Effects of ulipristal acetate on human embryo attachment and endometrial cell gene expression in an in vitro co-culture system

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STUDY QUESTION: Does ulipristal acetate (UPA) used for emergency contraception (EC) interfere with the human embryo implantation process?

SUMMARY ANSWER: UPA, at the dosage used for EC, does not affect human embryo implantation process, in vitro.

WHAT IS KNOWN ALREADY: A single pre-ovulatory dose of UPA (30 mg) acts by delaying or inhibiting ovulation and is recommended as first choice among emergency contraceptive pills due to its efficacy. The compound has also been demonstrated to have a dose-dependent effect on the endometrium, which theoretically could impair endometrial receptivity but its direct action on human embryo implantation has not yet been studied.

STUDY DESIGN, SIZE, DURATION: Effect of UPA on embryo implantation process was studied in an in vitro endometrial construct. Human embryos were randomly added to the cultures and cultured for 5 more days with UPA (n = 10) or with vehicle alone (n = 10) to record the attachment of embryos.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Endometrial biopsies were obtained from healthy, fertile women on cycle day LH+4 and stromal and epithelial cells were isolated. A three-dimensional in vitro endometrial co-culture system was constructed by mixing stromal cells with collagen covered with a layer of epithelial cells and cultured in progesterone containing medium until confluence. The treatment group received 200 ng/ml of UPA. Healthy, viable human embryos were placed on both control and treatment cultures. Five days later the cultures were tested for the attachment of embryos and the 3D endometrial constructs were analysed for endometrial receptivity markers by real-time PCR.

MAIN RESULTS AND THE ROLE OF CHANCE: There was no significant difference in the embryo attachment rate between the UPA treated group and the control group as 5 out of 10 human embryos exposed to UPA and 7 out of 10 embryos in the control group attached to the endometrial cell surface (P = 0.650). Out of 17 known receptivity genes studied here, only 2 genes, HBEGF (P = 0.009) and IL6 (P = 0.025) had a significant up-regulation and 4 genes, namely HAND2 (P = 0.003), OPN (P = 0.003), CALCR (P = 0.016) and FGF2 (P = 0.023) were down-regulated with the exposure of UPA, compared with control group.

LIMITATIONS, REASONS FOR CAUTION: This proof of concept study was conducted with a few human embryos, as their availability was limited. Although the 3D model used for this study is well established and the artificial endometrial luminal epithelium shown to express progesterone regulated markers of endometrial receptivity it is still an in vitro model, lacking all cell types that constitute the receptive endometrium in vivo.

WIDER IMPLICATIONS OF THE FINDINGS: This study provides new insights on the mechanism of action of UPA on human embryo implantation, demonstrating that UPA in a dosage used for EC does not affect embryo viability and the implantation process of embryo.

† Equal author contribution.
Introduction

Effective and safe methods for contraception are a prerequisite for reproductive health in preventing unplanned pregnancy, unsafe abortions and reducing maternal mortality and morbidity. Unwanted pregnancies resulted in an estimated 43.8 million abortions worldwide in 2008, of which 21.6 million were unsafe. The numbers of unsafe abortions are increasing due to the growing population of women of reproductive age and substantially contribute to maternal morbidity and mortality (Sedgh et al., 2012). The ability to control fertility through the use of effective and safe contraception is thus essential in preventive medicine. A recent study calculated that fulfilling the unmet contraception need worldwide would reduce maternal mortality by nearly a third (Ahmed et al., 2012). This includes making emergency contraception (EC) easily available and acceptable. However, in many countries effective contraceptive methods are withheld from women due to political and religious reasons and ideas that these drugs act on events that occur after fertilization.

Emergency contraception includes contraceptive methods that can be used after unprotected sexual intercourse (UPSI), failed contraception or sexual assault to prevent an unwanted pregnancy. Oral hormonal EC pills (ECPs) have almost no medical contraindications, are safe and highly accepted by women and may reduce the risk of unintended pregnancy up to 75% (Orihuela, 2010). However, there are many barriers to widespread access and use of ECPs including lack of knowledge on the mechanisms of action, especially with regard to effect on the endometrium and embryo implantation.

It is only during a short period of time in the menstrual cycle that the endometrium attains receptivity necessary for successful embryo implantation. This period is called the window of implantation (WOI), and occurs during cycle days 20–24 in the menstrual cycle. Steroid receptors including the progesterone receptor (PR) and their downstream target genes regulate uterine growth and differentiation, which controls the dynamic and highly coordinated event of molecular signalling between the receptive endometrium and the embryo, that also itself secretes various important factors (Norwitz et al., 2001; Pawar et al., 2014). The estimated rate of implantation in natural cycles is 15–30% (Miller et al., 1980).

