Expression of beta hCG and alpha CG mRNA and hCG hormone in human decidual tissue in patients during tubal pregnancy

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Introduction

hCG has been regarded as a pregnancy-specific hormone that is produced in the trophoblast. Later in the pregnancy, hCG is released in large quantities from the villous syncyto-trophoblast into maternal circulation. The glycoprotein hormone is composed of two non-covalently associated α and β subunits. The common α chorionic gonadotrophin (α-CG) subunit is encoded by a single α gene. Unlike α-CG, β-hCG is encoded by a cluster of six homologous genes localized in chromosome 19 (Fiddes and Goodman, 1979; Jameson and Hollenberg, 1993). The free α-CG and β-hCG proteins combine to form an intact biologically active hCG molecule. hCG bioactivity is dependent upon the glycoprotein side chain structure, which changes during pregnancy (Elliott et al., 1997; Mock et al., 2000). In the previous years, authors have reported that hCG is also secreted in non-trophoblastic healthy tissues and carcinoma (Yoshimoto et al., 1977; Braunstein et al., 1979; Marcillic et al., 1992). Recently, it was shown that gonadotrophin production appears to be associated with several different types of carcinomas (Dirnhofer et al., 1998; Hotokainen et al., 1999; Coleman et al., 2000).

We were the first group to demonstrate that the uterine glandular epithelium expresses hCG mRNA and produces hCG protein during the normal menstrual cycle (Alexander et al., 1998). hCG was also detected in the Fallopian tubes (Lei et al., 1993). Ovarian granulosalutein cells and testicular Leydig cells both express the hCG/LH receptor. This receptor binds hCG and relays the hormone message into the cell. Additionally, non-gonadal hCG/LH binding sites were identified in other tissues, such as the endometrium, decidua and the Fallopian tubes. Endometrial hCG/LH receptor expression is most pronounced in epithelial cells then stromal cells and finally vascular endothelial cells in the uterus (Reshef et al., 1990; Lei et al., 1992; Toth et al., 1994).

The maternal decidua is the transformed endometrium of a pregnant women. After conception, it develops from the pars functionalis of cyclic endometrium into two layers: the more superficial compact layer, which has glands with narrow lumen and the lower spongy layer, which has typical sawtooth-shaped papillar glands which are more dilated. The aim of our study was to examine whether the maternal decidua continues to produce the locally derived hCG, in addition to hCG produced by the trophoblast, in early pregnancy. A suitable early pregnancy model free of uterine cavity trophoblast tissue was needed for this experiment. Therefore, for our experiment we selected decidual tissue from patients with tubal pregnancy, which is free of villous or extravillous trophoblast hCG production.

RT–PCR and restriction enzyme analysis were used to demonstrate mRNA expression of α-CG and β-hCG subunits. Immunohistochemistry and the Western blotting method were used to demonstrate hCG secretion. Furthermore, we studied whether the amount of decidual hCG production depends on the degree of endometrial differentiation.

Key words: hCG/human decidua/immunohistochemistry/mRNA expression/Western blot
Materials and methods

Patients and decidual samples
To examine the α-CG and β-hCG expression and β-hCG protein production of uterine decidua lacking extravillous trophoblast, 24 cases of tubal pregnancies were identified for this study. Uterine decidual samples of extrauterine pregnancies serve as models of uterine mucosa during the pregnant state.

Decidua was collected by curettage during the laparoscopic extirpation of pregnancy. Informed consent was obtained from the patients. The gestational age was between weeks 6–11 of pregnancy. In each case, the ectopic pregnancy was verified by the clear localization of trophoblast in the Fallopian tube by ultrasound. Decidual specimens were only included in the study if the clinical analysis suggested the absence of other gynaecological pathologies. The collected decidual tissue samples were divided into two parts for routine paraffin embedding after they had been fixed in 4% neutral buffered formalin overnight, and for rapid processing or snap-freezing and storage at −80°C for subsequent RT–PCR analysis. Each of the decidual samples was examined histologically. Histological dating was confirmed by an experienced pathologist, who classified the decidual samples into three groups according to endometrial differentiation that took place prior to conception: (A) highly secretory transformed endometrium with distinct decidualized stromal areas, (B) diminished secretory transformed endometrium with partially decidualized stromal content, and (C) inferior and/or disturbed endometrial proliferation with minimal or no secretory transformation. Peripheral hCG concentrations were detected using the IMX-Totalβ-hCG MEIA (micro particle enzyme immunoassay) from Abbott.

