Transcriptome analysis reveals dialogues between human trophectoderm and endometrial cells during the implantation period

D. Haouzi¹,²,³, H. Dechaud¹,²,³, S. Assou¹,²,³, C. Monzo¹,²,³, J. de Vos¹,³,⁴, and S. Hamamah¹,²,³,⁴,*

¹CHU Montpellier, Institut de Recherche en Biothérapie, Hôpital Saint-Eloi, Montpellier F-34295, France ²CHU Montpellier, ART/PGD Division, Département de Biologie de la Reproduction, Hôpital Arnaud de Villeneuve, Montpellier F-34295, France ³INSERM U847, Hôpital Saint-Eloi, Montpellier F-34295, France ⁴UFR de Médecine, Laboratoire ‘Développement embryonnaire prêcoce et cellules souches embryonnaires humaines’, Université Montpellier1, Montpellier F-34000, France

*Correspondence address. ART/PGD Division, Département de Biologie de la Reproduction, Hôpital Arnaud de Villeneuve, Montpellier F-34295, France. Tel: +33 4 67 33 64 04; Fax: +33 4 67 33 62 90; E-mail: s-hamamah@chu-montpellier.fr

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BACKGROUND: Crosstalk between human trophectoderm (TE) and endometrial cells during the implantation window is a complex and not well-understood process. The aims of this study were (i) to evaluate the global gene expression profile in TE cells from Day 5 human blastocysts issued from IVF, (ii) to compare these data with the transcriptomic profile of endometrial cells in stimulated cycles for IVF and (iii) to identify potential early dialogues between maternal and embryonic cells during the implantation window.

METHODS: Endometrial biopsies (n = 18) from normal responder patients were performed on the day of embryo transfer (Day 5 after human chorionic gonadotrophin administration). TE biopsies from five blastocysts donated for research purposes were mechanically extracted. DNA microarray analysis was carried out to identify the specific gene expression profiles and the biological pathways activated during the implantation window in endometrial and TE cells.

RESULTS: Several cytokines (such as PDGFA, placenta growth factor, IGF2BP1 and IGF2BP3) were up-regulated in human TE cells, whereas some of the corresponding receptors (PDGFRα and KDR) were over-expressed in the receptive endometrium, suggesting that these molecules are involved in the early dialogue between blastocyst and maternal endometrial cells. In addition, several adhesion molecules and extracellular matrix proteins (MCAM, ITGAE and LAMA1) were also over-expressed in the TE, while others (ALCAM, CEACAM1, PECAM1, ITG8B and LAMA2) were restricted to the receptive endometrium.

CONCLUSION: The present study shows that several growth factors, cytokines, integrins and adhesion molecules are expressed in the TE and endometrium at the time of implantation. These results could contribute to the understanding of the mechanisms involved in the early dialogue between blastocyst and endometrium during implantation. Such results should be confirmed by further studies.

Key words: receptive endometrium / trophectoderm / blastocyst / microarray / early dialogue

Introduction

The high rate of implantation failure remains a major problem in IVF. More than 80% of such failures are associated with inadequate endometrial receptivity or with defects in embryo-endometrium dialogue (Paulson et al., 1990; Simon et al., 1998). Consequently, the understanding of the mechanisms that regulate implantation is a major issue in assisted reproductive technology (ART). Successful implantation requires a competent embryo, a receptive endometrium and a synchronized mother–embryo crosstalk that is regulated by endocrine, paracrine and autocrine interactions. During the early stages of human embryo implantation, the free-floating blastocyst acts on the endometrium, probably via soluble mediators such as cytokines and growth factors. Then, adhesive interactions between the trophectoderm (TE) (i.e. the outer cell mass of the blastocyst that gives rise to trophoblast and the extra-embryonic structures) and the endometrial surface epithelium occur during the implantation window (6–10 days after the luteinizing hormone peak) followed by embedding of the
blastocyst into the endometrium. Temporally- and spatially regulated changes in the expression of adhesion and extracellular matrix (ECM) molecules at the maternal–fetal interface during embryo implantation are crucial for blastocyst attachment, migration and invasion. These changes in gene expression profiles are known to be mediated by many molecules such as hormones, proteolytic enzymes, growth factors, cytokines and chemokines (Huppertz et al., 1998; Miller WL, 1998; Bischof et al., 2000; Seval et al., 2004; Chang et al., 2008; Hannan and Salamonsen, 2008).