Ulipristal acetate (UPA) is a selective progesterone receptor modulator (sPRM) with both agonistic and antagonistic effects on PR and has been specifically developed for EC as a 30 mg oral tablet (Chabbert-Buffet et al., 2005). Clinical trials comparing UPA and levonorgestrel (LNG) as an EC have demonstrated that UPA significantly reduces the pregnancy rate after UPSI and prevented more pregnancies than the gold standard LNG (Glasier et al., 2010). UPA is now recommended as the first choice of EC, where available, due to its efficacy (Lalitkumar et al., 2013a).

UPA has been demonstrated to act through delaying or inhibiting follicular development and ovulation. Recent pharmacodynamic studies that compared how different EC regimens prevented ovulation if administered in advanced follicular phase, demonstrated that UPA was superior to LNG (Croxatto et al., 2004; Brache et al., 2010, 2013). UPA interferes with the ovulatory process even in the presence of rising LH (Brache et al., 2013). However, if administered at or after the LH has peaked, UPA cannot prevent ovulation (Brache et al., 2010). The effect of UPA on the endometrium has been demonstrated to be dose-dependent and when given in a high dose or repeated doses it affects endometrial histology suggesting that UPA could theoretically impair the implantation of the embryo (Blithe et al., 2003; Chabbert-Buffet et al., 2007). In a prospective, randomized clinical trial, regularly cycling women were administered UPA in a single dose of 10, 50 or 100 mg or placebo after ovulation but within 2 days of the LH surge and the endometrium was studied with ultrasound as well as morphologically. The study showed that UPA caused inhibition of down-regulation of PRs, a decrease in peripheral node addressins and reduced endometrial thickness without altering hormone levels or cycle in a dose-dependent fashion but with no significant increase in delayed endometrial maturation compared with placebo (Stratton et al., 2010).

The mechanism of action of UPA as effective contraceptive agent is not completely understood, especially with regard to the effect on the endometrium (Thomin et al., 2014). Since it is ethically and technically difficult to study human embryo implantation in vivo we have developed a three-dimensional human endometrial culture system. Our model mimics the physiological endometrial environment to a large extent and allows experimental modulation by adding factors to the co-culture to investigate the role and function of individual molecules involved in the early stages of embryo implantation (Lalitkumar et al., 2007, 2013b; Meng et al., 2009).

The aim of this study was to investigate the effect of UPA in concentration relevant for EC on human embryo implantation in vitro.

Materials and Methods

Ethical consideration

This study, including the usage of human embryos was approved by the regional Ethics committee at Karolinska Institutet, Stockholm, Sweden. Written informed consent was obtained from all the participating subjects. Supernumerary embryos or embryos cryopreserved for >5 years, which were to be discarded otherwise, were donated by couples who had undergone IVF after obtaining written informed consent.

Endometrial biopsies

Endometrial samples were obtained from healthy volunteers (n = 20), aged 22–40 years, with normal menstrual cycles (25–35 days) and proven fertility. None of the women had used any hormonal contraception or intrauterine device for a minimum of 3 months prior to the biopsy. The subjects determined the urinary LH peak by testing urine samples twice daily from cycle between the receptive endometrium and the embryo, that also itself controls the dynamic and highly coordinated event of molecular signalling stream target genes regulate uterine growth and differentiation, which receptors including the progesterone receptor (PR) and their down mechanisms of action, especially with regard to effect on the endometrium and embryo implantation.

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day 10 to LH +2, using a rapid self-test (Clearplan, Searle Unipath Ltd, Bedford, UK). The endometrial biopsies were obtained from the fundal part of the uterine cavity without cervical dilation or local anaesthesia using a Pipelle aspirator (Cooper Surgical, USA) on cycle day LH +4. All women were instructed to use barrier methods for contraception if not sterilized (themselves or their partners).

**Cell isolation**
Endometrial biopsies were immediately processed for isolation of stromal and epithelial cells as per published protocol (Lalitkumar et al., 2013b). In brief, the biopsies were minced 1 x 1 mm with scalpel in fresh Ham F10 and incubated with pancreatin-trypsin EDTA (0.05 g/ml of trypsin-EDTA solution). The tissue was treated with a mixture of collagenase IV and DNAse (Worthington Biochemical, Lakewood, USA) and filtered using a 40 micron mesh cell strainer which allowed single stromal cells to pass through and epithelial glands to be retained in the strainer. Epithelial glands were then treated with a mixture of collagenase 3 (Worthington Biochemical) and DNAse and filtered by a 40 micron mesh cell strainer which yielded a single cell population of epithelial cells. Both stromal and epithelial cells were preserved in liquid nitrogen until used for in vitro endometrial cell culture.

