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Endometrial expression of receptivity markers subject to ovulation induction agents

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Abstract

Purpose Implantation rates differ according to ovulation induction agents in ART. This study investigates the different local endometrial effects of LH- versus hCG-induced ovulation.

Methods Endometrial stromal cells from healthy patients were cultured with hCG or LH in different concentrations, supplemented with 250 ng/mL hCG and progesterone after 2 and 5 days. In addition after decidualization induction, cells were treated with hCG (50 or 250 ng/mL) or LH (10 or 50 ng/mL) for 3 days. Receptivity markers expression was evaluated by real-time quantitative PCR on day 3 and 6.

Results On day 3, non-decidualized cells treated with LH showed an increased expression of IGFBP1, IL-8 and CXCL12 compared to hCG. The expression pattern changed on day 6, where cells treated with hCG showed higher expression of implantation markers compared to LH-treated cells. Furthermore, on day 3, decidualized cells treated with hCG250 showed an increased IL8 and CXCL12 expression compared to LH10.

Conclusions LH seems to modulate the local endometrial expression of receptivity markers earlier compared to hCG; however, the effect is not sustained over time in cells without prior decidualization. Though, in decidualized cells, pattern changed and an earlier positive effect of hCG was shown on IL-8 and CXCL12.

Keywords Endometrium implantation · hCG · LH · IGFBP1 · IL8 · CXCL12

Abbreviations

IGFBP1	Insulin-like growth factor binding protein 1
IL8	Interleukin 8
CXCL1	CXC-Motif-Chemokin ligand 1
CXCL12	CXC-Motif-Chemokin ligand 12, also stromal cell-derived factor 1 (SDF-1) or pre-B cell growth-stimulating factor (PBSF)
LH	Luteinizing hormone
hCG	Human chorionic gonadotropin

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Introduction

The blastocyst implantation into the uterine endometrium represents the key process for success a substantial restrictive stage for success in IVF. There is still a gap in the understanding of endometrial physiology and its role in implantation. For a successful implantation, a precise synchronization between embryo and endometrium is necessary. Through functional and structural changes, the endometrium decidualizes and during this short period of time, known as "window of implantation", it is receptive to the embryo [1]. During this stage, numerous implantation factors, cytokines, growth factors are produced by both the preimplanted embryo and the maternal cells [2, 3]. The human chorionic gonadotropin (hCG) and the luteinizing hormone (LH) have different functions in human reproduction: LH has a significant influence on gonadal steroidogenesis and ovulation [4], while hCG, in the absence of pathologies, is only produced during pregnancy [5, 6].

Controlled ovarian stimulation (COS) for assisted reproductive technologies (ART) has been used for decades to

obtain follicles for in vitro fertilization (IVF). Compared to natural cycles, lower implantation rates per embryo are seen in women undergoing COS [7]. Possibly, this effect is due to a suboptimal endometrial development. Mostly, the research on ovarian stimulation is focused on ovarian response, neglecting the impact on endometrial gene expression during the supraphysiological levels of steroid hormones exposure [2]. However, in order to achieve the optimal interaction between the mediators of maternal–fetal cross talk [1, 3], ovulation induction protocols with different stimulating agents need to be assessed in their impact on implantation factors within endometrial tissues. LH or hCG can be used for ovarian induction in stimulation protocols. Yet, it seems that they may have different modulation effects on gene profile of endometrial receptivity markers. hCG, when directly administered to the endometrium before embryo transfer, seems to improve implantation rates [8]. In the human endometrium, hCG binds to the luteinizing hormone (LH)/hCG receptor (hLHCGR) and is involved in several biological changes related to the decidualization of endometrial stromal cells (ESC) leading to implantation [1] and it is also associated with the uterine angiogenic adaptation in early pregnancy [9, 10]. LH appears to have an impact in embryo quality and on uterine receptivity [11]. Both hCG and LH seem to be helpful in improving controlled ovarian stimulation (COS) protocols [11, 12] but despite binding to the same receptor, they do not seem to induce a similar response within endometrium [13].

LH and hCG differ not just in their outcomes in assisted reproductive techniques (ART) nonetheless also in their side effects. In hCG-triggered cycles, more ectopic maternal pregnancies are observed [14]. The ovarian hyperstimulation syndrome (OHSS) is also more frequently seen in these cycles in comparison to the poorer implantation results under GnRH analogs treatments that lead to intrinsic LH secretion.

Even with decreased estrogen levels despite hormonal stimulation in hCG-triggered cycles, supplementation with hCG can improve the chances of pregnancy [15]. Moreover, better implantation rates have been demonstrated in vivo after intrauterine hCG application. [8, 16]. In order to investigate if the different outcomes of LH- versus hCG-induced ovulation could be caused by a shift in endometrial receptivity gene expression, we treated decidualized and non-decidualized cells with different dosages of LH and hCG.