The new high throughput genomic technologies have allowed the analysis of the shift in gene expression profile that characterizes the passage from the pre-receptive to the receptive stage of human endometrium in natural and stimulated cycles. They have thus provided the opportunity to understand the physiology of endometrial receptivity and to evaluate the impact on endometrial receptivity of controlled ovarian stimulation (COS) during IVF (Carson et al., 2002; Riezeuwijk et al., 2003; Mirkin et al., 2005; Talbi et al., 2006; Horcajadas et al., 2008; Liu et al., 2008; Haozui et al., 2009a, 2009b, 2010). By contrast, to date, the mechanisms that control the early dialogue between human blastocyst and endometrium during the implantation window have not been extensively studied as details of the implantation signalling cascades have been mainly investigated indirectly in animal models or in in vitro human models (Dey et al., 2004; Fukuda and Sughrha, 2008; Bazer et al., 2009).

The aim of this study was, therefore, to evaluate the global gene expression profile in human blastocyst TE cells and then to compare it with the transcriptomic profile of stimulated endometrium during the implantation window.

**Materials and Methods**

**Endometrial biopsies**

The study population included 18 normal responder patients (normal serum FSH, LH and oestradiol on Day 3), aged < 36 years and referred for IVF due to male infertility. The study was approved by the Ethical Committee of the Institut de Recherche en Biothérapie. Patients had COS with a combination of GnRH agonist long or antagonist protocols with either highly purified human menopausal gonadotrophin or recombinant FSH. After having collected the patient’s informed consent, one endometrial biopsy was carried out in conditions of the stimulated cycle and on the day of embryo transfer [human chorionic gonadotrophin (hCG + 5 days)]. Each biopsy was frozen at −80°C in RLT RNA extraction buffer (RNeasy Mini kit, Qiagen, Valencia, CA, USA).

**Blastocyst culture and TE biopsy**

Blastocysts were voluntarily donated for research by infertile couples undergoing IVF treatment. Both partners signed the informed consent form and the research protocol has been approved by national agency of biomedicine. After COS for ICSI, cumulus–oocyte complex (COC) retrieval was performed by vaginal puncture under ultrasound echo-guidance 36 h after administration of 5000 IU hCG. Immediately after ovum retrieval, COCs were placed at 37°C in G-MOPSPLUS medium (Vitrolife) for a few minutes and then transferred into G-IVFPLUS culture medium (Vitrolife). Oocytes were enzymatically denuded of cumulus cells with 80 UI/mL Hyaluronidase (SynVitroHyadase, Origio) to assess oocyte nuclear maturity. Mature MII oocytes were fertilized by ICSI. All oocytes were individually cultured in micro-drops of G-1PLUS medium (Vitrolife) under paraffin mineral oil. Three days after fertilization, supernumerary embryos were cultured in G-2 PLUS medium (Vitrolife) until the blastocyst stage (Day 5/6 post-fertilization). Only supernumerary embryos that could be donated for research purposes were used to obtain TE biopsies. Blastocyst morphological criteria were assessed according to Gardner’s classification: two blastocysts grade B1/B2 and eight blastocysts grade SBC or 6AB/6BB/6CC (Gardner et al., 1998). TE cells were mechanically extracted from individual blastocysts and immediately frozen at −80°C in RLT RNA extraction buffer. Five TE biopsies from blastocysts of two patients were used for microarray analyses; five additional TE biopsies from blastocysts of three other patients were used for qRT–PCR validation of the microarray data.