**Three-dimensional endometrial cultures**
Endometrial 3D co-cultures were constructed as described by Lalitkumar et al. (2007) with minor modifications. In brief, stromal cells were mixed with 3D gel composed of Bovine collagen solution Type I (Purecol 3 mg/ml, Advanced Biomatrix, San Diego, USA), 10X PBS and NaOH, pH 7.4 and overlaid on the collagen gel in Millicell cell culture inserts (EMD Millipore, Darmstadt, Germany). A thin layer of basement membrane extract (Trevigen, Maryland, USA) was coated on the stromal gel. Epithelial cells mixed with modified alpha medium supplemented with Amniomax (Life Technologies) and glutamate (Life Technologies) were distributed over the basement membrane extract coating and cultured for 5 days in complete alpha medium (final concentration of estrogen and progesterone in complete alpha medium was 0.3 and 902 nM respectively) with medium change every 2 days in a 5% CO2 incubator. After 5 days of epithelial confluence the cultures were treated (n = 10) with ulipristal acetate—200 ng/ml (Medchem Express LLC, Princeton, NJ, USA) in ethanol as solvent vehicle. The control cultures (n = 10) were cultured in alpha medium with only vehicle added. Healthy human blastocyst stage embryos (graded between 3 and 4; AA/AB/BA) were randomly added to the cultures after thawing and each construct received a healthy, viable human embryo after examination by a clinical embryologist. Vitellated blastocysts were warmed in Thermoblast™ Blast kit (Nidacon, Sweden) as per the manufacturers protocol and allowed to equilibrate for a minimum of 2 h in complete alpha medium before they were used on 3D cultures. A medium change was given every 2 days in both the control and UPA group and the cultures were observed for attachment. The cultures were examined for embryo every other second day until terminated on Day 5 and checked for attachment of blastocysts. The attachment of embryos were tested by mechanically shaking the cultures, followed by washing twice with PBS. The attached embryos were examined under light microscope and documented. The constructs were removed from the culture inserts and the embryo-attached portion along with the embryo was dissected out and the construct without the embryo was dissolved in 1 ml of Trizol reagent (Life technologies) and stored at −80°C.

**RNA extraction and real time PCR**
Total RNA was extracted from trizol dissolved 3D cultures using Arcturus PicoPure RNA isolation kit (Life technologies) as per the manufacturers protocol and all the samples were DNase treated to eliminate the contaminating genomic DNA for real-time PCR experiments (RT-PCR). RNA quality check was done on Agilent 2100 bioanalyzer using RNA Pico chip as per the manufacturers protocol. 20 μl first strand cDNA was synthesized using Superscript VILO mastermix (Life Technologies), composed of Superscript III Reverse Transcriptase, RNaseOUT Recombinant ribonuclease inhibitor, random primers, MgCl2 and dNTP’s. All the thermal steps were performed in a Biorad Mycycler Thermal cycler and cDNA synthesis was done as per the manufacturers protocol. A no template control (NTC) and RT control were also prepared simultaneously as controls in the RT-PCR.

Real-time PCR analysis was performed for 17 endometrial receptivity markers and PR using 18S as housekeeping gene on StepOne Plus instrument (Life technologies). All runs were performed in triplicates using Taqman Universal PCR Master mix (Life technologies) in a microamp fast optical 96 well reaction plate (Life technologies) with relative quantification method and the results were analysed by ΔΔCt method. The details of Taqman primers used for RT-PCR are summarized in Supplementary Table SI. RT-PCR was performed according to the manufacturers protocol using the following thermal cycling conditions: 50°C for 2 min, 95°C for 10 min and 40 cycles with 95°C for 15 s and 60°C for 1 min. The results are expressed in terms of fold change (2−ΔΔCt).

**Statistics**
Fisher’s exact test was applied to evaluate the difference in blastocyst attachment rate between groups. Statistical analysis for RT-PCR data and blastocyst attachment rate for 3D cell cultures were done using XLStat 2014 (AddinSoft SARL). Based on the homogeneity and distribution of the data, independent t-test or non-parametric Mann–Whitney’s test were performed for comparing the difference between control group and UPA treated group. The results are expressed as mean ± SE and P-value < 0.05 is considered as significant.

**Results**

**Embryo attachment**
A total of 26 embryos were warmed in this study of which 20 embryos survived. Ten embryos were randomly allocated to each group. Out of the 10 blastocysts exposed to UPA at 200 ng/ml dosage, 5 attached to the cultures. In the control group, out of 10 blastocysts, 7 attached to the cultures (Figs. 1 and 2). There was no significant difference in

![Figure 1](https://academic.oup.com/humrep/article-abstract/30/4/800/616120/16.120)

**Figure 1** No significant difference was observed in the number of human embryos attached to the in vitro endometrial construct exposed to 200 ng/ml ulipristal acetate (UPA) compared with control group.
the blastocyst attachment rate ($P = 0.650$) between the control and UPA exposed groups.