In reproductive medicine, the expression of potential endometrial receptivity factors that may be modulated during ovary stimulation is of special interest [17]. A comprehensive gene scanning has been previously performed [18]. Several different pathways seem to be modulated in endometrium during the window of implantation: Growth factors (IGF-1 and 2, IGFBP-1, VEGF, HB-EGF), metalloproteinases, chemokines (CXCL1; CXCL12), interleukins (IL-1, IL-8) are some among several others [19].

In the present study, we investigated the effect of different doses of hCG and LH on endometrium implantation markers in non-decidualized and decidualized endometrial stromal cells. Specifically, we evaluated IGFBP1, IL8, CXC Motif-Ligand (CXCL)1 and CXCL12 mRNA levels in the endometrial stromal cells stimulated in vitro after exposure to recombinant LH or hCG.

Materials and methods

Patients

Endometrial biopsies of the proliferative phase (based on clinical and anamnestic information) were collected from 14 healthy, regularly cycling women (34.8 ± 4.5 years old) suffering from infertility after informed consent under the approved ethics protocol of the Ruprecht Karls University Heidelberg (S239/2005) during gynecologic surgery by curette. Exclusion criteria were hormonal stimulation within the preceding 3 months, endocrinopathies, endometriosis, cancerous lesions and irregular menstrual bleeding.

Isolation of endometrial stromal cells

The human endometrial primary stromal cells were collected according to the previously well-defined protocol [43]. Briefly, the endometrial tissues were digested with collagenase (Gibco, Karlsruhe, Germany) then separated by 40-µm filter and cultured in phenol red-free Dulbecco's modified Eagle's medium (DMEM; Gibco), MCDB-105 (Sigma-Aldrich, Taufkirchen, Germany), supplemented with 10% HyClone fetal bovine serum (FBS, Thermo Scientific, UT, USA), 1% penicillin-streptomycin (Gibco), 0.1% gentamicin (Gibco), 1% nystatin (Gibco) and 5 µg/mL bovine insulin (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) at 37 °C in a humidified atmosphere with 5% CO_2. The cells were cultured until confluent.

Culture conditions of non-decidualized endometrial stromal cells

The cells were seeded at a density of 2×10^5 cells/mL into 6-well plates (Sarstedt AG & Co. KG, Germany) and cultured for 2 days with serum-free medium supplemented with 17β-estradiol (10 nM) and EGF (20 ng/mL). Thereafter, considered as day 0, the cells were treated according to the different groups: control (serum-free medium supplemented with 17β-estradiol and EGF—standard medium), hCG 50 (standard medium supplemented with hCG 50 ng/mL), hCG 250 (standard medium supplemented with hCG 250 ng/

mL), hCG 1000 (standard medium supplemented with hCG 1000 ng/mL), LH 10 (standard medium supplemented with LH 10 ng/mL), LH 50 (standard medium supplemented with LH 50 ng/mL), LH 100 (standard medium supplemented with LH 100 ng/mL). On day 2, the medium was changed (serum-free medium supplemented with 17β-estradiol, EGF, hCG 250 ng/mL and progesterone 1 mM). For the short-term cultures, the RNA was isolated on day 3 ($n=7$). However, for the long-term cultures, the medium (serum-free medium supplemented with 17β-estradiol, EGF, hCG 250 ng/mL and progesterone 1 mM) was changed again on day 5, and the RNA was isolated on day 6 ($n=4$). All hormones were purchased from Sigma-Aldrich.

Culture conditions of decidualization-induced endometrial stromal cells

Initially, the cells from 5 patients were seeded at a density of 1.5×10^5 cells/mL into 6-well plates and cultured during 2 days in DMEM/MCDB medium supplemented with 10% Hyclone FBS and 1% penicillin-streptomycin. After 2 days, in vitro decidualization was initiated as previously described [44]. Briefly, the medium was changed to phenol red-free DMEM/MCDB supplemented with 2% Hyclone FBS, 1% penicillin-streptomycin, 10 nM 17ß-estradiol (E2) mixed with 1 µM medroxyprogesterone-17-acetate (MPA—Sigma-Aldrich, USA) and 0.5 mM 8-bromoadenosine 3′,5′-cyclic monophosphate (cAMP, Sigma-Aldrich, USA). The negative control was cultivated with the same medium, however, supplemented only with the diluents, ethanol and $NaHCO_3$. If decidualization initiation was confirmed, on day 3 by evaluation of prolactin levels in the supernatant, cells were treated according to the different groups: positive control, hCG 50 ng/mL, hCG 250 ng/mL, LH 10 ng/mL and LH 50 ng/mL. Three days later (on day 6 of decidualization), the supernatant was collected and the cells were harvested for RNA isolation.