Isolation of mRNA and RT–PCR
mRNA was extracted from the decidua. Approximately 20 mg of fresh specimens were washed in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco), were disrupted using an ultra-turrax. The mRNA was isolated using the Quick prep mRNA kit (Pharmacia) with mRNA binding to oligo dT-cellulose, and 50 μl of eluate was stored in small portions at −80°C.

Reverse transcription
mRNA was reverse transcribed using standard methods. A 2.5 μl aliquot of mRNA solution was heated at 65°C for 5 min, followed by cooling at room temperature. A 7.5 μl aliquot of cDNA mixture was added to give a final concentration of 10 mmol/l Tris–HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl2, 0.2 mmol/l of each dNTP, 2.5 U/μl Taq DNA polymerase and 20 pmol/μl of each primer pair of different β-hCG, α-CG and β-actin-oligonucleotides. Alternatively, for 300 bp products, 0.5% (v/v) dimethylsulphoxide (DMSO) was included in the total PCR mixture volume to raise the efficiency, as well as 10 mmol/l NaHCO3, and 125 μg/50 μl transferrin to protect DNA polymerase activity. All PCR amplifications started with a denaturation step of 2 min at 95°C and finished with an elongation step of 5 min at 72°C.

Amplifications of cDNA were performed using four different primer pairs specific for β-hCG gene 3, 5, 7 and 8 products respectively (all forward then reverse primer): β-hCGI 5′-TGGCGGTACGGCCCTTCTC-3′ and 5′-CCCGAGAACCCCTGCAAGCA-3′ (562 bp product) with 1 min denaturation at 95°C, 2 min annealing at 60°C and 3 min extension at 74°C for 35 cycles (Bo et al., 1992); β-hCG2 5′-TTCTTACACCTCTTCCTGTG-3′ and 5′-CCCGAGAACCCCTGCAAGCA-3′ (511 bp) with 60 s at 95°C, 40 s at 61°C and 45 s at 72°C for 35 cycles (Jameson and Hullegen, 1993; Dirdhorst et al., 1996); β-hCG3 5′-TCACCTACCGGTTTGTCTC-3′ and 5′-TGGAGCAGGGTGTCATGGTT-3′ (423 bp) with 30 s at 95°C, 30 s at 60°C and 30 s at 72°C for 35 cycles (Miller-Lindholm et al., 1997); β-hCG4 5′-TGGCTGGTGAGGGAAGGAGCGGC-3′ and 5′-GGAAAGCGGGGCTCATCACAGGTCT-3′ (300 bp) with 30 s at 95°C, 30 s at 65°C and 60 s at 72°C for 35 cycles (Talmadge et al., 1984; Krichevsky et al., 1995). In addition, the primer α-CG 5′-TGCCAGATTGGCGCAATGC-3′ and 5′-CCGTTGTTCTCCA-CCTTG-3′ (233 bp) with 30 s at 94°C, 30 s at 56°C and 60 s at 72°C for 35 cycles (Fiddes and Goodman, 1979) and β-actin 5′-GTGGGGGCCCG-CAAGGCACCA-3′ and 5′-CTCTTTAATGTCAGCGACGATTCC-3′ (547 bp) with 30 s at 95°C, 30 s at 65°C and 60 s at 72°C for 35 cycles (Stewart et al., 1998) were used for PCR amplification. All the oligonucleotide primer pairs were synthesized by Applied Biosystems. Aliquots of 9 μl PCR products were run on a 2.0% agarose gel in 0.50 mol/l Tris-buffered 0.15 mol/l saline (TBS) buffer, pH 7.4, to prove the efficiency and fidelity of the β-hCG and α-CG-DNA fragment production, using 0.01% ethidium bromide for UV identification and photographic documentation.