**Endometrial receptivity markers**

To study the effect of UPA on endometrial receptivity markers, 17 genes relevant to endometrial receptivity and embryo implantation were selected for RTPCR analysis (Table I). All the cultures, irrespective of treatment or attachment of embryo, expressed mRNA for progesterone receptor (PGR) and the expression of PGR with UPA showed a significant decrease compared with control group ($P < 0.001$, Table I). However, within the group, the groups with embryo attached and non-attached did not show any significant difference. Eleven genes studied had

**Table I** Expression levels of selected genes involved in endometrial receptivity, embryo implantation process and decidualisation analysed by real-time PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control $\Delta C_T$ mean ± SE</th>
<th>UPA $\Delta C_T$ mean ± SE</th>
<th>$\Delta C_T$</th>
<th>Fold change</th>
<th>$P$-value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALCR</td>
<td>28.002 ± 0.19</td>
<td>29.336 ± 0.26</td>
<td>1.334</td>
<td>−2.522</td>
<td>0.016*</td>
<td>Li et al. (2008) and Kumar et al. (1998)</td>
</tr>
<tr>
<td>COUP-TFI</td>
<td>18.777 ± 0.04</td>
<td>19.970 ± 0.04</td>
<td>1.193</td>
<td>−2.288</td>
<td>0.306</td>
<td>Kurihara et al. (2007) and Takamoto et al. (2005)</td>
</tr>
<tr>
<td>CSF1</td>
<td>18.888 ± 0.10</td>
<td>18.899 ± 0.10</td>
<td>0.011</td>
<td>−1.008</td>
<td>0.48</td>
<td>Wurzel et al. (2010)</td>
</tr>
<tr>
<td>FGF2</td>
<td>16.790 ± 0.04</td>
<td>17.832 ± 0.05</td>
<td>1.041</td>
<td>−2.058</td>
<td>0.023*</td>
<td>Taniguchi (1998)</td>
</tr>
<tr>
<td>FOXO1</td>
<td>16.861 ± 0.05</td>
<td>17.870 ± 0.05</td>
<td>1.009</td>
<td>−2.012</td>
<td>0.055</td>
<td>Kim et al. (2005) and Christian et al. (2002)</td>
</tr>
<tr>
<td>HAND2</td>
<td>17.653 ± 0.05</td>
<td>19.105 ± 0.058</td>
<td>1.452</td>
<td>−2.736</td>
<td>0.003*</td>
<td>Li et al. (2011)</td>
</tr>
<tr>
<td>HBEGF</td>
<td>20.660 ± 0.055</td>
<td>19.350 ± 0.03</td>
<td>−1.31</td>
<td>2.479</td>
<td>0.009*</td>
<td>Xie et al. (2007) and Chobotova et al. (2002)</td>
</tr>
<tr>
<td>HOXA10</td>
<td>20.406 ± 0.03</td>
<td>20.499 ± 0.05</td>
<td>0.093</td>
<td>−1.067</td>
<td>0.307</td>
<td>Lim et al. (1999) and Taylor et al. (1998)</td>
</tr>
<tr>
<td>IGFBP1</td>
<td>23.196 ± 0.07</td>
<td>23.614 ± 0.131</td>
<td>0.418</td>
<td>−1.336</td>
<td>0.635</td>
<td>Kim et al. (2005), Matsumoto et al. (2008) and Telgmann and Gellersen (1998)</td>
</tr>
<tr>
<td>IL1A</td>
<td>18.128 ± 0.06</td>
<td>17.792 ± 0.08</td>
<td>−0.336</td>
<td>1.262</td>
<td>0.065</td>
<td>Simon et al. (1994)</td>
</tr>
<tr>
<td>IL6</td>
<td>17.990 ± 0.04</td>
<td>16.970 ± 0.06</td>
<td>−1.396</td>
<td>2.632</td>
<td>0.025*</td>
<td>Robertson et al. (2000)</td>
</tr>
<tr>
<td>LIF</td>
<td>16.417 ± 0.03</td>
<td>16.893 ± 0.02</td>
<td>0.476</td>
<td>−1.391</td>
<td>0.107</td>
<td>Lalitkumar et al. (2013b) and Stewart et al. (1992)</td>
</tr>
<tr>
<td>MUC1</td>
<td>17.096 ± 0.05</td>
<td>17.617 ± 0.035</td>
<td>0.5213</td>
<td>−1.435</td>
<td>0.391</td>
<td>Meseguer et al. (2001)</td>
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<tr>
<td>OPN</td>
<td>16.455 ± 0.05</td>
<td>18.414 ± 0.025</td>
<td>1.959</td>
<td>−3.887</td>
<td>0.0036*</td>
<td>Apparao et al. (2001) and von Wolff et al. (2001)</td>
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<tr>
<td>PRL</td>
<td>21.756 ± 0.153</td>
<td>22.311 ± 0.149</td>
<td>0.554</td>
<td>−1.469</td>
<td>0.427</td>
<td>Matsumoto et al. (2008) and Telgmann and Gellersen (1998)</td>
</tr>
<tr>
<td>SGK1</td>
<td>28.083 ± 0.333</td>
<td>29.336 ± 0.117</td>
<td>1.253</td>
<td>−2.383</td>
<td>0.055</td>
<td>Salker et al. (2011)</td>
</tr>
<tr>
<td>VEGFA</td>
<td>18.340 ± 0.054</td>
<td>18.215 ± 0.047</td>
<td>−0.125</td>
<td>1.09</td>
<td>0.596</td>
<td>Halder et al. (2000) and Torry et al. (1996)</td>
</tr>
<tr>
<td>PGR</td>
<td>22.922 ± 0.107</td>
<td>24.910 ± 0.174</td>
<td>2.288</td>
<td>−4.885</td>
<td>0.001*</td>
<td>Lydon et al. (1995)</td>
</tr>
</tbody>
</table>