Protein–protein interaction network construction

A comprehensive literature search about genes involved in the endometrial receptivity has been previously performed and the Search Tool for the Retrieval of Interacting Genes/Protein (STRING) database online (http://string-db.org/) was used to retrieve the predicted interactions for the identified genes and allows visualizing complex networks [35]. We set the required interaction score at 0.7 and up to 10 interactions as the threshold used for analysis.

Microscopic morphology

Endometrial stromal cells were cultured until a monolayer formation with 80% confluency was obtained. Decidualization-induced was confirmed after 6 days cells treated either with vehicle or 10 µM E2, 1 mM MPA and 50 mM cAMP. Inverted microscope was used to observe the morphology of endometrial stromal cells. Pictures were acquired using 10× magnification with a Zeiss AxioVert 40 CFL microscope. Rabbit monoclonal anti-vimentin-Alexa Fluor-594 (1:500) antibody (ab154207, Abcam, UK) was used overnight according to the manufactures instructions to stain stromal cytoskeletal and DAPI to stain nucleus. Immunofluorescent images were acquired using Leica microscope equipped with a Spectral Imaging 2.6 digital camera and the FISHView Expo 2.0 software.

Prolactin assessment

Prolactin was assessed using chemoluminescenceimmunoassay (CLIA) with Centaur XPT (Siemens healthineers) and reagent from Siemens healthineers.

mRNA analysis

The RNA was extracted using TRIZOL (Gibco) according to the manufacturer's protocol. The complementary DNA (cDNA) was synthesized from 1 µg of total RNA using the Reverse Transcription Using AMV Reverse Transcriptase (Promega, Germany) following to the manufacturer's instructions. The mRNA expression of IGFBP-1 (Hs00236877_m1), CXCL1 (Hs00236937_m1), CXCL12 (Hs00171022_m1) and IL8 (Hs99999034_m1) were performed by real-time PCR using TaqMan detection (TaqMan 2X Universal PCR Master Mix, Applied Biosystems by Thermo Fischer Scientific, Woolston, Warrington, UK) with 40 cycles (10 min 95 °C, 15 s 95 °C, 1 min 60 °C) on a 7500 Fast real-time PCR System (Applied Biosystems). For all reactions, HPLO (Hs99999902_m1) was used as a housekeeping gene and the values were calculated using the ΔΔC(t) method and expressed as a fold change.

Statistical analysis

Quantitative data are represented as the mean ± SEM of at least five independent experiments using the ΔCt values and expressed as a fold change. SPSS version 24 (IBM SPPS, Armonk, NY, USA) was used to perform a generalized estimating equation test or paired student's *t* test, as appropriated. $P\leq0.05$ was considered statistically significant.

Results

IGFBP1, IL8, CXCL1 and CXCL12 are functional partners

After a comprehensive literature search about implantation markers, IGFBP1, IL8, CXCL1 and CXCL12 were selected and a gene/protein interaction network was constructed using the database STRING. As shown in Fig. 1, IGFBP1, IL8, CXCL1 and CXCL12 are functional partners exhibiting functional associations and molecular action as activation, inhibition, binding, catalysis with the predicted partners IGF1, CXCR2, IGF2, RELA, CXCR1, DARC, INS, NFKB1 and JUN.

LH and hCG affect the expression of implantation markers differently in non-decidualized cells

To evaluate the direct effects of hCG and LH on endometrial receptivity, we assess the RNA expression of important implantation markers in non-decidualized endometrial stromal cells over time. Figure 2 shows the significant enhancing effect, on day 3 of the different LH concentrations in the expression of IGFBP1, IL-8 and CXCL12 compared to different hCG concentrations. Therefore, LH 100 enhanced the IGFBP1 expression by 36% compared to cells treated with hCG 50 ng/mL ($P < 0.01$). The IL8 expression was increased by 17% after LH 10 ng/mL addition compared to hCG 250 ng/mL group ($P = 0.05$) and the CXCL12 expression by 24% compared to hCG 1000 ng/mL group ($P = 0.02$). The LH 50 ng/mL group increased the expression of CXCL12 by 30% compared to cells treated with hCG 1000 ng/mL ($P = 0.02$). Furthermore, LH 100 ng/mL showed a 30% higher expression of CXCL12 compared to hCG 1000 ng/mL ($P = 0.04$). However, after 6 days of initial treatment with hCG versus LH, the expression pattern changed and the cells treated with hCG hormone showed a higher expression of the examined implantation markers IGFBP1, IL8, CXCL1 and CXCL12 compared to cells treated with LH, although this difference was only noticed at the highest doses of hCG (250 and 1000 ng/mL). Cells treated with hCG 250 ng/mL showed an increased expression by 33% of IL8 compared to the cells treated with LH 100 ng/mL ($P = 0.01$). Moreover, the highest hCG dose slightly affected the implantation markers expression, the cells treated with hCG 1000 ng/mL showed 25% and 15% enhanced IGFBP1 expression compared to LH 10 ng/mL and LH 50 ng/mL, respectively, ($P = 0.05$ and $P = 0.02$), 30% of IL8 compared to LH 50 ng/mL ($P = 0.04$), 46% and 44% of CXCL1 compared to LH 10 ng/mL and LH 50 ng/mL ($P = 0.01$ both), as well as 23% and 48% of CXCL12 compared to LH 50 ng/mL and LH 100 ng/mL treatments ($P = 0.03$ and $P = 0.01$) (Fig. 2). Considering the effect between the different concentrations of each hormone, cells treated with LH showed a 38% higher expression of IGFBP1 in the highest concentration group

