in 2720 Thermal Cycler (Applied Biosystem, Waltham, MA, USA) applying the program: 94 °C for 1 min followed by 35 cycles of amplification (94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s) and 72 °C for 3 min as final extension step. Positive control of all reactions was performed by amplifying 2 μl of cDNA resulted from FSH-stimulated primary cultured hGCs. Additional controls on the specificity of gonadotrophin receptors pair of primers employed in RT-PCRs reactions were adopted by amplifying 2 μl of cDNA resulted from the IPLB-LdFB insect cell line applying the same thermal profile and PCR general conditions used for endometria samples. The obtained PCR products were fractionated through a 2% agarose gel in 1X tris-acetic acid-EDTA (TAE) buffer and images captured using Gel Doc XR (Bio-Rad, Hercules, CA, USA). The resulted amplicon bands were semi-quantified by comparison with known molecular weight marker (Marker VIII, Roche, Mannheim, Germany) using Quantity One software (Bio-Rad, Hercules, CA, USA).

Semi-quantified PCR's products (10 ng) for each single reaction were cleaned-up by enzymatic digestion using Illustra™ ExoProStar™ (GE Healthcare, UK) according the manufacturer's protocol, then mixed with 1 μl of 10 μM of either single primer forward or reverse and finally dry-pelleted for 20 min at 65 °C. Purified amplicons were sequenced applying the Sanger's standard protocol by BMR genomics laboratories (Padua, Italy). The resulted sequences were analyzed by bioinformatics methods using Basic Local Alignment Search Tool (BLAST, National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD, USA) (http://www.ncbi.nlm.nih.gov/BLAST/), then multiple aligned using CLUSTALWPROF tool of the on line free-software SDSC Biology WorkBench 3.2 (University of California, San Diego, CA, USA).

RT-quantitative PCR

To evaluate if gonadotrophin treatments were able to modulate the endometrial genes expression through the gonadotrophin receptors activation, we analyzed the relative expression of two genes notoriously regulated by gonadotrophins in hGCs, namely *cytochrome P450 family 19 subfamily A member 1* (*CYP19A1* also known as *aromatase*) and *cytochrome P450 family 11 subfamily A polypeptide 1* (*CYP11A1* also known as *P450 cholesterol side-chain cleveage*, *P450scc*) [23]. The cDNAs obtained from gonadotrophin-treated cultured endometria samples as previously described were employed (2 μl) as template in reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR). Samples were evaluated in triplicate and expressed as mean average to allow gene expression analysis. Reactions were carried out on ice using SsoAdvanced Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) following the manufacturer's datasheet. Primers employed (Table 1) were designed where possible to anneal across the intron to avoid aspecific amplification due to

Table 1 List of primers employed in PCRs and sequencing reactions

Gene	Protein name	Sequence 5′-3′	Amplicon lenght (bp)	NCBI Ref. sequence
ACTB	β-actin	F: GCAGAAGGAGATCACTGCCCT R: GCTGATCCACATCTGCTGGAA	135	NM_001101.3
LHCGR	Luteinizing hormone/choriogonadotropin receptor	F: GAGGCAATAAAGGAGCTCACC R: GATGATCTCTTCTTTTGCTTCACAT	128	XM_0115328
FSHR	Follicle-stimulating hormone receptor	F: CATGGTGATGGGCTGGATTT R: CAAAGGCCAGGACATTGAGC	111	X91747.1
CYP19A1	Aromatase	F: CCCTTCTGCGTCGTGTCAT R: GATTTTAACCACGATAGCACTTTCG	86	NM_000103.3
P450scc (*CYP11A1*)	Cholesterol side-chain cleavage enzyme	F: ACCAAGAACTTTTTGCCCCT R: ATGTCCCCCGAGTAATTTCC	127	NM_000781.2

genomic DNA contaminations. Primers shared a similar melting temperature, hence the general thermal profile applied was the same for each gene analyzed: 30 s at 95 °C for the initial activation, followed by 40 cycles of 5 s denaturation at 95 °C and annealing/extension at 60 °C for 20 s performed on StepOne Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). Results were normalized by using *β-actin*, a stable gene across endometrial stages as reference gene. Negative controls of reactions were performed in triplicate substituting templates with water corresponding volumes. The specificity of each assay was evaluated by separating resulted amplicons through a 2% agarose gel in 1X TAE buffer and by the dissociation curve analysis.