Restriction enzyme analysis
The identity of the 300 bp PCR product was verified by restriction enzyme digestion. Several β-hCG-specific restriction enzymes (Slyl, Bsp1286, HaeIII and AvalII from Roche) were included in cleavage experiments to confirm the PCR-derived DNA sequence for β-hCG as opposed to β-LH. The enzyme concentration and optimal buffer conditions for DNA digestion were chosen according to the manufacturer’s instructions. The RT–PCR products were separated electrophoretically on an agarose gel and the 300 bp β-hCG DNA amplicate was extracted using a DNA purification kit (Biozyn). DNA digestion fragments resulting after overnight incubation at 37°C were demonstrated on a 2% agarose gel. The patterns of cleavage products obtained from digestion of the 300 bp amplify with Slyl (300 bp), Bsp1286 (175/125 bp), HaeIII (300 bp) and AvalII (94/87/43/33/33 bp) would be consistent with the β-LH DNA sequence.

Immunohistochemistry
The divided parts of fresh decidua collected from tubal pregnancies were used in parallel for PCR studies as well as histological embedding in paraffin. The tissue blocks of decidua, and as a control, paraffin blocks of early pregnancy placenta, were cut at 5 μm serial tissue sections, mounted on superfridge slides, deparaffinized, cleared in xylene and a gradient of ethanol, and rehydrated and incubated for 10 min in 0.05 mol/l TBS with 0.1% Tween-20, pH 7.6 (TBST). After rehydration, the specimens were incubated with 0.3% hydrogen peroxide in methanol (30 min) to block endogenous peroxidase activity. Triplet sections were stained for all 24 samples and controls.

Immunolocalization of hCG
For hCG staining using polyclonal antibody, after brief washes in TBS, the tissue sections were placed in a humidified chamber and then sequentially overlaid and incubated with 100 μl of the following reagents: TBS with 0.2% Triton X-100 as antigen demasking procedure for 10 min; the biotin and then avidin blocking solution (Dako) for 10 min each for endogenous biotin suppression; 10% normal goat serum (NGS) in TBS for 30 min to block the non-specific staining; after removing excess NGS blocking serum, the anti-hCG rabbit A0231 (Dako) primary antibody (diluted 1:500 in TBST/10% NGS) was left overnight at 4°C; Vector Elite ABC kit (Vector) with biotinylated goat anti-rabbit IgG as second antibody diluted 1:200 in TBST/NGS for 30 min; POD-conjugated avidin–biotin complex (Vectorstain ABC) for 30 min; finally, diaminobenzidine (DAB) from Vector for 5 min to develop a brown reaction product. Negative controls were performed by omitting the use of primary hCG or other primary antibodies. Initially, the specimens were mounted on aqueous-based histogel and later dehydrated, cleared in xylene and mounted with a non-aqueous permanent mounting medium.

For hCG staining in endometrial sections using mouse monoclonal anti-β-hCG antibody (INN2 or INN22, Serotec) diluted 1:5000 or 1:200 in TBST/normal rabbit serum (NRS), the Catalyzed Signal Amplification (CSA) system of Dako was required and was used according to manufacturers’ instructions. After POD blocking of sections with hydrogen peroxide in methanol, biotin/avidin blocking and blocking non-specific antibody binding with TBST/NRS as described, incubation with the primary antibody was continued at 4°C overnight, followed by the secondary biotinylated rabbit anti-mouse IgG (Vector) antibody diluted 1:200 in TBST/NRS and the POD-conjugated avidin–biotin complex (Dako), each for 30 min. In the CSA system a supplementary amplification step was included to increase biotin signals localized at the antibody-binding site. Finally, the incubation with the POD–avidin conjugate allowed the amplified DAB staining reaction. The
intensity of hCG staining scaled semi-quantitatively from 3-fold positive to negative compared with controls.