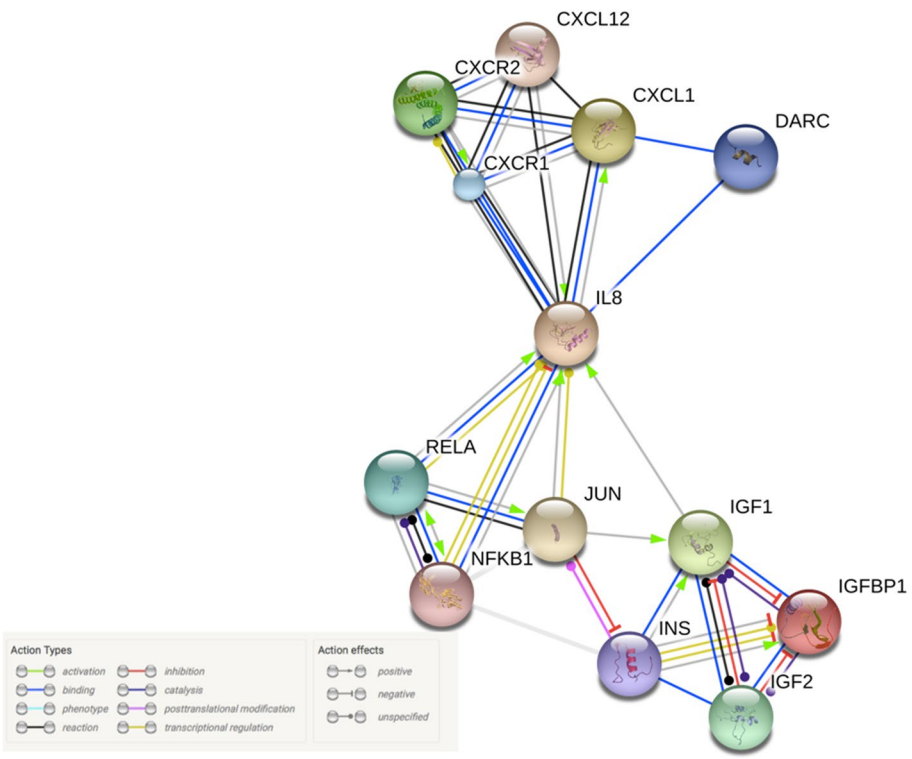

Fig. 1 Protein–protein interaction network between the implantation markers IGFBP1, IL8, CXCL1 and CXCL12 obtained from the STRING. Threshold used: interaction score at 0.7 and 10 interactions

