Expression of transcription factor Oct-4 and other embryonic genes in CD133 positive cells from human umbilical cord blood

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Summary
A significant number of hematopoietic stem/progenitor cells (HSPC) in human umbilical cord blood could serve as a reservoir for the placental vasculature, yet, their morphological and functional features are not completely understood. Here, we describe the characterization of purified CD133+ progenitor cells from umbilical cord blood, a subset of CD34+ hematopoietic progenitors that were grown in proliferation medium containing Flt3-ligand, thrombopoietin and stem cell factor. Following isolation and enrichment of the CD133+ cells by immunomagnetic cell sorting, they remained non-adherent for up to 40 days in culture and expressed different pluripotency markers including Sox-1, Sox-2, FGF-4, Rex-1 and Oct-4. Oct-4 expression was confirmed by laser-assisted single cell picking with subsequent quantitative real-time RT-PCR. The expression of Oct-4 indicates a pluripotent phenotype of CD133+ cells and appears to be of functional relevance: After three weeks in endothelial differentiation medium, suspended cells became adherent, developed an endothelial cell-like morphology, bound fluorescein isothiocyanate-labeled Ulex europaeus agglutinin-1, took up acetylated Di-LDL, and expressed other endothelial markers such as PECAM-1 or VEGFR-2. Concomitantly, Oct-4 expression was significantly reduced. Moreover, following treatment with retinoic acid, CD133+ cells exhibited neural morphology associated with the expression of βIII-tubulin. CD133+ cells were found to express the luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor, detected by RT-PCR and immunocytochemistry. The recombinant human chorionic gonadotropin induced proliferation of the CD133+ cells in a dose-specific manner. Our results indicate that CD133+ HSPC from umbilical cord blood may have a greater differentiation potential than previously recognized and give rise to proliferative endothelial cells participating in placental vasculogenesis.

Keywords
CD133+ cells, umbilical cord blood, Oct-4, pluripotency

Introduction
The placental vascular system develops through two distinct processes: vasculogenesis and angiogenesis. During vasculogenesis, endothelial progenitor cells – angioblasts – form a primitive vascular network. It has been shown that human umbilical cord blood contains a significant number of primitive hematopoietic stem/progenitor cells (HSPC) or “hemangio-

blasts”, which may be a reservoir for placental vascular formation. Their proliferation, differentiation and developmental plasticity are not fully characterized. Evidence emerged that fetal and/or adult stem cells may have a greater differentiation potential than previously known (1) and distinct molecular markers have been associated with the undifferentiated pluripotent state of stem cell populations.
Among these, Oct-4 is the only gene whose specific function in pluripotent cell populations from embryonic origin or from bone marrow has been confirmed. Its product, the transcription factor Oct-4, constitutes a class I molecular marker and identifies undifferentiated pluripotent cells, is turned off after differentiation (2). Oct-4 is expressed in embryonic stem cells, germ cells as well as cells from amniotic fluid (3). Analysis of the Oct-4 expression pattern suggested that it may be involved in the maintenance of an undifferentiated pluripotent state and self-renewal of the stem cell populations. It also may play a role in the initiation of the pathways controlling the early differentiation of the primitive endoderm (4). As a member of the family of POU transcription factors Oct-4 contains a bipartite DNA binding domain (POU domain), allowing it to bind to the ATGCAAT octamer motif in promoter regions of housekeeping and immunoglobulin genes (5). Although identification of specific target genes has proved difficult, different genes including fibroblast growth factor-4 (FGF-4), Utf-1, platelet-derived growth factor-α receptor, osteopontin or human chorionic gonadotropin (hCG) are regulated by Oct-4 (6). These considerations prompted us to look for the expression of Oct-4 and other pluripotency markers (7) in HSPC from umbilical cord blood. Since our previous studies indicated that hCG may act as a new angio-regulatory factor in the feto-maternal unit (8) and CD133+ cells isolated from umbilical cord blood exhibit a phenotype similar to hemangioblasts, which mediate vasculogenesis in the embryo and take part in the angiogenesis in extraembryonic tissues, we analysed the effect of the reproductive tract specific angiogenic factor hCG on CD133+ cells.