**Trophoblast immunolocalization**

In order to exclude the presence of extravillous trophoblast cells we examined the decidua specimens with the monoclonal human cytokeratin antibody (MNF116, Dako). It is specific for cytokeratin 5, 6, 7, 17 and probably 19, and binds to epithelial cells and all trophoblast cells. Before staining, the paraffin-embedded sections were treated as described.

However, cytokeratin antigen staining required demasking pretreatment with target retrieval citrate solution, pH 6.2 (Dako), for 30 min at 95°C in a water bath followed by 20 min of cooling. The hydrogen peroxide and biotin/avidin-blocked and NRS-preincubated slides were incubated with cytokeratin antibody diluted 1:1000 overnight at 4°C, followed by biotinylated secondary rabbit anti-mouse IgG antibody (Dako). The immunohistochemical staining procedure was performed according to the CSA method as given above, visualized by application of VIP chromogen (Vector).

**Western blotting**

Approximately 50 mg of fresh decidual tissue specimens were cut with scissors into small pieces and washed repeatedly with RPMI 1640 containing 50 IE/ml penicillin and 50 μg/ml streptomycin. The separated tissue was taken up in 2 ml of 50 mmol/l Tris–HCl reducing lysis buffer containing 150 mmol/l NaCl, 1.0% Nonidet P-40, 0.5% sodium deoxycholate, 1 Complete Protease Inhibitor tablet (to 1 mmol/l EDTA)/10 ml (Roche), 1 μmol/l pepstatin, inhibiting acid proteases, and disrupted by the ultra turrax method at 4°C. Clarified lysate supernatant was prepared by centrifugation at 12 000 g for 5 min at 4°C. To reduce non-specific binding to the nitrocellulose membrane, the lysate supernatant was precleared using protein A±agarose incubation at 4°C overnight (Roche). The lysate proteins and a low molecular weight protein standard mixture (Pharmacia) were size separated by reducing 10% sodium dodecyl sulphate±polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose by electroblotting. Resulting filters were incubated with polyclonal primary rabbit anti-β-hCG antibody (A0231, Dako) at a dilution of 1:500 or monoclonal anti-β-hCG antibody (INN22, Serotec) at a dilution of 1:100 at 4°C overnight followed by incubation with biotinylated secondary goat anti-rabbit (1:500) or anti-mouse (1:500) antibodies respectively, for 1 h at room temperature and then reaction with ABC complex (Vector). The molecular forms of hCG were detected by visualization with DAB staining using the same conditions as immunohistochemistry and correlated to molecular weight markers. Dimeric hCG products were obtained from Sigma, Biotrend and Serono. The staining of the biotinylated secondary antibodies without any hCG binding could be localized by omission of the first hCG antibody.

**Statistical analysis**

All hCG data were expressed as the mean ± SEM. Statistical analyses were performed using the SPSS statistical software program. The data were checked for normal distribution (Kolmogorov–Smirnov test) and are distributed normally. The two-tailed t-test reflects a significant difference at a value of P < 0.050.
Results

We examined mRNA transcription and corresponding hCG production in decidual samples from patients during tubal pregnancy. β-hCG and α-CG mRNAs were detected with the RT–PCR technique (Figure 1) and β-hCG quantified with respect to β-actin (Figure 2). β-LH expression was excluded with restriction enzyme analysis (Figure 3). hCG production in the decidua was determined in decreasing stages of differentiation (Figure 4) and with different hCG antibodies by immunohistochemical staining. The absence of extravillous trophoblast cells in the decidua was verified by cytokeratin staining (Figure 5). The molecular weights of hCG subunit proteins were determined using SDS–PAGE and Western blotting (Figure 6).