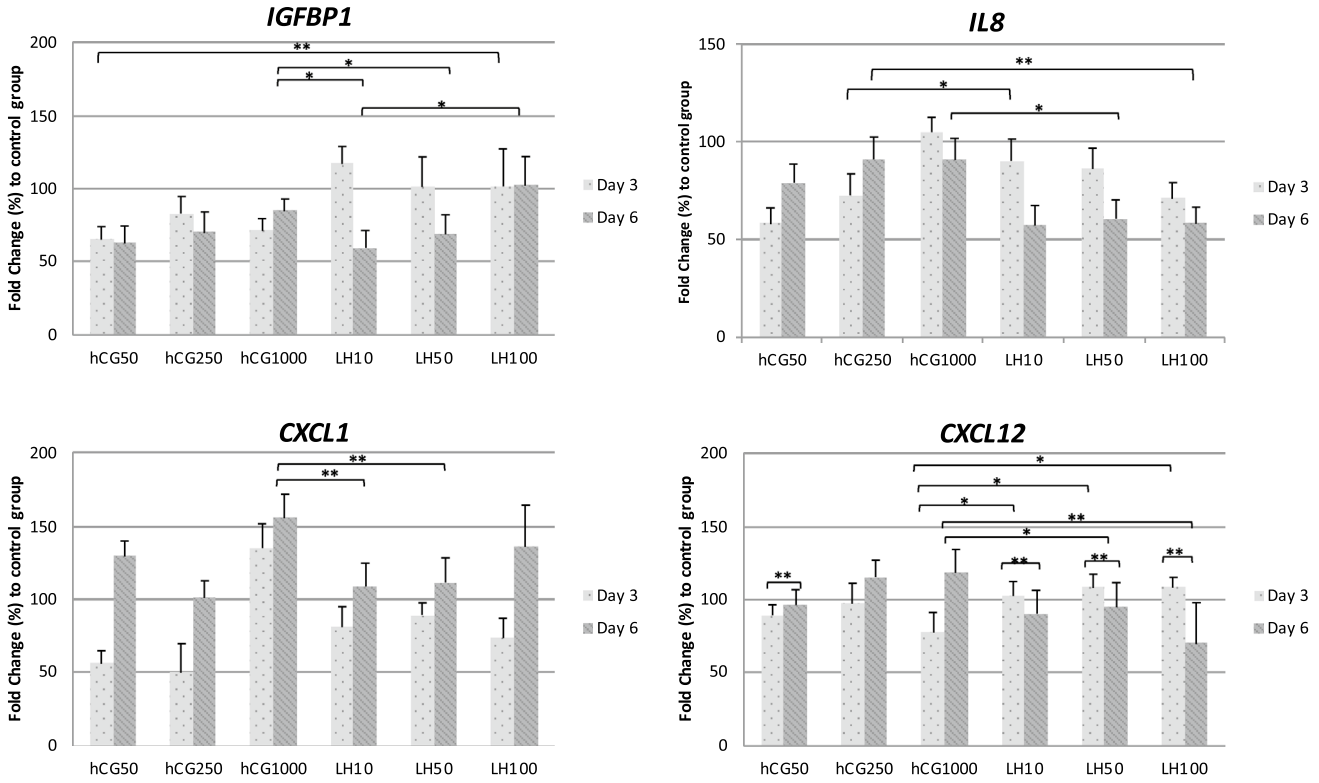

Fig. 2 Non-decidualized endometrial stromal cells implantation markers gene expression levels in response to different concentrations of hCG and LH compared to control group. Relative mRNA levels of IGFBP1, IL8, CXCL1 and CXCL12 to the housekeeping gene HPLO in response to hCG 50, 250 and 1000 ng/mL as well as LH 10, 50 and 100 ng/mL after 3 and 6 days of treatment followed by progesterone and hCG addition 2 days thereafter, depicted as percentage mean ± SD of fold change normalized by the control group. $^{*}P \leq 0.05$; $^{**}P \leq 0.01$

(LH 100 ng/mL) compared to the cells treated with the lowest LH concentration (LH 10 ng/mL) ($P = 0.05$).

Additionally, we compared the individual effect of each treatment on day 3 to day 6 and despite the fact that most groups tend to show a higher expression of IGFBP1, IL8 and CXCL1 implantation markers on day 6 compared to day 3, we only found a statistically significant difference in CXCL12 expression within the different treatment groups, showing a diminished expression over time (Fig. 3). Hence, cells treated with hCG 50 ng/mL, LH 10, LH 50 and LH 100 ng/mL showed a reduced expression by 45%, 55%, 55% and 67% of CXCL12 on day 6 compared to day 3 ($P = 0.01$, $P < 0.01$, $P = 0.01$, $P < 0.01$).

hCG treatment increases the expression of implantation markers in pre-decidualized cells

In vitro decidualization was performed to mimic physiologic conditions, and the effect of hCG and LH hormones on endometrial receptivity markers was evaluated. Firstly, the cells were pre-decidualized during 3 days, and prolactin levels from the supernatant were measured to confirm decidualization. Therefore, on day 3, the supernatant prolactin level from negative control was 12 ± 2 mU/L, while the positive control showed prolactin levels of 62.8 ± 34 mU/L. Afterward, the cells were treated for 3 days with low doses of hCG (hCG 50 and 250 ng/mL) or LH (LH 10 and 50 ng/mL) to provide physiologic concentrations. However, after 6 days decidualization-induced and 3 days treatment the prolactin levels were similar between the groups (Fig. 4).

Furthermore, microscopic morphology examination of negative and positive decidualization-induced cells by E2, MPA and cAMP (EPC) treatment was observed. Morphological shifts associated with in vitro decidualization were visualized in all groups of decidualized cells as shown in Fig. 5. The characterized cellular enlargement is visualized in Fig. 5a. Vimentin stain was performed to confirm the stromal cytoskeletal organization during decidualization. Upon decidualization, endometrial stromal cells undergo cytoskeletal rearrangement from elongated, fibroblastic cells to rounded epithelioid cells as well as changes within the nucleus, remodeling to rounding morphology (Fig. 5b).

Additionally, after 3 days of stimuli, the cells treated with hCG 250 ng/mL showed an increased IL8 and CXCL12 expression by 36% and 32% compared to the LH 10 ng/mL group, respectively, ($P = 0.03$ and $P = 0.01$) (Fig. 6).