Materials and methods

Source, collection and isolation of CD133+ cells

Cord blood samples were obtained from normal full-term deliveries using a blood collection bag containing citrate-phosphate-dextrose-anticoagulant solution (Maco Pharma, Langen, Germany) and were immediately diluted 1:4 with phosphate-buffered saline (PBS). Mononuclear cells were isolated on a Ficoll density gradient (Ficoll-Paque™ Plus, Amersham Biosciences, Freiburg, Germany), washed twice in PBS containing 0.5% bovine serum albumin (Fraction V, Sigma, Taufkirchen, Germany) and 2 mM EDTA. CD133+ cells isolated from the mononuclear cell fraction using the magnetic bead separation column (MACS) (Miltenyi Biotech, Bergisch Gladbach, Germany). Briefly, at least 1 × 10⁶ mononuclear cells in 500 μl BSA-EDTA were incubated with 100 μl Fc receptor-blocking reagent for 15 min at 4°C before monoclonal mouse anti-human CD133 antibody conjugated colloidal superparamagnetic microbeads were added for 30 min at 4°C. Cells were then washed and applied to a separation column in a magnetic field and allowed to pass through. Cells labelled with conjugated antibodies bound to the column were eluted by rinsing the column with PBS. A sample fraction of the purified CD133+ cells was checked for viability, cell number, morphology and immunophenotype. Patients were informed about the objectives and procedures of the clinical study and signed a written consent before being included in the clinical study. The study protocol was approved by the Ethics Committee of the Medical Faculty, Justus-Liebig-University, Giessen, Germany.

Source of endothelial cells

Human umbilical cord vein endothelial cells (HUVEC) were isolated and characterized based on established methods (9). Human uterine microvascular endothelial cells (UMVEC) were purchased from BioWhittaker (Verviers, Belgium) and maintained in the selection medium. Endothelial cells, which were characterized based on the presence of von Willebrand factor (vWF) and CD31 (PECAM-1) and the absence of α-actin, were grown in EBM-2 medium containing 5% fetal calf serum (FCS) as described earlier (8).

Flow cytometry

Phenotypic characterization of cells was carried out with a fluorescence activated cell sorting (FACS) analyser (FACScalibur; Becton Dickinson, Heidelberg, Germany). Cells were incubated for 45 min at 4°C with the following monoclonal antibodies (mAb), respectively: CD31-FITC, CD14-FITC, CD45-PerCP, CD62E-APC (all from Becton Dickinson); CD133-PE, CD34-FTC (Milteny Biotech). Anti-Oct-4 mAb was purchased from Santa Cruz (Temecula, CA). Untreated cells and cells incubated with isotype-matched FITC- or PE-conjugated immunoglobulins were used as controls for flow cytometric analysis and concentrations of mAb applied followed the manufacturers’ instruction. For Oct-4 nuclear staining, a standard protocol for FACS detection of human cyclin D1 was employed (www.bdbiosciences.com/pharmingen/protocols). HUVEC and UMVEC were used as phenotype control.

Ex vivo cell expansion in liquid cultures

CD133+ cells were cultured in 25 ml cell culture flask (Becton Dickinson) in Iscove-s-modified Dulbecco’s medium (IMDM; Cambrex, Verviers, Belgium) containing 10% FCS. One tested FCS batch was used throughout all experiments. Cultures were supplemented with 50 ng/ml Flt-3-ligand, 10 ng/ml thrombopoietin and 20 ng/ml stem cell factor (all from TEBU, Offenbach, Germany). Culture medium was renewed twice a week and viable cells were counted after trypan blue staining following standard cell culture procedure.

Differentiation of CD133+ cells

CD133+ cells were cultivated on chamberslides coated with human extracellular matrix (BD Biosciences, Heidelberg, Germany) in endothelial differentiation medium (EBM-2...
medium, Promocell, Heidelberg, Germany), supplemented with 20% FCS, 0.2 µg/ml hydrocortisone, 1 µg/ml ascorbic acid, 50 ng/ml vascular endothelial cell growth factor, 20 ng/ml insulin-like growth factor-1, 10 ng/ml basic fibroblast growth factor, and 5 ng/ml epidermal growth factor (all from Promocell). Immunocytochemistry was performed after 18 days cultivation of CD133+ cells in IMDM and 10 days in EBM-2 medium.