**Complementary RNA (cRNA) preparation and microarray hybridization**

Total RNA (between 50 and 100 ng) was used to prepare twice amplified and labelled cRNA for hybridization with HG-U133 plus 2.0 arrays (AffymetrixTM) as described in Haozui et al. (2009b). Each TE and endometrial biopsy were processed individually on a DNA microarray chip.

**Data processing**

Scanned GeneChip images were processed using the AGCC (Affymetrix GeneChip Command Console) software. Microarray data were analysed using the Affymetrix Expression Console software and normalization was performed with the RMA (Robust Multiarray Averaging) algorithm to obtain an intensity value signal for each probe set. The microarray data were analysed at our facility following the minimal information about microarray experiment recommendations (Brazma et al., 2001).

**Microarray data analysis**

We first selected 4 137 probe sets that presented a fold ratio > 5 or < 0.2 of the mean signal intensity between TE and endometrial cells samples and performed an unsupervised hierarchical cluster with the Cluster and Treeview software packages. Then, the Significant Analysis of Microarrays software (SAM, Stanford University, USA; Tusher et al., 2001) was used to identify genes whose expression varied significantly in the TE and endometrial groups. SAM provides the mean or median fold change values (FC) and a false discovery rate (FDR) confidence percentage based on data permutation. Selected genes (FC > 2 and FDR < 5%) were then analysed using Ingenuity (http://www.ingenuity.com) and FatigO+ (http://bachelomics2.bioinfo.cipf.es) to identify the biological pathways/functions that are specific to each tissue group.

**Quantitative RT–PCR analyses**

Amplified RNA (0.35 μg) from endometrial (n = 3) and TE (n = 5) biopsies was used for quantitative reverse transcriptase polymerase chain reaction (qRT–PCR) according to the manufacturer’s recommendations (Applied Biosystems). The 10 μl reaction mixture for quantitative PCR consisted of first-strand DNA (2 μl of a 1:1 dilution), 2.5 μlM of each primer and 5 μl of 2 × LightCycler 480 SYBR Green I Master mix (Roche). The amplification was measured during 45 cycles with annealing temperature at 62°C using the Light Cycler 480 detection system (Roche) and normalized to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) expression for each sample using the following formula: 

\[
E = \frac{\text{GAPDH}_{\text{unknown}}}{\text{GAPDH}_{\text{standard}}} \\
\Delta C_t = C_t \text{control} - C_t \text{unknown}, \quad \text{where } E \text{ corresponds to the efficiency of the PCR reaction. The } E \text{ value is obtained by a standard curve that varies in function of the primers used. One endometrial or TE sample was used as control. Each sample was analysed in duplicate and multiple water blanks were included.}
Statistical analyses

Statistical analyses of qRT–PCR data were performed using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). A repartition difference between sample groups was considered significant when the Kruskal–Wallis non-parametric test gave a P value < 0.05.

Results

Gene expression profiles of TE and endometrial cells in stimulated cycles during the implantation window

To identify possible dialogues between TE and endometrial cells during the implantation window, a DNA microarray analysis was carried out using TE biopsies from in vitro matured blastocysts and receptive endometrial cells from women in stimulated cycles. Unsupervised clustering (Fig. 1) clearly separated all TE and endometrial samples, suggesting that these two groups are characterized by distinct expression profiles. SAM analysis of the gene expression profiles of the TE and the endometrial groups identified 2737 genes that were differentially expressed in the two groups. Specifically, 1889 (5 ≤ FC ≤ 718.5) genes were up-regulated in endometrial cells and 848 (5 ≤ FC ≤ 529.6) in the TE group (Fig. 2A). The 10 most up-regulated genes in the two groups are listed in Fig. 2B. In addition to these, the TE group was characterized by over-expression of GCM1 (62.8, FDR < 0.0001), HERV-FRD (12.6, FDR < 0.0001), CYP19A1 (34.4, FDR < 0.0001), PPIA (5.4, FDR < 0.0001), HSD17B1 (21.9, FDR < 0.0001), DNMT3A (6.6, FDR < 0.0001) and DNMT3B (29.1, FDR < 0.0001).