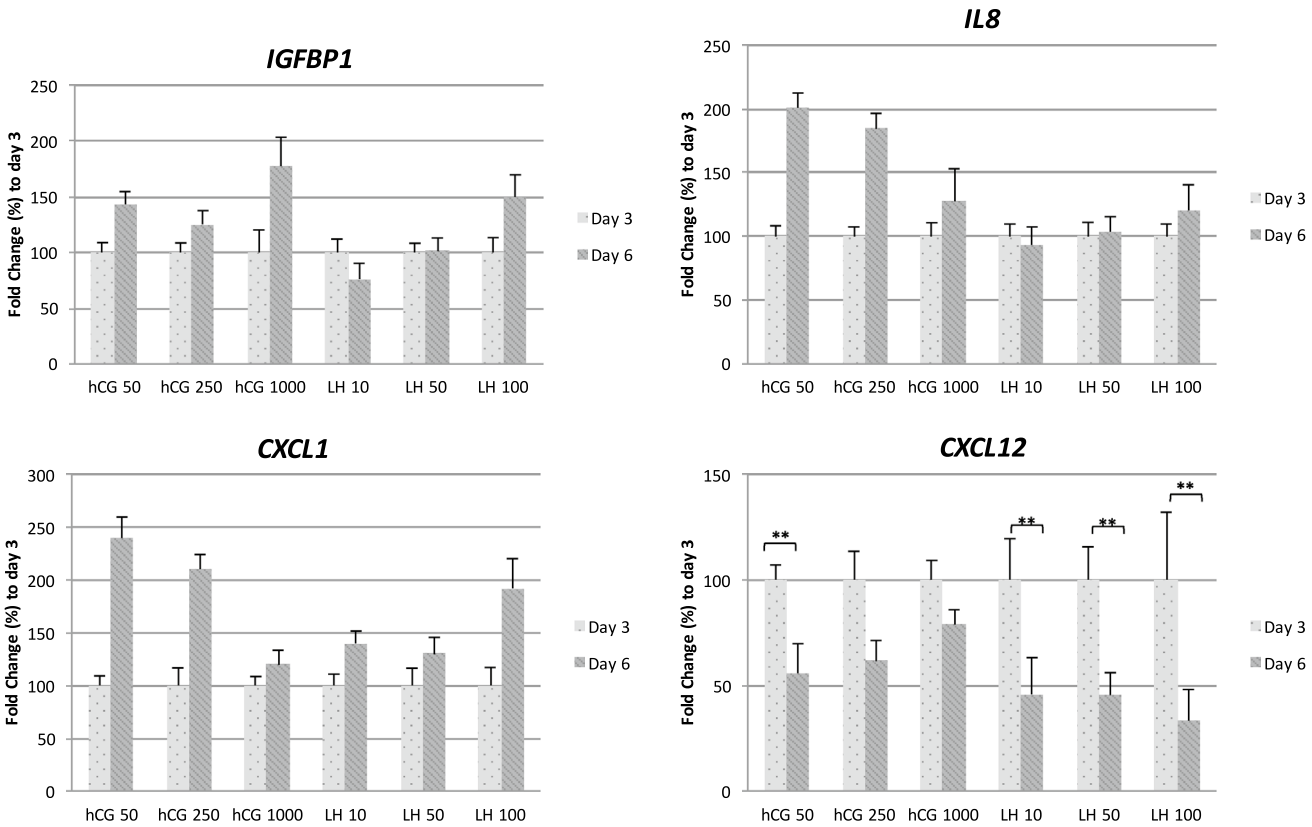

Fig. 3 Relative gene expression levels of implantation markers over time (day 6 compared to day 3) in response to a one time exposure to different concentrations of hCG and LH in non-decidualized endometrial stromal cells followed by progesterone and hCG addition on day 2 and 5. Gene levels of IGFBP1, IL8, CXCL1 and CXCL12 of each individual treatment, hCG 50, 250 and 1000 ng/mL, LH 10, 50 and 100 ng/mL, examined 3 days versus 6 days after exposure, considering day 3 as 100% expression are depicted as percentage mean ± SD normalized to day 3 of each individual group. *$P \leq 0.05$; **$P \leq 0.01$

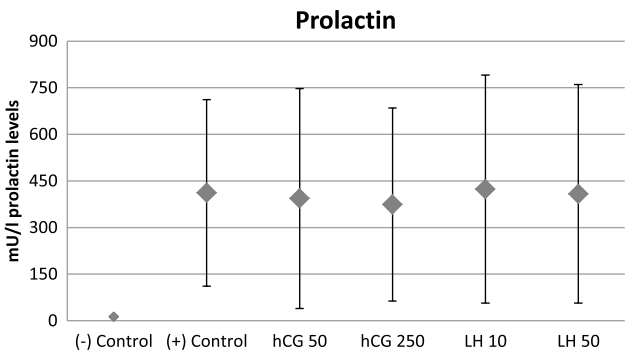

Fig. 4 Prolactin levels secreted by in vitro decidualized endometrial stromal cells in response to different concentrations of hCG and LH. Measurement of prolactin levels in the supernatant of in vitro decidualized cells after 6 days of decidualization-induced and in response to the different treatment groups: (−) control = negative control; (+) control = positive control; hCG 50 ng/mL; hCG 250 ng/mL; LH 10 ng/mL; LH 50 ng/mL. Values presented in medians and ranges (min–max) of mU/L prolactin levels of five independent experiments