Freshly isolated CD133+ cells were grown in methylenecellose medium (StemCell Technologies, Vancouver, Canada) containing 30% FCS, 1% BSA, 0.1 mM β-mercaptoethanol, 2 mM sodium L-glutamate, 50 ng/ml Flt-3-ligand, 40 ng/ml stem cell factor, and 10 ng/ml thrombopoietin to achieve confluence. After 10 days in culture, the expanded cell population of CD133+ cells was transferred to α-MEM (Invitrogen) supplemented with 20% FCS. These cells were exposed to 10-6 M all-trans retinoic acid (Sigma) for further 14 days.

**Proliferation assays**

CD133+ cells were seeded in quadruplicates on 24-well plates (approximately 11000 cells/well) in IMDM containing 10% FCS. The cells were washed the next day and were further cultured in medium without supplements for 12 hr use. Recombinant hCG (Serono, Munich, Germany) was diluted in physiological sodium chloride solution, and cells were incubated with hCG (0.5-100 U/ml) for 24 hr. Total cell number was measured with a Casy Cell Analyser (Schräfe System, Reutlingen, Germany). As mitogenic stimuli, 20% FCS or 10 ng/ml VEGF-A (R&D Systems, Wiesbaden, Germany) was applied to the cells, while medium containing 10% FCS was used as baseline control. Proliferation index was calculated considering basal proliferation as 1.

**RNA preparation and gene expression analysis**

Total RNA from MACS-isolated CD133+ cells was extracted using RNeasy Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. RNA from mouse ES R1 cells, as positive control for Oct-4 expression, was a generous gift from Dr. G. Breier (Max-Planck-Institute, Bad Nauheim, Germany). Analysis of Oct-4, Sox-1, Sox-2, FGF-4 and Rex-1 mRNA expression by RT-PCR was performed by One-step-RT-PCR (Qiagen) using 40 ng of total RNA. The Oct-4 specific primer sequences (12) were synthesized (Sigma) and One-Step-RT-PCR was performed for Sox-1 (848 bp fragment and annealing temperature 56°C) with primers 5'-GGG CTC GAG GAG GTA GTG GTT CTT CTG TGT TCA GCC GTT TCC-3' and 3'-TGC CCT GGT CTG CCT TCA GCC GTT TCC-5'; for Sox-2 (448 bp fragment and annealing temperature 60°C) with primers 5'-CCC CCG GCG GCA ATA GCA GCA-3' and 3'-TCG GCC GCG GGG AGA TAC AT-5'; for FGF-4 (370 bp fragment and annealing temperature 55°C) with primers 5'-CTA CAA CGC CTA CGA GTC CTA CA-3' and 3'-GTT GCA CCA GAA AAG TCA GAG TTG-5'; and for Rex-1 (306 bp fragment and annealing temperature 56°C) with primers 5'-GGC TAC GCA AAT TAA AGT CCA GA-3' and 3'-CAG CAT CCT AAA CAG CTC GCA GAA T-5'. Primers for B-actin controls were purchased from BD Biosciences. The RT-PCR product of Oct-4 was cloned into pGEM-T vector (Promega, Mannheim, Germany) and was sequenced with standard primers (Seqlab, Göttingen, Germany). Obtained sequences were blasted against NCBI data base (www.ncbi.nlm.nih.gov).

mRNA isolated from HUVEC and UMVEC were used as control.

For analysing LH/hCG receptor mRNA expression a Two-Step RT-PCR was performed. Up to 5 µg total RNA was reverse transcribed in 20 µl with SuperScript II (Invitrogen, Karlsruhe, Germany) according to manufacturer's instruction, with the exception that 1 mM dNTPs (Eurogentec, Seraing, Belgium) and 500 ng of random primers (Promega) were used. The primers used for amplification of a 474 bp fragment of human hCG/LH receptor coding region were 5’-GGA AAC CAC TCT CTC ACA GTG-3’ and 5’-GGT GGA TTG AGA AGG CTT ATT TG-3’ as recently described (10) using the following conditions: 60 sec at 94°C, 90 sec at 56°C, 120 sec at 72°C for 30 cycles and finally 10 min at 72°C. Primers for B-actin were used as controls.