Out of 17 genes analysed, 11 genes did not show any significant change with UPA treatment. Relevant references against each gene about its role in endometrial receptivity or embryo implantation process are mentioned under references.

*Statistically significant.
no significant difference in the expression with UPA treatment (Fig. 3A and B). Real-time PCR analysis showed six genes differentially expressed with UPA exposure compared with control (Fig. 4). The genes studied include cytokines, growth factors, integrins and transcription factors.

Cytokines: With the exposure to UPA, a significant up-regulation in the gene expression of interleukin-6 (IL6) fold change (FC) \(= 2.632, P = 0.025\) was observed whereas interleukin-1A (IL1A, FC \(= 1.262, P = 0.065\)) did not show any significant difference. There was no change in the expression of leukaemia inhibitory factor (LIF, FC \(= -1.391, P = 0.107\)) with UPA exposure.

Transcription Factors: Of the transcription factors studied, heparin-binding EGF-like growth factor (HBEGF FC \(= 2.479, P = 0.009\)) and heart- and neural crest derivatives-expressed protein 2 (HAND2, FC \(= -2.736, P = 0.003\)) showed a significant up- and down-regulation respectively with UPA treatment. Forkhead box protein O1 (FOXO1, FC \(= -2.012, P = 0.055\)), COUP transcription factor 2 (COUP-TFII, FC \(= -2.288, P = 0.306\)) and homeobox-A10 (HOXA10, FC \(= -1.067, P = 0.307\)) showed no significant difference in their expression between the groups.

Among other known receptivity markers, a significant down-regulation was observed in the gene expression of calcitonin receptor (CALCR, FC \(= -2.522, P = 0.016\)), osteopontin (OPN) (FC \(= -3.887, P = 0.003\)) and fibroblast growth factor 2 (FGF2, FC \(= -2.058, P = 0.023\)) with UPA exposure. Serum/glucocorticoid-regulated kinase 1 (SGK1, FC \(= -2.383, P = 0.055\)), mucin1 (MUC1, FC \(= -1.435, P = 0.391\)), colony stimulating factor 1 (CSF1, FC \(= -1.008, P = 0.480\)), vascular

**Figure 3** (A) Real-time PCR analysis showed no difference in the expression of cytokines IL1A, VEGFA, LIF, CSF1 involved in endometrial receptivity and no significant difference was observed in the expression of decidualization factors PRL and IGFBP1 on exposure of ulipristal acetate (UPA) to the endometrial construct in vitro. (B) Transcription factors (FOXO1, SGK1, COUP-TFII) Homeobox gene (HOXA 10) and cell adhesion molecule (MUC1) known to be involved in endometrial receptivity showed no significant change with the exposure of ulipristal acetate (UPA) to the in vitro endometrial construct. The boxes indicate 25–75 percentile and the whiskers spread from the smallest to largest data points.
endothelial growth factor A (VEGFA, FC = 1.09, \(P = 0.596\)) and decidualization factors prolactin (PRL, FC = -1.469, \(P = 0.427\)) and Insulin like Growth Factor Binding Protein 1 (IGFBP1, FC = -1.336, \(P = 0.653\)) showed no significant difference in expression after UPA exposure.

The analysis between the cultures with the embryo attached and non-attached in either control or treatment groups did not show any significant difference (\(P > 0.05\)) in the expression of the 17 genes studied (data not shown).