Immunohistochemical analysis

Biopsies of original pieces of endometrium were fixated at 4 °C overnight in paraformaldehyde solution (4% paraformaldehyde dissolved in 1X PBS at pH 7.4). Samples were dehydrated by ascending scale of ethanol, starting from ethanol 70% up to ethanol absolute by increasing ethanol percentage after every 1-h incubation. Samples were ultimately clarified with Xylene (Sigma, St. Louis, MO, USA) and included in paraffin blocks. Slices of endometrium were obtained using manual microtome (10 μM section's size) then deposed onto a glass slide and dried overnight at 37 °C. Antigen retrieval was performed by treatment with protease (Pronase 1:20, DakoCytomation, CA, USA) for 7 min at 37 °C. Slides were treated with 3% bovine serum albumin (BSA) in 1X PBS for 30 min at room temperature, then incubated with the primary polyclonal antibodies (rabbit anti-human LHR, rabbit anti-human FSHR, Santa Cruz, Dallas, TX, USA) diluted 1:25 in 1X PBS containing 3% BSA for 1 h at room temperature. After being washed three times with 1X PBS, the slides were incubated for 1 h at room temperature with the secondary antibodies diluted 1:20 in PBS containing 3% BSA respectively, goat anti-rabbit tetramethyl-rhodamine isothiocyanate (TRITC) conjugated, and goat anti-rabbit fluorescein isothiocyanate (FITC) conjugated (Sigma, St. Louis, MO, USA). After three final washes with 1X PBS to remove the unbound antibodies, the samples were counterstained with 1 mg/ml of 4′,6-diamidino-2-phenylindole (DAPI, Life technologies, UK) in distilled water and mounted with anti-fading medium (0.21 M 1,4-diazabicyclo[2.2.2]octane (DABCO) and 90% glycerol in 0.02 M Tris, pH 8.0). Negative control samples were not incubated with the primary antibody. The confocal imaging was performed on a Leica TCS SP2 AOBS confocal laser scanning microscope. Excitation and detection of the samples were carried out in sequential mode to avoid overlapping of signals. Sections were scanned with laser intensity, confocal aperture, gain, and black level setting kept constant for all samples. Optical sections were obtained at increments of 0.3 mm in the *z*-axis and were digitized with a scanning mode format of 512 × 512 or 1024 × 1024 pixels and 256 gray levels. The confocal serial sections were processed with the Leica LCS software to obtain three-dimensional projections. Image rendering was performed by Adobe Photoshop software.

Intracellular cAMP stimulation protocol and detection

Samples of primary cultured endometria were pre-incubated with 5 μM 3-isobutyl-1-methylxanthine (IBMX, Sigma Aldrich, St. Louis, MO, USA) dissolved in starvation buffer for 1 h at 37 °C to prevent cAMP degradation before gonadotrophin stimulations. Gonadotrophin treatments were performed by adding either rhLH or rhFSH (range 0–100 ng/ml) to single-cultured endometrium in the presence of 5 μM IBMX up to 2 h at 37 °C in duplicate. IBMX-treated control samples omitted gonadotrophins. Extra controls to demonstrate the basal effect of IBMX were performed by incubation without IBMX. Subsequently, endometria samples were processed for the intracellular cAMP assay using DetectX direct cyclic–AMP ELISA kit (Arbor assay, Ann Arbor, MI, USA) according to manufacturer's protocol. Briefly, samples were frozen in liquid nitrogen and grinded in a stainless mortar under liquid nitrogen until the samples became a fine powder. After that, samples were weighted and incubated for 10 min on ice with the right amount of 1X sample diluent (1 ml of sample diluent for 100 mg of tissue). Samples were centrifuged at 700×*g* at 4 °C for 15 min and the collected supernatants were immediately run in the assay according the protocol. Negative controls without templates or DetectX cAMP antibodies were processed following the datasheet instructions. Optical density generated from each well was read at 450 nm wavelength using MultiSkan FC plate reader (Thermo

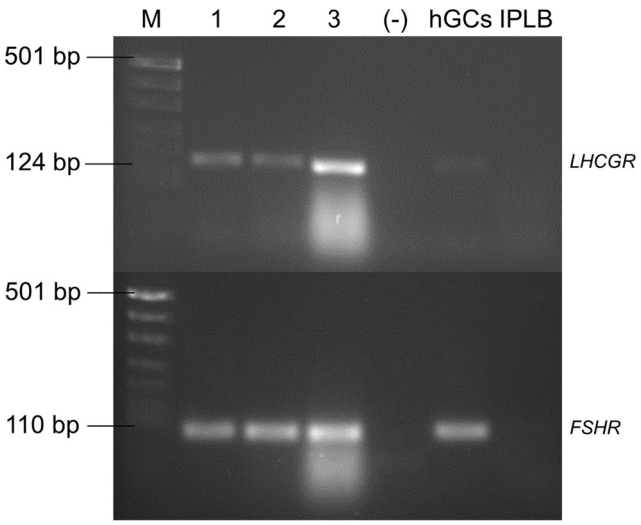

Fig. 1 Lines 1–3 refer to samples of original endometria recovered from three different patients. The template was omitted from the negative control (−). *M* DNA standard ladder, *hGCs* human granulosa cells, *IPLB* insect cell line

Fisher, MD, USA). Sensitivity and limit of detection were calculated according to the datasheet by comparing the OD's for the maximum binding wells (B_0) and the most diluted standard (sensitivity = 0.67 pmol/ml; limit of detection = 0.31 pmol/ml; coefficients of variation R^2 = 0.9974). The results were normalized by corresponding cAMP standard curve obtained through serial dilutions of cAMP stock solution. Results and coefficient of variations were obtained using the online tool MyAssay to calculate the concentrations of intracellular cAMP generated by gonadotrophin treatment.