Differential expression of genes with specific biological functions between TE and endometrial cells during the implantation window

A FatiGO+ analysis of the genes that were specifically up-regulated in the TE group was then carried out to identify the biological functions/pathways in which they are involved. The majority of the TE-specific genes were associated with processes linked to transcriptional and translational control of gene activation, such as 'mitotic phase of the
cell cycle’ (81.1% in the TE and 18.9% in the endometrial group, adjusted $P$ value = 0.0003), ‘translation’ (73.6 and 26.4%, adjusted $P$ value = 0.0003), ‘DNA replication’ (78.4 and 21.6%, adjusted $P$ value = 0.02) and ‘RNA metabolism’ (100 and 0%, adjusted $P$ value = 0.03). Moreover, most of these genes were localized in ribosomes (88.7% in the TE compared with 11.3% in the endometrial group, adjusted $P$ value = 0.0001), chromosomes (75.3 and 24.7%, adjusted $P$ value = 0.002), nucleus (55 and 45%, adjusted $P$ value = 0.006) and the eukaryotic 48S initiation complex (100 and 0%, adjusted $P$ value = 0.04).

Differential up-regulation of specific growth factors in TE and endometrial cells during the implantation window

To identify potential dialogues between embryonic and maternal cells, we then focused on the growth factors that were specifically over-expressed in each tissue group during the implantation window. Many growth factors and growth factors receptors were found to be differentially up-regulated in TE or receptive endometrial cells (see Table I for a complete list). Particularly, placenta growth factor

### Table I Growth factors, growth factor receptors, adhesion and extracellular matrix molecules that are over-expressed in (A) human blastocyst TE and (B) receptive endometrium cells during the implantation window.

<table>
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<tr>
<th>A</th>
<th>Gene name</th>
<th>FC</th>
<th>FDR</th>
<th>B</th>
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(PGF) and its receptor KDR were respectively over-expressed in TE and endometrial cells. A similar profile was reported for PDGFA (TE cells) and its receptor PDGFRA (endometrial cells).

**Differential expression of adhesion and extracellular matrix molecules at the maternal–fetal interface**

During the implantation window, only one member of the Integrin family, ITGAE was over-expressed in TE cells, whereas ITGAV, ITGB1 and ITGB8 were specifically up-regulated in receptive endometrial cells (FCs and FDR were shown in Table I). Similarly, only COLEC12, among the Lectin family members, was over-expressed in the TE group compared with LGALS1, LGALS3, LGALS3BP and LGALS8 in endometrial cells (Table I; data not shown). Concerning cell adhesion molecules (CAM), only MCAM was up-regulated in the TE group, whereas in endometrial cells ALCAM, CEACAM1 and PECAM1 were over-expressed. In addition, LAMA1 and LAMC1, two members of the laminin family, were over-expressed in the TE group compared with only LAMA2 in the endometrial group. Several members of the Collagen family, as well as other ECM components, were exclusively up-regulated in the endometrium (Table I).

Among the proteoglycans, HAPLN1 (×19.1, FDR < 0.0001) and hyaluronan-mediated motility receptor (HMMR; 7.1, FDR < 0.0001) were over-expressed in TE cells and CD44 (67.1, FDR < 0.0001) and SDC2 (115.5, FDR < 0.0001) were over-expressed in receptive endometrial cells (Table I).

**Validation of gene expression**

Some of the results of the microarray analysis were validated by quantitative RT–PCR using independent TE and endometrial biopsies (Fig. 3).