The immunohistochemical and clinical findings of the decidual samples were classified into three groups according to the morphological developmental stage as described above (Table I). The peripheral hCG levels of patients were recorded immediately before termination of the tubal pregnancy. We found a significant difference between groups A and C (P < 0.001). In group A (high secretory transformation and decidualization), the mean serum hCG was 1829 ± 292 mU hCG/ml (mean ± SEM), in group B (diminished secretory transformation and partial decidualization), 1072 ± 354 mU hCG/ml, and in group C (inferior or disturbed proliferation and minimal or no secretory transformation), 349 ± 98.7 mU hCG/ml. Uterine bleeding, indicative of steroid hormone deficiency, occurred mainly in group C with poor proliferation. Haematosalpinx (haemorrhagia into the tubal lumen) was found in all three groups. A likely positive correlation between secretory transformation and immunospecific glandular hCG formation in both the decidual compact and non-decidualized spongy layers was demonstrated by the strength of the glandular immunohistochemical hCG staining (from 3-fold positive to negative).

Using specific oligonucleotide primer pairs in the PCR, which have been described previously (Bo and Boime, 1992; Dinnohofer et al., 1996; Miller-Lindholm et al., 1997), we found the expected β-hCG cDNA amplification products of 423, 511 and 562 bp. This confirmed decidual β-hCG gene expression. Early pregnancy placenta specimens were used as a positive control (Figure 1A, B). While the abovementioned primer pairs covered the full-length exon 1 to exon 3 cDNA, a further primer pair (Krichevsky et al., 1995) produced a 300 bp amplification of exon 2 to exon 3. The expression of α-CG mRNA was found in decidual tissue from the patients with tubal pregnancy as well as the placental control (Figure 1C). We also compared the extent of β-hCG mRNA expression in the samples with varying decidual differentiation.

We were able to semi-quantitatively demonstrate that the 300 bp β-hCG nucleotide products are expressed in varying amounts relative to the constitutive β-actin mRNA expression (Figure 2). β-hCG messenger RNA is lacking or poorly expressed in decidual tissue that is only poorly proliferated or has not undergone secretory transformation. To verify the identity of β-hCG amplificates, DNA cleavage experiments were performed. Digestion of the 300 bp β-hCG cDNA with different restriction enzymes (Styl, Bsp1286, HaeIII, AvaII) resulted in the anticipated smaller fragments, which characterize the β-hCG cDNA origin and proved that β-LH mRNA was not present (Figure 3A, B).

Serial decidual tissue sections were evaluated immunohistochemically for hCG and correlated to decidual immune cells to identify competent decidual cells for hCG expression and release (data not shown). The immunolocalization indicates that glandular epithelial cells seem to be the major site of uterine hCG expression, in both the non-decidualized spongy layer and the more decidualized compact layer of decidua that is closed to the luminal epithelium. However, it is likely that immunostaining of glandular hCG in decidualized compact areas and non-decidualized spongy layer is more prominent in the high secretory transformation group (Figure 4A, B) than in the reduced secretory group, where only singular stained cells in the spongy glands can be found (Figure 4C, D). In contrast, hCG protein expression could not be detected in inferior and disturbed secretory endometrium sections using the immunohistochemical hCG staining method (Figure 4E, F). We used placenta specimens from early pregnancy as a positive control for hCG, and decidua sections that were not treated with hCG antibody as a negative control (Figure 5C, D). In order to exclude the presence of interstitial extravillous trophoblast cells in the studied decidua specimens of tubal pregnancy, serial tissue sections were stained using trophoblast-specific cytokeratin antibody.

Extravillous trophoblast cells in the decidual stromal or spiral artery regions, which could have been responsible for the β-hCG production, were not found in any of the investigated tissue (Figure 5A, B). In contrast to intrauterine pregnancies, decidual extravillous trophoblast cells should not occur.