Statistical analysis

Statistical analysis was performed using the Kruskal-Wallis test followed by the Dunn-Bonferroni's test, as appropriate ($P < 0.001$) set for statistical significance. Statistical analysis for cAMP detection was performed according to the online tool (https://www.myassays.com/arbor-assays-cyclic-amp-direct-eia-kit-non-acetyl.assay) The relative expression of each gene has been evaluated using the $2^{-\Delta\Delta Ct}$ method [24] and calculated as the relative ratio in comparison to the first control sample, arbitrarily set to 1.

Results

FSHR and LHCGR are expressed in primary cultures of endometrium

As shown in Fig. 1 and Fig. 1 supplemental, the presence of LHCGR and FSHR transcripts was confirmed in all the endometria tested. The primers employed in RT-PCR reactions fail to amplify invertebrate cDNA supporting the high affinity of primers with their target region. The sequencing of the PCR resulted amplicons matched specifically the sequences of their respective gonadotrophin receptor after alignment (Fig. 2).

Fig. 2 Nucleotide sequences of **a** *LHCGR* and **b** *FSHR* cDNA. Lines are namely according to the resulted sequence names' or with the accession number of corresponding sequence. Asterisks underneath alignments mark the matching between sequences

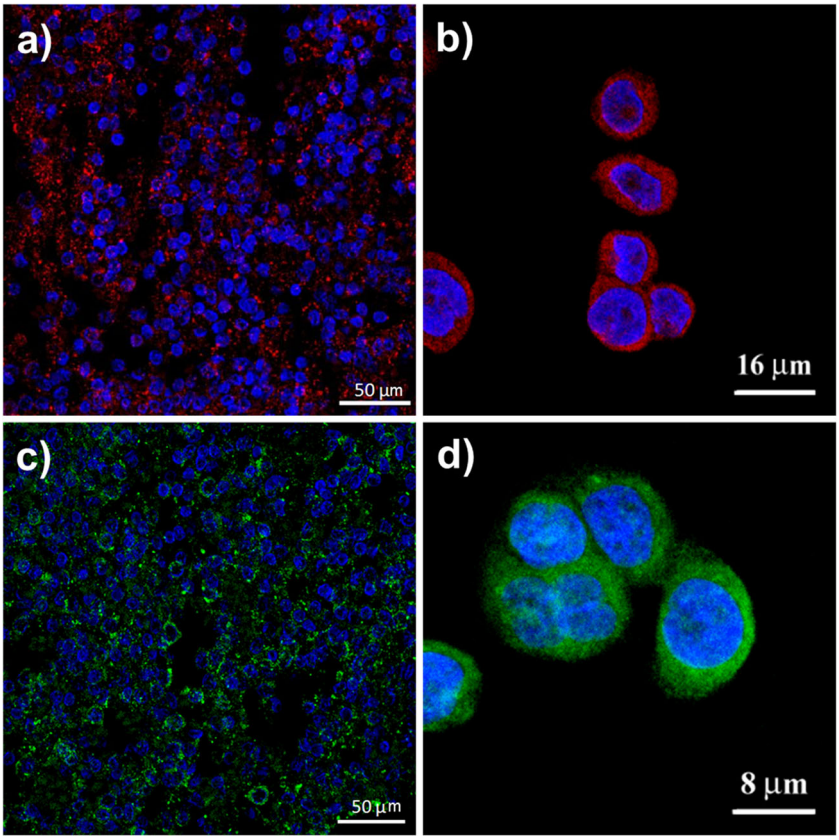

Fig. 3 **a** LHCGR are depicted in red color **b** higher magnification. **c** FSHR are depicted in green color; **d** higher magnification. Controls in which the primary Abs were omitted were negative (not shown)

Figure 3 shows the presence and the localization of gonadotrophin receptors on the membrane of endometrial glands cells throughout the glandular epithelium, although confocal imaging cannot distinguish stromal from epithelial cells stained. Regardless of the primary antibody used, erythrocytes and neutrophils exhibiting typically nonspecific staining are present.