**Discussion**

The initial steps of the implantation process involve a complex dialogue between embryonic and endometrial cells. In the current study, we report the differential expression of growth factors and growth factor receptors in the TE and endometrial cells during the implantation window, suggesting a potential role of these molecules in the early dialogue between blastocyst and uterus. In addition, we also found tissue-specific differences in the expression profiles of adhesion and extracellular matrix molecules that might play a role in blastocyst attachment and its subsequent invasion of the endometrium. Most of these results are novel and offer the opportunity to better understand the mechanisms, which control the early stages of human embryo implantation.

**The gene expression pattern of human blastocyst TE cells: embryonic genome activation**

In the present study, the analysis of the transcriptome of blastocyst TE cells revealed the presence of many over-expressed genes involved in DNA replication, transcription, RNA metabolism and translation, confirming previous studies performed in whole blastocysts (Zeng et al., 2004; Sudheer and Adjaye, 2007; Bell et al., 2008; Jaroudi et al., 2009). In mammals, after fertilization, the genome of the newly formed embryo is at first transcriptionally inactive and embryo development relies on maternal proteins and transcripts synthesized during oogenesis. At later cell cycles, following activation of the zygotic genome, newly synthesized RNA and proteins are crucial for further embryonic development (Bettegowda et al., 2008). Epigenetic chromatin remodelling, including post-translational modifications (such as methylation and acetylation) of histones, non-histone proteins and DNA, is now proposed to be a key mechanism by which the embryonic genome is activated. In particular, de novo methylation has been observed in the blastocyst and it involves two DNA methyltransferase, DNMT3A and DNMT3B, as well as the DNA methyltransferase-like DNMT3L, which are all essential for mammalian development and regulation of genomic imprinting (Okano et al., 1999; Chedin et al., 2002). Here we found a strong expression of DNMT3A, DNMT3B and DNMT3L in blastocyst TE cells. This finding is in agreement with...
the study by Rahnama et al. (2006) showing that depletion of both DNMT3A and DNMT3B in a choriocarcinoma cell line affected human trophoblast migration (Rahnama et al., 2006). In addition, the offspring from female Dnmt3L−/− mice are non-viable presumably due to placental defects (Li et al., 1992). It has to be noted, however, that these enzymes present species-specific features. For instance, in mouse blastocysts, de novo methylation is restricted to the inner cell mass (Watanabe et al., 2002), whereas in bovine preimplantation embryos, methylation is higher in TE cells (Hou et al., 2007).

Potential paracrine interactions between human TE and endometrium during the implantation window

The differential expression of various growth factors and their receptors in endometrium and TE during the preimplantation period suggests that some of these molecules are important for implantation. Particularly, PDGFA, a member of the platelet-derived growth factor family, was strongly expressed in TE cells, whereas its receptor PDGFRα was up-regulated in endometrial cells. PDGFA transcripts have been previously found in human unfertilized oocytes and embryos from the 8-cell to blastocyst stage, while PDGFRα has been detected in 4-cell and 8-cell embryos and blastocysts but not in oocytes (Osterlund et al., 1996). Although it has been shown that, in vitro conditions, human blastocysts respond to PDGF by promoting cell growth and differentiation of early human embryos (Lopata and Oliva, 1993), we do not think that PDGFRα signalization is associated with autocrine signalling. Rather, since our findings suggest that PDGFA is produced by the blastocyst TE, we suggest a potential paracrine action of PDGFA from the blastocyst to the endometrium, which expresses strongly PDGFRα. Moreover, our microarray analysis shows for the first time that PGF, a member of the vascular endothelial growth factor family of pro-angiogenic factors, is strongly expressed in TE cells of early human embryos. Indeed, previously PGF has been detected in human choriocarcinoma cell lines, term placenta, isolated term cytotrophoblast cells and in vitro differentiated syncytiotrophoblast cells (Hauser and Weich, 1993; Khaliq et al., 1996; Shore et al., 1997; Athanassiades et al., 1998; Desai et al., 1999). Our finding is supported by the TE-specific expression of GCM1 (glial cells missing homologue 1), which transcriptionally regulates PGF in human choriocarcinoma cell lines as well as trophoblast-specific genes, including syncytin and aromatase (Yamada et al., 1999; Chang et al., 2008). The early expression of PGF suggests a possible role for PGF in trophoblast function via an autocrine mechanism. Moreover, the concomitant expression of KDR, a PGF receptor, in endometrial cells (Hauser and Weich, 1993), but not in TE cells (Clark et al., 1996; Vuckovic et al., 1996; Shore et al., 1997), implies that PGF could act also in a paracrine fashion to modulate angiogenesis and vessel remodelling during implantation and placentation.