**Laser microdissection and real-time RT-PCR**

Laser microdissection from cytospin preparations of CD133+ cells was performed using a Laser Microbeam system (P.A.L.M., Bernried, Germany) as described in detail previously (11). Briefly, 1 ml cell suspensions of CD133+ cells were spun down for 8 min at 1000 rpm on glass slides (SuperFrost Plus, Menzel-Glaeser, Germany) using a Cytospin 2 centrifuge (Shandon, Astmoor, UK). After hemalaun staining for 45 sec, the cytospins were immersed in 70%, 96% ethanol and stored in 100% ethanol until use. One hundred CD133+ cells each were isolated from the slide after removing cell fragments and non-rounded cells using the laser microbeam. The cells were isolated with a sterile 30 G needle on a micromanipulator (P.A.L.M.) and transferred into a reaction tube containing 10 µl first-strand buffer (11), followed by incubation on ice for 5 min and snap-freezing of specimens in liquid nitrogen. cDNA synthesis and real-time PCR was performed as described earlier (11). For realtime PCR, 25 µl of a universal master mix (qPCRxMD Mastermix for SYBR®, Eurogentec) was used. Human porphobilinogen deaminase (PBGD) oligonucleotides were synthesized by MWG Biotech (Ebersberg, Germany), using published PBDG primer sequences (12). The same Oct-4 primers as mentioned...
above were used. Oligonucleotide primers (final concentration 300 nmol/l) and 1.5 µl SYBR® were added to a final volume of 50 µl. Cycling conditions were 6 min at 95 °C, followed by 55 cycles for 60 s, and finally 5 sec at 62°C and 15 sec at 72°C. The real-time PCR was performed on an ABI Prism 7700 Sequence Detection System (PE Biosystems, Foster City, CA). The Ct values of each PCR product were verified by melting curve analysis (13). Serial dilutions of PCR products served for generating PBGD and Oct-4 standard curves and for calculation of the PCR efficiencies. For relative mRNA quantitation, the comparative Ct method (delta Ct) was used to normalize the number of target gene copies (Oct-4) to the PBGD housekeeping mRNA reference (11).

**Immunocytochemistry**

Fresh isolated CD133+ cells were fixed for 10 min in Zamboni’s solution, washed, and dried on glass slides pre-coated with Vectabond (Vector Labs, Burlingame, CA). Non-specific immunoreactions were blocked with 10% donkey serum in PBS for 30 min. Cells were first incubated overnight at 4°C with mouse anti-Oct-4 (clone 9E3, Chemicon; Hofheim, Germany; dilution 1:50), KDR/Flk-1 (Sigma), PECAM-1 (Becton Dickson) or
anti-β-III-tubulin (Chemicon) in PBS containing 0.1% Triton X-100 and then 1 hr at room temperature with FITC-labelled donkey anti-mouse antibody (Dianova, Hamburg; Germany; dilution 1:400) as described previously. Cell nuclei were counterstained with 4’,6-diamidino-3-phenylindole (DAPI, blue).

Immunocytochemistry of hCG/LH receptor was performed as described previously (8, 14) using the avidin immunoperoxidase method with a 1:500 dilution of a polyclonal hCG/LH receptor antibody raised against an N-terminal sequence recognizing amino acids 15-38. For control, receptor antibody was reabsorbed with an excess of receptor peptide, or receptor antibody was replaced with non-specific IgG. The controls showed no immunostaining.

For analysing the induced endothelial phenotype, CD133+ cells were first grown in IMDM containing 10% FCS, 50 ng/ml Flt-3, 20 ng/ml thrombopoietin and 10 ng/ml stem cell factor for 18 days before they were transferred into EBM-2 for 10 days.
After transfer onto chamber slides, the medium was supplemented with fluorescent-labelled acetylated low density lipoprotein (1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarboxyanine perchlorate) (Dil-Ac-LDL) (Biomedical Technologies, Stoughton, MA). After incubation for 4 hr the medium was removed, the cells were fixed and visualized by indirect fluorescence microscope (Leica, Wetzlar, Germany). Verification of the endothelial phenotype was performed by incubating the fixed cells with *Ulex europaeus* lectin (Sigma, 1:2000) labelled with fluoresceine isothiocyanate.

**Statistical analysis**

Normality (after Kolmogorov-Smirnov) of the data was confirmed by SigmaSTAT (SPSS Inc. Chicago). For statistical analysis, a student’s *t*-test was performed using Excel software from Microsoft (Redmond, WA). A *p* < 0.05 on the basis of at least four independent sets of experiments was considered to be statistically significant.