FSHR and LHCGR expressed in endometrium are functional and mediate the response of endometrial cells to gonadotrophins

Figure 4 shows the results of dose-response experiment in which all the gonadotrophin treatments tested (10, 50, and

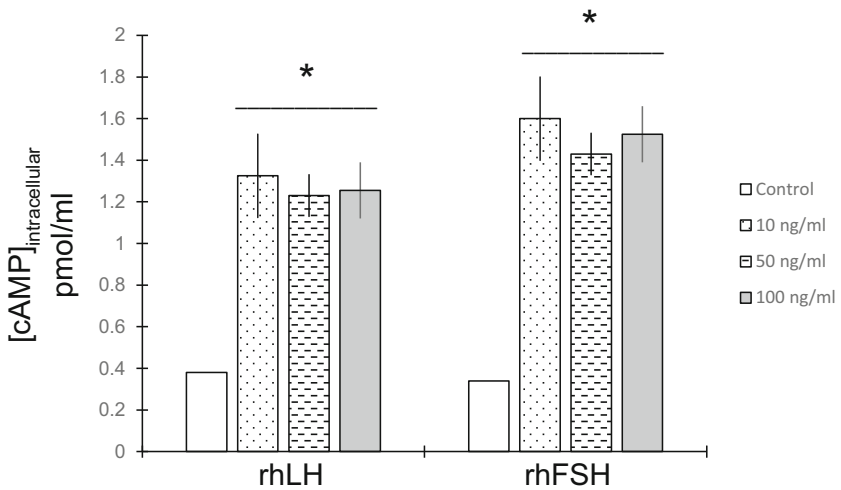

Fig. 4 Representative ($n = 1$) dose-response production of intracellular cAMP in primary cultured endometria collected during the proliferative phase of menstrual cycle stimulated by increasing concentrations of rhLH or rhFSH, measured after 2 h of incubation in the presence of 5 µM IBMX. The cAMP measurement were conducted in duplicate and expressed as mean. Bars represent the two values used for to calculate the mean value. Significant differences between treated cases and untreated controls were marked by * ($P < 0.001$), Kruskal-Wallis test followed by the Dunn-Bonferroni's test

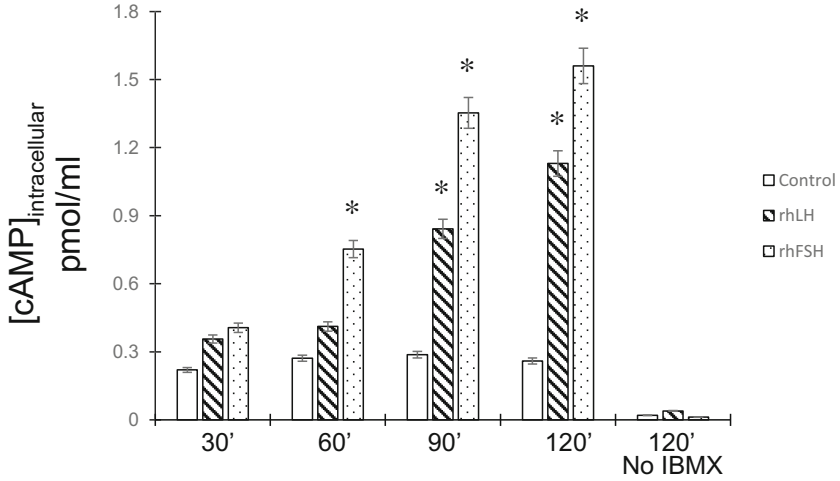

Fig. 5 Intracellular cAMP production in primary cultures of endometrium induced by 10 ng/ml of rhLH or rhFSH in time-course experiment ($n = 4$). The cAMP measurements were conducted in duplicate and expressed as mean and standard deviation. Significant differences between treated cases and untreated controls were marked by * ($P < 0.001$), Kruskal-Wallis test followed by the Dunn-Bonferroni's test

100 ng/ml of rhLH or rhFSH) were able to increase the cAMP intracellular content if compared to endometria control, without any differences among treatments. The chosen dose of 10 ng/ml for each gonadotrophins was also analyzed in time course experiment (Fig. 5) which shows a progressive accumulation of measurable intracellular cAMP generated by both gonadotrophin. The cAMP production was ultimately expressed (Fig. 6) as fold change in comparison to the mean average of controls set arbitrarily to 1 to apply statistical significance.

Figure 7 confirmed the presence of both *CYP19A1* and *P450scc* genes transcription in three different untreated endometria collected during the proliferative phase demonstrated by RT-PCR.

The effect of 24 h of incubations with rhLH or rhFSH on endometria was evaluated by RT-qPCR and presented as normalized ratio using the reference gene *β-actin* (Fig. 8). rhLH and rhFSH (10 ng/ml) were able to significantly induce the expression of aromatase *CYP19A1* (Fig. 8a) and *P450scc* (Fig. 8b) genes, possibly through the activation of their specific gonadotrophin receptors.