Finally, Syncytin-1 (ERVV1) and Syncytin-2 (HERV-FRD), which encode highly fusogenic retroviral envelope proteins that are expressed in the syncytiotrophoblast layer generated by fusion of mononuclear cytotrophoblast cells at the maternal–fetal interface (Lyden et al., 1994; Blond et al., 2000; Frendo et al., 2003; Prudhomme et al., 2005; Dunlap et al., 2006), as well as specific aromatases, such as CYP19A1 (an enzyme involved in the oestrogen biosynthesis), CYP11A1 and HSD3B1 (two enzymes controlling progestosterone biosynthesis) were also up-regulated in blastocyst TE (data not shown; Fig. 2). Their expression was previously reported in human placenta (Mahendroo et al., 1991; Yamada et al., 1995; Harada et al., 2003; Rama et al., 2004; Yashwanth et al., 2006), which is the primary site of oestrogen and progestosterone synthesis in pregnant women after the first trimester of pregnancy. The early presence of these genes is in agreement with previous studies which further showed that trophoblast-associated hormones induce blastulation and neurulation during early human embryogenesis (Gallego et al., 2009).

Adhesion and extracellular matrix molecules expressed in TE and endometrium: potential role in blastocyst attachment during implantation

The initial attachment of TE to the uterine walls involves low-affinity carbohydrate ligand-binding molecules such as selectins, galectins, heparin sulphate proteoglycans and heparin-binding growth factors (Bazer et al., 2009). Among the selectin family, only COLEC12 was up-regulated in TE cells, while several other selectin members were strongly expressed in the receptive endometrium. COLEC12 is part of the C-lectin family that is characterized by the presence of collagen-like sequences and carbohydrate recognition domains. These structural features suggest that this molecule might be involved in the initial TE attachment to the receptive endometrium, which expresses many collagen types (see Fig. 4).

Galectins are a newly defined family of galactose-binding lectins with conserved carbohydrate recognition domains that can bind to several glycoconjugates such as the basement membrane glycoproteins, laminin and fibronectin, and are involved in many biological events including development, differentiation, cell–cell adhesion, cell–matrix interaction and growth regulation. Galectin-3 was previously found in both the endometrium and all trophoblastic lineages, including villous cytotrophoblasts and extravillous trophoblasts, while galectin-1 was restricted to the endometrium (Maquoi et al., 1997; Lei et al., 2009). Galectin-8 expression is reported here for the first time in the endometrium. The implication of endometrial galectins in the initial steps of the implantation process has not yet been demonstrated. However, the expression of laminin in TE cells suggests a possible interaction with endometrial galectins.