**Results**

**Isolation and characterization of CD133+ cells from umbilical cord blood**

In this report, we present a method for purification, characterization and *in vitro* functional analysis of CD133+ cells from umbilical cord blood. After Ficoll gradient centrifugation mononuclear cells were subjected to immunomagnetic cell sorting with anti-CD133 antibody. Purity of the cells was determined by flow cytometry. FACS analysis indicated that the mononuclear cell fraction contained about 0.4% CD133+ cells, whereas after magnetic separation this population reached approximately 94.2% and decreased to about 45% or 10% after 5 or 40 days *ex vivo* expansion, respectively. The CD133+ cells also expressed CD45 (>99%) and CD14 (1% of the population on day 0 and up to 20% on day 40). The expression of CD31 was unchanged during the *ex vivo* expansion and ranged from 78-88% of the CD133+ cells, whereas the expression of CD62E (E-selectin) increased significantly from <1% at day 0 up to 35% on day 40. The detailed characteristics and growth dynamics of the cells are summarized in Figure 1.

**Expression of pluripotency markers in CD133+ cells**

RT-PCR analysis of mRNA isolated from freshly isolated as well as from *ex vivo* expanded CD133+ cells demonstrated that human CD133+ HSPC from umbilical cord blood retained the expression of Oct-4 pluripotency marker (Fig. 2A). The PCR product was cloned and sequenced and shown to be identical to human Oct-4 (accession number Z11898). Oct-4 protein was also expressed in nuclei from CD133+ cells as evidenced by FACS analysis and immunocytochemistry (Fig. 3). Moreover, Oct-4 mRNA could be detected in laser-microdissected cytospin preparations of CD133+ cells with rounded cell phenotype (data not shown). On day 7 the relative Oct-4 mRNA expression measured by quantitative real-time RT-PCR amounted to 90.8 ± 43.1 (K x Oct-4 copies/1 copy human PBGD), and on day 23 to 89.0 ± 81.7 (K x Oct-4 copies/1 copy human PBGD), respectively. Both values were not statistically different and indicated that the Oct-4 mRNA concentration did not change during the course of the experiment. Oct-4 expression in CD133+ cells was...
significantly reduced when cells were cultivated in endothelial differentiation medium for up to three weeks. Endothelial cells (HUVEC, UMVEC) did not express Oct-4 at all. Loss of Oct-4 expression paralleled the down regulation of CD133. In addition, using RT-PCR other pluripotency markers such as Sox-1, Sox-2, FGF-4 or Rex-1 were detected in undifferentiated CD133+ cells (Fig. 2B).

**Differentialion of CD133+ cells**
The expression of the pluripotency markers were of functional relevance: Isolated CD133+ cells were cultivated on extracellular matrix material containing laminin, collagen IV and heparan sulphate proteoglycan in endothelial differentiation medium. After 24 hr the initial cell suspension became adherent, developed an endothelial cell-like morphology including typical cobble-stone-shaped phenotype (Fig. 4A), bound fluoresceine isothiocyanate-labeled *Ulex europaeus* agglutinin-1 (data not shown) and took up acetylated Dil-LDL (Fig. 4B). Differentiated CD133+ cells also expressed other endothelial cell markers like KDR/Flk-1 or PECAM-1. We also found that these cells retained a normal karyotype during in vitro expansion (data not shown). Alternatively, stimulation of CD133+ cells with all-trans retinoic acid after cultivation in methylcellulose medium induced a neural morphology of the progenitor cells associated with the expression of β-III-tubulin (Fig. 4C and D).

**hCG-induced proliferation of CD133+ cells**
Recently, hCG was characterized as an angiogenic factor of extraembryonic tissues (9). We demonstrate here that the LH/hCG-receptor is not only expressed e.g. on endothelial cells of the reproduction tract (15) but also on CD133+ cells (Fig. 5). Furthermore, hCG was found to stimulate proliferation of the precursor cells in a dose-dependent manner (Fig. 6). The highest proliferative response was obtained with hCG concentration of 50 U/ml compared to VEGF-A and 20% FCS as positive controls.

**Discussion**
Umbilical cord blood raised great hopes as an alternative source of transplantable haematopoietic stem/progenitor cells to adult bone marrow or growth factor-mobilized leukapheresis harvest (16). Moreover, HSPC from umbilical cord blood are a potential source of stem/progenitor cells for genetic therapies, cancer treatment or as a model system in developmental biology. These cells are easy to obtain and are far less controversial than embryonic stem cells in terms of moral, religious, ethical and legal considerations. Issues regarding the developmental plasticity of these cells remain unsolved, and here we present the detection and analysis of Oct-4, a pluripotency marker, that has been recently described on undifferentiated progenitor cells from embryonic origin and from bone marrow (4).