Discussion

This study demonstrated the presence of gonadotrophin receptors in endometria fragments, chosen as experimental model in order to preserve the morphology and the functionality of the tissue. In particular, using RT-PCR followed by sequencing of amplicons and immunohistochemistry methods, the expression of FSHR and LHCGR in human endometrium during the proliferative phase of menstrual cycle was confirmed at both gene and protein level. Both receptors were spatially expressed in cells of endometrial glands. The gonadotrophin receptors identified in the endometrium are functional since their stimulation with gonadotrophin was able to promote intracellular cAMP accumulation, as well as the up-regulation of at least two steroidogenic genes. These findings open up many implications on possible effects mediate by FSH and LH/hCG on endometrial physiology.

The main well-known physiological function of the glycoprotein FSH is to stimulate the follicular maturation and estrogen production through aromatase activation, by binding to FSHRs present on granulosa cells in the ovary. Several studies

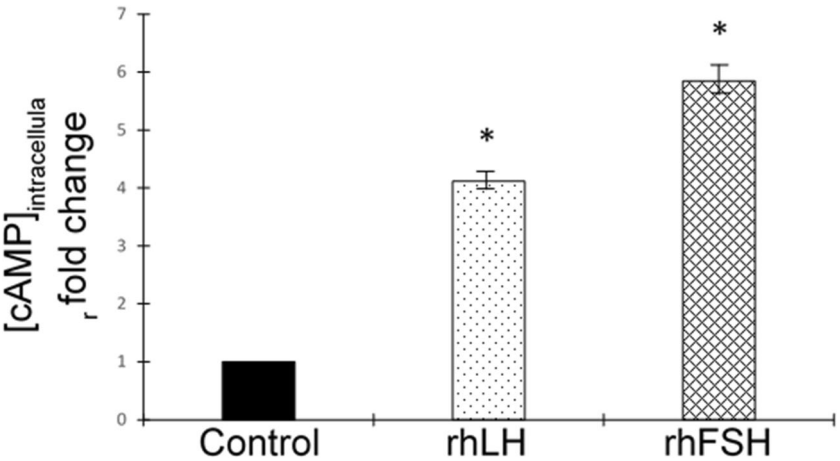

Fig. 6 Gonadotrophin-stimulated intracellular cAMP content expressed as fold change in comparison to untreated control proliferative endometria set arbitrarily to 1 ($n = 6$). Significant differences versus untreated controls were marked by * ($P < 0.001$), Kruskal-Wallis test followed by the Dunn-Bonferroni's test

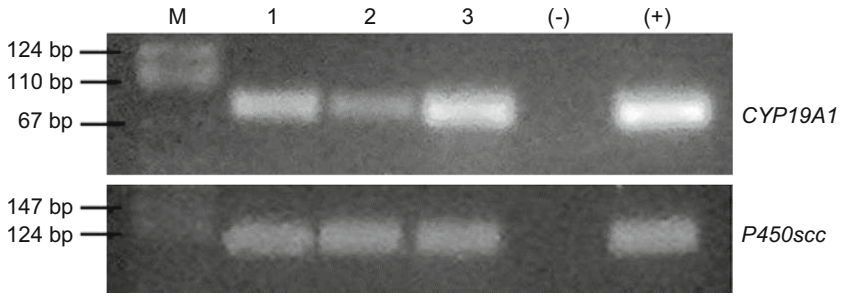

Fig. 7 *Aromatase CYP19A1* and *cytochrome P450scc* expression in endometria collected during the proliferative phase of the menstrual cycle. Lines 1–3 refer to individual original samples obtained from three different patients. *M* DNA standard ladder

has demonstrated that FSHRs are also present in extragonadal non-reproductive tissues and throughout the human female reproductive system, in addition to the ovary [14, 16, 18, 19]. Aromatase is physiologically expressed in a variety of tissues, including the ovary, placenta, skin, adipose tissue and brain, and the local estrogen biosynthesis is thought to be integral to the pathophysiology of a variety of uterine and extrauterine disorders, such as adenomyosis, fibroids, and endometriosis [25–28]. Aromatase transcription was also detected in the endometrium of infertile patients, although the levels varied considerably among samples [29]. Hence, FSH could act on the endometrial aromatase, inducing a local estrogen production.

According to some authors [30], locally produced estrogens may direct site-specific remodeling of the endometrium, to confer endometrial receptivity during the period of implantation or to influence estrogen-dependent epithelial production of factors related to embryo implantation. A possible influence of locally produced estrogen on endometrial vasculature cannot be excluded. At least, in animals, supraphysiological estrogen concentrations are associated with a low endometrium receptivity [31] and some evidences from IVF cycles seem to indicate that high levels of estradiol, which are typically associated with overstimulated ovaries, may indeed cause a relative reduction in the embryo implantation rate [32].