**Discussion**

Due to the worldwide debate regarding the mechanism of action of various ECs and especially UPA-EC, there is an urgent need to understand the effect of ECPs on preimplantation or implanted embryos, so that women can be provided with correct information. This study demonstrates for the first time that UPA in the dose used for EC does not affect human embryo or implantation process as the rate of blastocyst attachment to the UPA exposed endometrial construct did not significantly differ from that of controls.

It is impossible to study the effect of EC, including UPA in human embryo implantation process in vivo, due to ethical, legal and technical reasons. Most of the currently available data on the effect of UPA on embryo implantation has been derived from animal studies (Reel et al., 1998; Hild et al., 2000; Singh et al., 2011). Non-human primate models also have contributed in understanding the mechanism of antiprogestin action (Lalitkumar et al., 1998; Sengupta et al., 2003). Although there are similarities to human reproductive events in non-human primates, the process of human embryo implantation is a unique process and studies of this event in different animal models have limitations (Koot et al., 2012). We have developed and described a 3D endometrial culture system that mimics endometrial cell environment to a large extent and provides the possibility of studying human embryo implantation process including its regulation by progesterone as well as cytokines...
(Lalitkumar et al., 2007; Meng et al., 2009). Although this culture system is complex, it is now well established and has proven to be robust and reproducible. This model has also been tested and validated for the studies on human embryo attachment using LNG, mifepristone and LIF inhibitor (Lalitkumar et al., 2007, 2013b; Meng et al., 2009).

In this in vitro study, we have used a dose of UPA, equivalent to the conventional dose of UPA when used for EC. UPA at a dose of 30 mg orally has a maximum mean serum concentration ($C_{\text{max}}$) of 176 ± 89 ng/ml, which is observed approximately after one hour of administration (Kim and Bridgeman, 2011). However, in our study, the treatment group of cultures were continuously exposed to UPA 200 ng/ml, which is slightly higher than the observed serum concentration as it is known that the endometrial concentration of steroids is higher than the serum levels. Despite using a pharmacological dose, we do not see any significant difference in the attachment of embryos following treatment with UPA compared with controls. Also, there were no observable degenerative changes in the embryos. This further substantiates that UPA at this dose, does not compromise embryo viability nor compromise its ability to attach.

Both, in vivo and in vitro studies have shown that UPA which is a sPRM binds to progesterone receptor (Attardi et al. 2002, 2004). This further impacts the signal transduction mediated by PR and affects endometrial receptivity according to its binding region in the hormone response element. Not all sPRMs act in the same manner on endometrial

**Figure 4** Six out of eleven genes suggested to be involved in endometrial receptivity in the endometrial construct, namely HAND2, OPN, HBEGF, CALCR, FGF2 and IL6 showed significant difference in their expression levels as analysed by real-time PCR on exposure with ulipristal acetate (UPA). The boxes indicate 25–75 percentile and the whiskers spread from the smallest to largest data points.
receptivity. Although implantation itself arguably would be the best marker of endometrial receptivity, it is of great importance to evaluate the endometrium at the molecular level for understanding how endometrial receptivity and implantation is regulated by different sPRMs. Important markers of endometrial receptivity include cell adhesion molecules, cytokines, growth factors and prostaglandins (Zhang et al., 2012). Previous studies on the effect of UPA on human endometrium were restricted to studies on histological evaluation with endometrial dating and by measuring its thickness (Bliithe et al., 2003; Williams et al., 2012; Huang et al., 2014). These parameters only roughly correlate with endometrial receptivity and fertility (Coutafaris et al., 2004). Other important studies on UPA and endometrial receptivity have only examined a very limited number of receptivity markers (Stratton et al., 2000, 2010). Although no single endometrial receptivity marker has yet been defined in humans, a large number of differentially expressed genes and proteins that are potential markers of endometrial receptivity present in the endometrium during WOI, have been identified (Lessey et al., 1992; Chobotova et al., 2002; Kao et al., 2002; Horcajadas et al., 2004; Mirkin et al., 2005; Popovici et al., 2006). However, the vast amount of data and information generated from global gene expression studies show a large degree of variability and low reproducibility (Altmae et al., 2014). There is not much information in the literature on the effect of UPA on different endometrial receptivity markers. Here, we examined the expression of 17 genes belonging to different functional groups including cytokines, growth factors and transcription factors involved in endometrial receptivity. We chose to perform the gene expression analysis, as most of the earlier reported studies had performed gene expression analysis of the selected molecules (Kao et al., 2002; Riesewijk et al., 2003; Horcajadas et al., 2004; Mirkin et al., 2005; Popovici et al., 2006; Hess et al., 2007; Zhang et al., 2012; Garrido-Gomez et al., 2013).