The microarray analysis also identified over-expression of HMMR and hyaluronan and proteoglycan link protein 1 (HAPLN1) in TE, and up-regulation of the receptor for hyaluronic acid (CD44) and of syndecan 2 (SDC2) in the endometrium. It was recently reported that up-regulation of CD44 is coincident with the period in which the endometrium is receptive to embryo implantation (Affy et al., 2006), while SDC2 is the most strongly expressed syndecan throughout the menstrual cycle (Germeyer et al., 2007). In addition to its contribution to the endometrial glyocalyx in the secretory phase, CD44 might play a role in blastocyst attachment via its interaction with sulphated proteoglycans expressed in early human embryos (Weber et al., 1996; Horne et al., 2002). Hamilton et al. (2007) reported that CD44 can act with HMMR leading to high basal motility of invasive breast cancer cells, suggesting their potential implication in blastocyst attachment to the receptive endometrium via the TE.
Stable adhesion through integrins expressed in blastocyst TE and in the endometrial luminal epithelium and their ECM ligands are required for implantation. Here we show that blastocyst-stage TE cells express only few genes of this family, including integrin αE, which was previously described to be preferentially expressed in human intestinal intraepithelial lymphocytes (Ebert et al., 1998). On the other hand, we could not confirm previous findings showing that human blastocysts express the integrin subunits αV, α3, β1, β3, β4 and β5 (Campbell et al., 1995).

MCAM is a membrane-bound glycoprotein that mediates cell–cell adhesion and plays a role in several biological processes, including tumour progression, and possibly in implantation and placentation (Shih et al., 1998; Guezguez et al., 2007). MCAM is expressed in extra-villous trophoblasts in both human choriocarcinoma cell lines and human choricarcinoma explant cultures, but not in the villous trophoblast, suggesting a role of MCAM in trophoblast differentiation (Shih and Kurman, 1996; Higuchi et al., 2003). Here, we report a strong MCAM expression in TE cells suggesting its implication also in other functions, for instance trophoblast invasion as already proposed by Liu et al. (2004).

Limitations
Finally, we cannot exclude the possibility that the expression profiles we identified as potentially involved in the early dialogues between blastocyst and endometrium in IVF conditions do not completely reflect the conditions in natural cycles due to the impact of the COS protocol on endometrial receptivity (Horcajadas et al., 2008; Haouzi et al., 2009b, 2010) and of the in vitro manipulation on oocytes. Indeed human IVF embryos often show abnormal or delayed cell division (Trounson and Bongso, 1996) and data from animal models indicate that blastocysts produced in vitro show altered gene expression (Rizos et al., 2002; Lonergan et al., 2003; Gutiérrez-Adán et al., 2004; Whitworth et al., 2005; Corcoran et al., 2006, 2007; Balasubramanian et al., 2007; Girirhan et al., 2007; Bauer et al., 2010). Specifically, genes involved in transcription and translation are often down-regulated in in vitro-produced compared with in vivo bovine embryos (Lonergan et al., 2003; Corcoran et al., 2006, 2007). Girirhan et al. (2007) reported that genes involved in development, cell division and apoptosis are up-regulated following IVF in mouse blastocysts. Bauer et al. (2010) recently showed up-regulation of genes involved in transcription, translation and development and down-regulation of genes involved in protein glycosylation and phosphorylation, cell structure and motility, fatty acid and steroid metabolism and transcription regulation in porcine blastocysts produced in vitro. On the other hand, Whitworth et al. (2005) reported less dramatic effects on pig embryos at the blastocyst stage than at the 4-cell stage, suggesting that differences in gene expression do not persist throughout embryonic development.

Conclusion
The transcriptomic pattern of endometrial and TE cells at the time of implantation under IVF conditions reveals differential expression of specific growth factors and their receptors as well as ECM molecules that might play a role during the early stages of human embryo implantation. This study contributes to the understanding of the mechanisms that control human embryo implantation after IVF. Moreover, some of these genes could be used as biomarkers of the synchronization between maternal and embryonic tissues by checking either expression of these genes from blastocyst TE biopsy and/or from endometrial biopsy at Day 5 post-fertilization. However, additional studies need to be carried out in order to determine the relevance of such biomarkers.

Authors’ roles
H.D. involved in concept design, analysis and interpretation of data and participated in manuscript preparation. D.H. performed endometrial biopsies. A.S. and D.J. involved in concept design. M.C. gave technical assistance. H.S. involved in concept design, manuscript preparation and approved the final version.
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