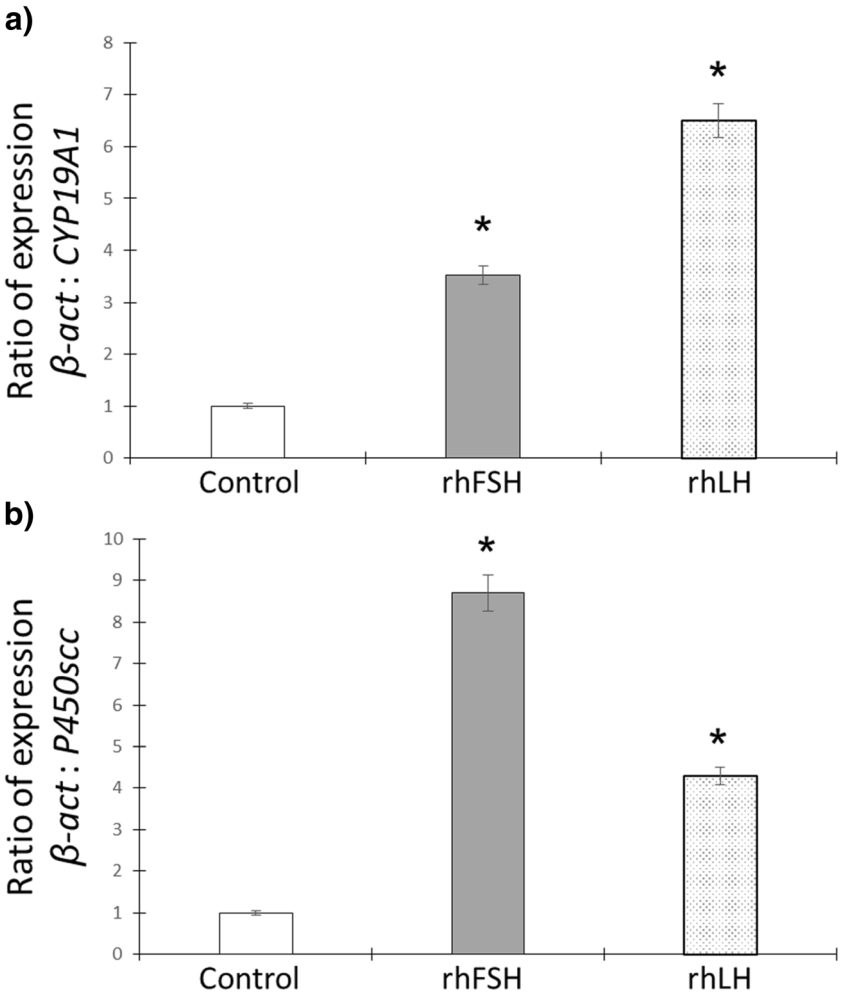

Fig. 8 **a** *Aromatase CYP19A1* and **b** *cytochrome P450scc* gene expressions in primary culture of proliferative endometria after 24-h incubation with 10 ng/ml of gonadotrophins ($n = 6$). Negative controls were cultured only with culture medium. Significant differences between treated samples and untreated controls were marked by * ($P < 0.001$), Kruskal-Wallis test followed by the Dunn-Bonferroni's test

The main physiological function of LH in the ovary during the luteal phase is to promote luteinization and progesterone production by granulosa cells through binding to LHCGR. LHCGRs have been previously identified in many extragonadal tissues and across the female reproductive tract [11–14]. In the current study, we could confirm the presence of functionally active LHCGR and FSHR in the human endometrium. The presence of these receptors in endometrium led some authors to propose a possible role of LH and human choriogonadotrophin (hCG) in the endometrial physiology.

The possible physiological significance of the presence of LHCGR was evident from the observation that hCG induced the expression of some genes such as steroidogenic acute regulatory protein (StAR) and 3β-Hydroxysteroid dehydrogenase/Δ5-4 isomerase (3β-HSD), with increased synthesis of local progesterone in the mammals endometrium [33]. The role of locally produced progesterone may be related to the paracrine regulation of some processes related to endometrial metabolism and growth. Several authors also proposed different paracrine functions of LH and hCG in the endometrium, such as prostaglandin and cytokine biosynthesis [34, 35] and modulation of embryo implantation [36, 37]. A recent study showed that prolonged endometrium exposure to low-dose hCG abrogated extracellular signal-regulated kinases (ERK) 1/2 phosphorylation, adhesion to extracellular matrices, and changes in tight junction integrity, suggesting that precocious or prolonged hCG exposure may detrimentally affect endometrial receptivity [38].