Discussion

Controlled ovarian stimulation plays an important role in human reproduction therapies, controlling quality and number of oocytes [20]. The hormones hCG and LH are most used to induce ovulation and support early pregnancy [21]. Several clinical studies have been performed to evaluate the beneficial effect of the different COS protocols [8, 22, 23]. However, in general the endometrium has received much less attention in the reproductive research, and the local effects of hCG and LH in the endometrial receptivity remain controversial. The present study evaluated the direct effects of different doses of hCG and LH on endometrial implantation markers in stromal cells in vitro with and without prior decidualization.

Endometrial decidualization is a key step to successful pregnancy. Several signaling cascades have to interact in order to provide an adequate environment for the implantation process of the trophoblast [24, 25]. An adequate ESCs decidualization is necessary for implantation, and its disruption may compromise the female fertility [26]. In in vitro

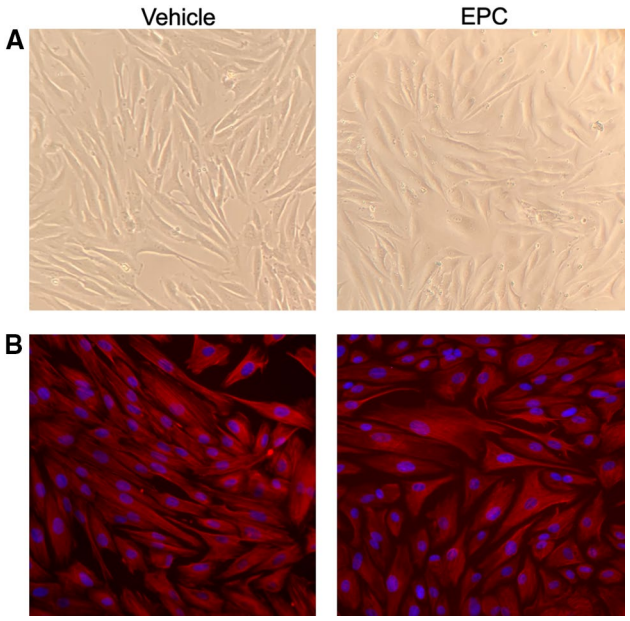

Fig. 5 Cellular changes of stromal endometrial cells during in vitro decidualization. Human endometrial stromal cells were cultured for 6 days in media containing either vehicle or E2, MPA and cAMP (EPC). **a** Cellular morphology changes from fibroblastic to epithelioid cells. Phase contrast microscope; 100 µm. **b** Cytoskeletal changes (red) and nucleus changes (blue) are shown using immunofluorescence: vimentin (red), DAPI (blue); 100 µm

studies, prolactin is a well-known decidualization marker for stromal cells [27]. Our data showed no difference in the effect of hCG and LH on the in vitro decidualization of ESCs, suggesting that both hormones contribute equally to the endometrial preparation for the arrival of the developed embryo. It is well defined that hCG and LH bind to the same receptor; however, it is important to note that the half-life of hCG is much longer than that of LH [28, 29]. Licht et al. [30] reported that hCG/LH receptors undergo alternative splicing as regulatory mechanism by which endometrium controls the expression of full-length and functional receptors. A pattern of full-length LHCGR mRNA expression was observed up to mid-secretory phase being downregulated during late secretory phase and early pregnancy decidua, supporting the concept of positive direct role for LH and hCG during early secretory changes in the endometrium.

Moreover, endometrial biopsy of ART patients demonstrated that pro-inflammatory cytokines are upregulated during implantation: macrophage inflammatory protein, tumor necrosis factor alpha (TNFa), CXCL1, osteopontin (OPN) and interleukins, as well as the influx of specific immune cells. These implantation markers can be secreted by endometrial cells as well as by immune cells recruited during the window of implantation [31]. It seems that these immune cells trigger endometrial stromal cells to secrete chemokines (IL-8, CXCL1, CCL8) that act synergistically with uterine-specific natural killer (NK) cells inducing trophoblast migration. This inflammatory reaction may facilitate embryo apposition and attachment to the uterine wall [32]. Furthermore, similar to prolactin, IGFBP-1 is a marker for endometrial stromal cell in vitro decidualization [27] and it also participates in the cell-to-cell communication between fetal trophoblasts and maternal decidual cells through interaction with IGF-II secreted by the embryo [33].