In the present study we found that the level of genes of several factors believed to be vital for embryo implantation remain unaltered in the endometrial construct after exposure to UPA. There were no changes in the expression of 9 previously well-studied genes reported as endometrial receptivity markers, namely LIF, CSF1, FOXO1, HOXA10, MUC1, SGK1, IL1A, VEGFA and COUP-TFII (Kao et al., 2002; Gargiulo et al., 2004; Kurihara et al., 2007; Meng et al., 2009; Salker et al., 2011; Garrido-Gomez et al., 2013; Kajihara et al., 2013). There are several studies showing the importance of endometrial LIF in implantation (Stewart et al., 1992; Cheng et al., 2002; Wu et al., 2013). Low endometrial LIF was reported in women with infertility (Wu et al., 2013). Our group has earlier reported that LIF is essential for human embryo survival and implantation process using the same in vitro model (Laitkumar et al., 2013b). Furthermore administration of CSF1 improved pregnancy rate in women with repetitive implantation failure (Wurfel et al., 2010) and thus we chose to study the effect of UPA on this molecule. Decidualization is one of the immediate post-implantation reactions seen in endometrium and higher expression of IGFBP1 and prolactin (PRL) in endometrial cells are markers of decidualization (Telgmann and Gellersen 1998; Matsumoto et al., 2008). IGFBP1 is also up-regulated by FOXO1 in endometrial cells (Kim et al., 2005) and IGFBP1 binds to trophoblast-specific integrins and modulates trophoblast migration and invasion in vitro (Giudice, 2003). Thus we studied the effect of UPA on the above decidualization markers, which are secretory proteins synthesized by decidualised endometrial stromal cells (Gellersen et al., 2007). There were no significant changes in the expression of IGFBP1 or PRL on exposure to UPA compared with controls. The lack of change in the expression of decidualisation markers could be due to the failure of trophoblast cell invasion into the endometrial construct, reflecting the difference in culture conditions compared with the in vivo system. Thus, further fine-tuning of the 3D culture model is required to overcome such limitation and allow trophoblast cell invasion into it. The progesterone regulated transcription COUP-TFII with a functional link between the epithelial and stromal compartments is also of importance for endometrial stromal decidualization. Kurihara et al. (2007) demonstrated that COUP-TFII knockout mice are infertile due to loss of decidualization potential and failure of embryo attachment. The expression of COUP-TFII remained unchanged after exposure to UPA. We also looked into the expression of MUC1, a cell adhesion molecule seen during the luteal phase and regulated by progesterone in the endometrial epithelial cells (Meseguer et al., 2001). Deficiency of maternal glucocorticoid-inducible kinase SGK1 in endometrium leads to failure in embryo implantation (Salker et al., 2011). None of the above mentioned genes of interest in endometrial receptivity or embryo implantation process did not show any significant difference with UPA treatment. These findings at the molecular level of endometrial cells are in line with the functional outcome of the study, enabling attachment of the embryos and thus explain that UPA does not affect the embryo attachment.

Six genes were differentially expressed after UPA exposure compared with controls. Gene expression profiling of human endometrial stromal cells has shown an elevation of IL6 in response to trophoblast-conditioned medium and is in line with our findings as an elevation within the limits would not inhibit endometrial receptivity and implantation (Hess et al., 2007). We also found a significant up-regulation of HBEGF after UPA exposure compared with controls. This is in contrast to a previous study where a single high dose of mifepristone inhibited embryo implantation without altering the expression of HBEGF in the endometrium during the receptive phase (Sun et al., 2006). Therefore the effect of UPA on the expression of HBEGF would not be expected to negatively influence on embryo attachment and invasion as it is also supported by the observations that HBEGF regulates endometrial cell proliferation and glandular epithelial secretion and induces invasiveness in human endometrium (Martin et al., 1998; Simon et al., 2000; Chobotova et al., 2002). There was a significant down-regulation of the transcripts HAND2, OPN, FGF2 and CALCR in the UPA exposed group compared with the control group in our study. Among these, the receptivity marker OPN has consistently been up-regulated during the receptive phase in previous studies and thus it is suggested to be a valid marker of receptivity (Kao et al., 2002; Riesewijk et al., 2003; Horcajadas et al., 2004; Mirkin et al., 2005; Zhang et al., 2012). However, Zhang et al. (2012) commented that OPN might not be a unique marker for WOI, since it still rose in the late secretory phase, when the progesterone level declines. The mRNA expression of OPN was down-regulated but was still present in the cells. It is possible that other molecules expressed in cells compensate the impact caused by the down-regulation of the above studied molecules, as endometrial receptivity is an interplay of many molecules. Progesterone is known to up-regulate Hand2 in endometrium and this further down-regulates FGF leading to decreased estrogen mediated cell proliferation (Li et al., 2011). In our study we see a down-regulation of Hand2 with UPA treatment reflecting the function of progesterone receptor modulation by UPA. This study further substantiates the fact that endometrial receptivity is not regulated by one single gene but by a combination of genes in a network of parallel pathways.
and there seems to be a functional redundancy where one member of a gene family can compensate for the function of another. We presume that the impact of UPA on the endometrial co-culture causing the described changes in levels of some receptivity markers is thus counteracted by regulatory mechanisms and alterations of other markers involved in implantation and therefore UPA in the dose used for EC does not prevent embryo attachment.