The present study confirmed that the addition of hCG/LH to the endometrium led to intracellular cAMP accumulation and activation of some steroidogenic key enzymes such as *P450scc* and aromatase *CYP19A1* showing a possible relevant role of hCG/LH in the paracrine control of human endometrium.

In conclusion, the results here presented showed that FSHR and LHCGR are present and functionally active in human endometrium and may mediate cellular functions. It was also demonstrated that gonadotrophins may play a role in the gene expression across the endometrial stages, possibly affecting the cellular environment during embryo implantation process.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Vaitukaitis JL, Ross GT, Braunstein GD, Rayford PL. Gonadotropins and their subunits: basic and clinical studies. Recent Prog Horm Res. 1976;32:289–331.
2. Ji TH, Grossmann M, Ji I. G protein-coupled receptors. I Diversity of receptor-ligand interactions. J Biol Chem. 1998;273(28):17299–302.
3. Fritz IB. Sites of action of androgens and follicle-stimulating hormone on cells of the seminiferous tubule. In: Litwack G, editor. Biochemical actions of hormones, vol. 5. New York: Academic; 1978. p. 249–81.
4. Kangasniemi M, Kaipia A, Toppari J, Perheentupa A, Huhtaniemi I, Parvinen M. Cellular regulation of follicle-stimulating hormone (FSH) binding in rat seminiferous tubules. J Androl. 1990;11(4):336–43.
5. Dankbar B, Brinkworth MH, Schlatt S, Weinbauer GF, Nieschlag E, Gromoll J. Ubiquitous expression of the androgen receptor and testis-specific expression of the FSH receptor in the cynomolgus monkey (Macaca fascicularis) revealed by a ribonuclease protection assay. J Steroid Biochem Mol Biol. 1995;55(1):35–41.
6. Richards JS. Maturation of ovarian follicles: actions and interactions of pituitary and ovarian. Physiol Rev. 1980;60(1):51–89.
7. Tisdall DJ, Watanabe K, Hudson NL, Smith P, McNatty KP. FSH receptor gene expression during ovarian follicle development in sheep. J Mol Endocrinol. 1995;15(3):273–81.
8. Zheng W, Magid MS, Kramer EE, Chen YT. Follicle-stimulating hormone receptor is expressed in human ovarian surface epithelium and fallopian tube. Am J Pathol. 1996;148(1):47–53.
9. Ascoli M, Fanelli F, Segaloff DL. The lutropin/choriogonadotropin receptor, a 2002 perspective. Endocr Rev. 2002;23(2):141–74.
10. Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics. 2014;13(2):397–406.
11. Rahman NA, Rao CV. Recent progress in luteinizing hormone/human chorionic gonadotrophin hormone research. Mol Hum Reprod. 2009;15(11):703–11.
12. Licht P, von Wolff M, Berkholz A, Wildt L. Evidence for cycle-dependent expression of full-length human chorionic gonadotropin/luteinizing hormone receptor mRNA in human endometrium and decidua. Fertil Steril. 2003;79(Suppl 1):718–23.
13. Reshef E, Lei ZM, Rao CV, Pridham DD, Chegini N, Luborsky JL. The presence of gonadotropin receptors in nonpregnant human uterus, human placenta, fetal membranes, and decidua. J Clin Endocrinol Metab. 1990;70(2):421–30.
14. Popovici RM, Kao LC, Giudice LC. Discovery of new inducible genes in in vitro decidualized human endometrial stromal cells using microarray technology. Endocrinology. 2000;141(9):3510–3.
15. Altmäe S, Tamm-Rosenstein K, Esteban FJ, Simm J, Kolberg L, Peterson H, et al. Endometrial transcriptome analysis indicates superiority of natural over artificial cycles in recurrent implantation failure patients undergoing frozen embryo transfer. Reprod BioMed Online. 2016;32(6):597–613.
16. La Marca A, Carducci Artenisio A, Stabile G, Rivasi F, Volpe A. Evidence for cycle-dependent expression of follicle-stimulating