In the present study, we used the STRING database, which aims to collect and integrate all information, providing a critical assessment and consolidating known and predicted protein–protein association data, including direct (physical) as well as indirect (functional) associations [34, 35] to visualize the gene interaction network among the implantation markers selected in this study. There seems to be a functional link between the assessed well-known implantation markers IGFBP1, IL8, CXCL1 and CXCL12. The knowledge of all functional interactions between the expressed proteins is required to achieve a system-wide understanding. Furthermore, information about its specific interaction partners is an important prerequisite for a full protein's function description. Likewise, using combined algorithms to analyze gene interactions provide a new

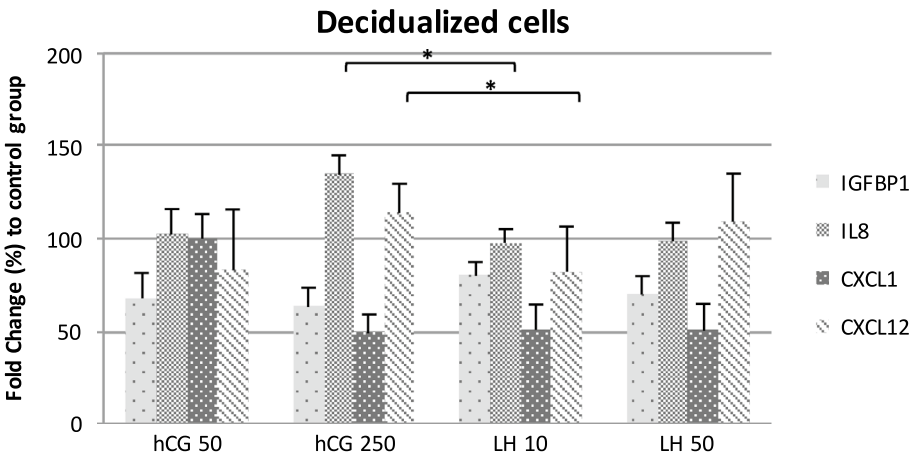

Fig. 6 In vitro decidualized endometrial stromal cells relative implantation marker gene expression in response to different concentrations of hCG and LH. Levels of *IGFBP1*, *IL8*, *CXCL1* and *CXCL12* mL gene expression after 6 days of decidualization-induced and in response to 3 days of hCG 50 and 250 ng/mL, LH 10 and 50 ng/treatment depicted as percentage mean ± SD of fold change normalized by the control group. $*P \leq 0.05$; $**P \leq 0.01$

perspective for network-based analysis may reveal new insights into the molecular mechanisms [36].

During ART cycles, while hCG is directly applied, LH is indirectly released from the pituitary after application of a GnRH agonist injection and is used to prevent the ovarian hyperstimulation syndrome (OHSS) [37]. Relevant for ART, however, is that the use of GnRH agonist ovulation trigger prevents OHSS compared to hCG. However, many have shown that it leads to lower implantation rates [14]. It is believed that the sole GnRHa trigger leads to a compromise of the endometrial function, which can be overcome by adding low dose HCG after oocyte retrieval [38]. Even though hCG and LH are similar in structure and bind to a common receptor, the downstream effects may differ enormously [37, 39]. Our data showed that in an endometrial environment not well arranged for implantation (non-decidualized cells); LH showed a higher effect increasing the IGFBP1, IL-8 and CXCL12 expression on day 3 compared to hCG. However, over time this pattern changed, and on day 6 hCG showed a greater effect on the implantation markers expression compared to LH, suggesting an earlier local effect of LH while this effect is overcome by hCG later on. Furthermore, in an endometrial environment arranged for implantation (decidualized cells), hCG showed superior favorable effects over LH regarding the expression levels of IL-8 and CXCL12. This may explain the positive results seen after local intrauterine hCG applications before embryo transfer, improving the implantation and pregnancy rates [8, 40]. Although, the results are promising, there are still no conclusions about the benefits regarding the other complications after hCG exposure [41].

Our data suggest that hCG might have a greater direct effect on endometrial receptivity when compared to LH and it may explain the negative impact seen in ART cycles after GnRH agonist ovulation induction when blastocyst transfers are performed, showing low live birth rate, lower ongoing pregnancy rate and higher rate of early miscarriage compared to hCG, suggesting beneficial local effects of hCG on endometrium regulation. However, for women who do not receive a fresh embryo transfer, donate oocyte or freeze their oocytes, GnRH agonist could be useful, as the influence on the endometrial function is irrelevant [42]. The present study improves the knowledge about molecular endometrial mechanism during implantation, increasing the understand of the inherent differences between hCG and LH effect on endometrium. However, further studies are needed to clarify the precise effect of both protocols in the endometrial receptivity.

Conclusions

Our results suggest that hCG might have a greater direct effect on endometrial receptivity when compared to LH.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest regarding this paper.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All patients provided written consent for the use of their tissue samples. The study was approved by the Ethics Committee of Ruprecht Karls University Heidelberg (S239/2005).

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