The probability of conception is related to the day of ovulation. UPSI may result in a pregnancy only from 5 days prior to 1 day after ovulation, with the highest rates of conception occurring within 2 days prior to ovulation due to lifespan of spermatozoa in the female reproductive tract as well as that of the oocyte after ovulation (Wilcox et al., 1995). The embryo thus is unable to adhere to the endometrium during the majority of days of the menstrual cycle in humans. Glasier et al. (2011) demonstrated that pregnancies occurred to a higher extent when an UPSI had taken place on the most fertile days prior to UPA intake. In addition there was also a high risk of failure if further acts of UPSI occurred after ECP intake, as the fertile window is delayed to later on in the cycle when women probably no longer perceive themselves of being at risk of becoming pregnant. This also emphasized by the normal function of the endometrium as soon as ovulation has occurred and the fact that an EC with a significant postovulatory effect would be more effective to prevent pregnancy than the currently available ECPs.

A recently published report from post-marketing surveillance monitoring the safety profile of UPA with special interest in the outcome among those who became pregnant does not suggest a teratogenic effect. However, only a small number of pregnancies have been reported after UPA intake so far, probably due to the fact that many women who conceive choose to terminate the pregnancy as well as that unexplained abortions occurred after UPA intake so far. This also emphasizes the normal function of the endometrium after ovulation and the fact that an EC with a significant postovulatory effect would be more effective to prevent pregnancy than the currently available ECPs.

Our finding that UPA in doses relevant for EC does not inhibit embryo attachment in vitro is consistent with the mechanism of action of UPA as EC in vivo where UPA does not have clinically significant post-ovulatory effects on the endometrium to prevent pregnancy (Creinin et al., 2006; Fine et al., 2010; Glasier et al., 2010). This new knowledge in understanding the mechanism of action of UPA in vitro contributes to the evidence that could influence the acceptability of EC in different societies and prevent unwanted pregnancies, thereby improving women’s health and reducing maternal morbidity and mortality. Effective postcoital contraception is highly accepted by women and can have advantages when regular contraceptive methods cannot be used.

In this study, we have examined the implantation process limiting to attachment phase, which is one of the important steps in the process of embryo implantation (Driedrich et al. 2007). Even though studying the attachment of human embryos gives sufficient information about the effect of UPA on receptivity and implantation, an in depth analysis of post attachment phase would be of importance. This is possible by further improving the culture conditions. The expression of receptivity genes was studied in the whole endometrial construct comprising epithelial and stromal cells. Data from this pooled cell population may not give the true picture of molecular expression in any cells present in the construct. Studying the receptivity markers in specific cell types namely stromal and epithelial cells may help us to understand the expression and regulation of receptivity markers in specific cell types.

This study provides new insights into the mechanism of action of UPA when used as EC. We report that continuous exposure of UPA at the dose of 200 ng/ml did not compromise the quality or the capability of human embryo attachment at a physical level. We conclude that the mechanism of action of UPA when used as EC does not disrupt the implantation process or affect the implanting embryo.

Supplementary data
Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors’ roles
N.R.B and P.G.L.L. performed the experiments and analysed the results. C.B. recruited volunteers, collected endometrial biopsies, helped with the experiments and analysis of results. K.G.D. and P.G.L.L. designed the study and gave critical inputs. K.G.D. conceptualized and funded the study, was overall responsible for conducting the study and collected endometrial biopsies. J.M. took care of embryo cultures and analysed their staging. All authors were involved in manuscript writing and critical intellectual inputs and gave their final approval for manuscript submission.

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Conflict of interest
Authors have no conflict of interests in connection to this manuscript.

References
Ulipristal acetate in embryo implantation process


Croxatto HB, Brache V, Pavez M, Cochon L, Forcelledo ML, Alvarez F, Massai R, Faundes A, Salvatierra AM. Pituitary-ovarian function following the standard levonorgestrel emergency contraceptive dose or a single 0.75-mg dose given on the days preceding ovulation. Contraception 2004;70:442–450.