hormone receptor in human endometrium. Gynecol Endocrinol. 2005;21(6):303–6.
17. Zhu LL, Blair H, Cao J, Yuen T, Latif R, Guo L, et al. Blocking antibody to the β-subunit of FSH prevents bone loss by inhibiting bone resorption and stimulating bone synthesis. Proc Natl Acad Sci U S A. 2012;109(36):14574–9.
18. Radu A, Pichon C, Camparo P, Antoine M, Allory Y, Couvelard A, et al. Expression of follicle-stimulating hormone receptor in tumor blood vessels. N Engl J Med. 2010;363(17):1621–30.
19. Stilley JA, Christensen DE, Dahlem KB, Guan R, Santillan DA, England SK, et al. Segaloff DL. FSH receptor (FSHR) expression in human extragonadal reproductive tissues and the developing placenta, and the impact of its deletion on pregnancy in mice. Biol Reprod. 2014;91(3):74.
20. Nordhoff V, Sonntag B, von Tils D, Götte M, Schüring AN, Gromoll J, et al. Effects of the FSH receptor gene polymorphism p.N680S on cAMP and steroid production in cultured primary human granulosa cells. Reprod BioMed Online. 2011;23(2):196–203.
21. Sacchi S, D'Ippolito G, Sena P, Marsella T, Tagliasacchi D, Maggi E, et al. The anti-Müllerian hormone (AMH) acts as a gatekeeper of ovarian steroidogenesis inhibiting the granulosa cell response to both FSH and LH. J Assist Reprod Genet. 2016;33(1):95–100.
22. Lynn DE, Dougherty EM, McClintock JT, Shapiro M. Comparative replication of Lymantria dispar nuclear polyhedrosis virus strains in three continuous-culture cell lines. Appl Environ Microbiol. 1989;55(5):1049–51.
23. Yong EL, Hillier SG, Turner M, Baird DT, Ng SC, Bongso A, et al. Differential regulation of cholesterol side-chain cleavage (P450scc) and aromatase (P450arom) enzyme mRNA expression by gonadotrophins and cyclic AMP in human granulosa cells. J Mol Endocrinol. 1994;12(2):239–49.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods. 2001;25(4):402–8.
25. Huhtinen K, Saloniemi-Heinonen T, Keski-Rahkonen P, Desai R, Laajala D, Ståhle M, et al. Intra-tissue steroid profiling indicates differential progesterone and testosterone metabolism in the endometrium and endometriosis lesions. J Clin Endocrinol Metab. 2014;99(11):E2188–97.
26. Robin B, Planeix F, Sastre-Garau X, Pichon C, Olesen TK, Gogusev J, et al. Follicle-stimulating hormone receptor expression in endometriotic lesions and the associated vasculature: an immunohistochemical study. Reprod Sci. 2016;23(7):885–91.
27. Tang B, Gurpide E. Direct effect of gonadotropins on decidualization of human endometrial stroma cells. J Steroid Biochem Mol Biol. 1993;47(1–6):115–21.
28. Hinshelwood MM, Dodson Michael M, Sun T, Simpson ER. Regulation of aromatase expression in the ovary and placenta: a comparison between two species. J Steroid Biochem Mol Biol. 1997;61(3–6):399–405.
29. Sebastian S, Takayama K, Shozu M, Bulun SE. Cloning and characterization of a novel endothelial promoter of the human CYP19 (aromatase P450) gene that is up-regulated in breast cancer tissue. Mol Endocrinol. 2002;16(10):2243–54.
30. Brosens J, Verhoeven H, Campo R, Gianaroli L, Gordts S, Hazekamp J, et al. High endometrial aromatase P450 mRNA expression is associated with poor IVF outcome. Hum Reprod. 2004;19(2):352–6.
31. Gibson DA, McInnes KJ, Critchley HO, Saunders PT. Endometrial intracrinology—generation of an estrogen-dominated microenvironment in the secretory phase of women. J Clin Endocrinol Metab. 2013;98(11):E1802–6.
32. Ma WG, Song H, Das SK, Paria BC, Dey SK. Estrogen is a critical determinant that specifies the duration of the window of uterine receptivity for implantation. Proc Natl Acad Sci U S A. 2003;100(5):2963–8.
33. Coleson MP, Sanchez NS, Ashley AK, Ross TT, Ashley RL. Human chorionic gonadotropin increases serum progesterone, number of corpora lutea and angiogenic factors in pregnant sheep. Reproduction. 2015;150(1):43–52.
34. Han SW, Lei ZM, Rao CV. Up-regulation of cyclooxygenase-2 gene expression by chorionic gonadotropin during the differentiation of human endometrial stromal cells into decidua. Endocrinology. 1996;137(5):1791–7.
35. Srisuparp S, Strakova Z, Brudney A, Mukherjee S, Reierstad S, Hunzicker-Dunn M, et al. Signal transduction pathways activated by chorionic gonadotropin in the primate endometrial epithelial cells. Biol Reprod. 2003;68(2):457–64.
36. Licht P, Russu V, Wildt L. On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. Semin Reprod Med. 2001;19(1):37–47.
37. Das SK. Cell cycle regulatory control for uterine stromal cell decidualization in implantation. Reproduction. 2009;137(6):889–99.
38. Evans J, Salamonsen LA. Too much of a good thing? Experimental evidence suggests prolonged exposure to hCG is detrimental to endometrial receptivity. Hum Reprod. 2013;28(6):1610–9.