HSPC were isolated using mAb against CD133 surface antigen instead of stromalucin CD34 which has been taken as “gold standard” for identification of HSPC for many years (17). CD133 antigen is a relatively novel marker for human hematopoietic stem/progenitor cells (18), and recent studies showed that CD133+ cells from bone marrow or umbilical cord blood constitute early and highly proliferating progenitor cells as compared to CD133- cells (19). Moreover, the expression of CD133 seems to be related to the stem cell capacity as indicated by the presence of CD133 transcripts in embryoid bodies derived from human embryonic stem cells (20).

Since pluripotent embryonic stem cells are characterized at the molecular level by the transcriptional factor Oct-4 and the presence of telomerase (21), Verfaillie proposed that for the stem cell to be pluripotent, one or several of these markers should be present (1). Here, we show for the first time that CD133+ HSPC from umbilical cord blood express the important class I stem cell marker Oct-4, indicating that greater pluripotency might persist in fetal stem cells from umbilical cord blood than previously recognized. We analysed Oct-4 expression in single CD133+ cells performing immunocytochemistry, FACS analysis as well as laser-assisted single cell picking with subsequent quantitative real-time RT-PCR. We were able to demonstrate that CD133+ cells expressed Oct-4 directly after isolation and up to day 40 during in vitro cultivation. In addition, other pluripotency markers such as Sox-1, Sox-2, FGF-4 or Rex-1 were also detected in freshly isolated CD133+ cells. Also, we detected mRNA of another progenitor cell marker, Nanog, in CD133+ cells (N. Baal, K. Reisinger et al., unpublished data).
Nanog, a recently identified homeobox protein, is so far attributed to pluripotent lineages in the embryo, including embryonic stem cells, blastocyst inner cell mass, epiblast before gastrulation, and primordial germ cells (22, 23).

There are additional prerequisite indicators of possible high pluripotent potential of HSPC as reported recently, including a low apoptotic activity (16) or self-renewing cell division capacity. The characteristic requirements of progenitor cells are fulfilled by their ability to repopulate the tissue of origin (26, 27) and the propensity to differentiate into more than one cell type (24, 25). Here we could demonstrate the expansion of CD133+ cells from two million to forty million cells within 30 days. Furthermore, the differentiation of CD133+ cells either into cells of endothelial or neural phenotype was attained, demonstrating their respective characteristics by analysing morphology, expression of specific cell determinants and their changed function. Moreover, Verfaillie and colleagues (28) pointed out the possibility that other mechanisms can dictate this apparent plasticity, including contamination with different tissue specific antigens such as SSEA-3, and the relevance of this observation has to be further explored.

After initial submission of this manuscript, Pochampally and colleagues published an interesting observation on the enhanced population of different embryonic genes, including Oct-4, in a subpopulation of human marrow stromal cells after serum deprivation (30). D’Ippolito et al. as well demonstrated Oct-4 expression in human bone marrow cells cultured on fibronectin under low oxygen tension (31). Our own experiments also revealed that Oct-4 mRNA is expressed in human mononuclear cells from peripheral adult blood (data not shown). Together, these reports support our findings and provides possible role of hCG in endothelial differentiation of CD133+ cells. Further studies are underway to analyse the respective conditions that may be sufficient to induce organ- or lineage-specific differentiation of these cord blood-derived progenitor cells.

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Abbreviations

FGF-4, fibroblast growth factor-4; hCG, human chorionic gonadotropin; HSPC, hematopoietic stem/progenitor cells; HUVEC, human umbilical cord vein endothelial cells; LH/hCG, luteinizing hormone/human chorionic gonadotropin; Oct-4, octamer binding protein; PBGD, porphobilinogen deaminase; Rex-1, zfp-42 (zinc-finger protein 42); RT-PCR, reverse transcriptase-polymerase chain reaction; SOX-1/-2, sty-type high mobility group box; UMVEC, uterine microvascular endothelial cells; VEGF-A, vascular endothelial growth factor-A; VEGFR-2, vascular endothelial growth factor receptor-2.

References