Figure 3. Cleavage of DNA amplificates of placental and decidual β-hCG by restriction enzymes. (A) Placental β-hCG amplificate of 300 bp without restriction enzyme (lane 2) and after digestion with enzymes Styl, Bsp1286, HaeIII and AvaII in lanes 3–6. (B) Decidual β-hCG amplificate was cleaved to identical fragments as the placenta specimen by Styl (261/39 bp), Bsp1286 (226/74 bp), HaeIII (196/104 bp) and AvaII (163/94/35/8 bp).
Epithelial hCG immunostaining of decidual glands was performed with a polyclonal β-hCG antibody as well as monoclonal β-hCG antibodies INN2 or INN22, characterizing the β-hCG chain epitope of cluster B I or B II from dimeric hCG respectively, as shown in Figure 5E–G (Lund and Delves, 1998).

To assign the detected immunohistochemical hCG to a molecular state, we examined the tissue extract fluid of decidua specimens and compared it with placental tissue extract from early pregnancy and pure dimeric hCG products. Western blots were obtained using polyclonal and monoclonal β-hCG antibodies, demonstrated in Figure 6A–D. The immunostained main band of decidual hCG appeared at ~30 kDa next to a faint band of 33 kDa, which was sometimes present using the polyclonal antibody. The bands were correlated to the expression profiles of N-glycosylated β-hCG molecular isoforms in placenta specimens or trophoblast cells (Elliot et al., 1997; Singh and Merz, 2000).

Discussion

The present study demonstrated that the β-hCG gene and α-CG gene are transcribed into mRNA in the uterine decidua of patients during tubal pregnancy. hCG is generally regarded as a trophoblast hormone. However, recently we identified hCG in glandular cells of the endometrium during the secretory phase of the menstrual cycle (Wolkersdörfer et al., 1998). In the present study, we investigated the role of decidua in hCG secretion. To our knowledge, this is the first demonstration of expression of α-CG and β-hCG subunit mRNAs and production of hCG protein in the decidua of patients during early pregnancy. We used tubal pregnancies, in contrast to normal intrauterine pregnancy, as a model that is free of extravillous trophoblast, which penetrates the decidua. We ruled out the presence of stromal or endovascular trophoblast cells by using cytokeratin antibody MNF116 for immunohistochemical trophoblast staining.
We demonstrated that the mRNA gene expression of α-CG and β-hCG is actually occurring in decidual mucosa. Moreover, different PCR primer pairs spanning exon 1 to exon 3 of the β-hCG DNA sequence established decidual, as opposed to trophoblast, expression. Additionally, the application of DNA restriction enzymes for subunit-specific digestion confirmed the expected β-hCG restriction sites. In our three patient groups, decidual β-hCG mRNA expression was related to the level of endometrial differentiation. This appears to be dependent on the degree of endometrial secretory transformation and stromal decidualization that took place before conception.

Furthermore, the immunohistochemical staining of the decidua demonstrated that in early pregnancy, hCG is produced in the epithelium but probably not in the stromal cells. This hCG production seems to correlate with the level of differentiation of the epithelium. The most intensive staining was found in the mono-layered glands with rather small-sized epithelial cells surrounded by decidual cells of a superficial compact layer. Also, the common papillary indented glands in the deeper spongy layer with their round to oval cell nuclei of epithelium were readily stained. hCG staining of glands near to the basal layer, which were not as differentiated with rather oval to longish nuclei, showed only slight staining that was restricted to single cells.

Haematoxylin counterstaining of the serial tissue sections was not shown. It seems that hCG protein is either not produced at all or only in small quantities in stratified epithelium of decidual glands with cylindrical to spindle-shaped nuclei (i.e., the hCG stain disappears in the epithelial structures of this tissue with inferior proliferation without secretory transformation). Therefore, we conclude that the glandular hCG production depends on the degree of the epithelial cell differentiation in early pregnancy.

hCG is primarily considered a specific placental hormone. However, numerous authors reported that hCG is present in normal and tumour tissues (Braunstein et al., 1979; Marcillac et al., 1992; Dinhofer et al., 1996, 1998; Yokotani et al., 1997; Coleman et al., 2000). We showed for the first time that normal cyclical secretory endometrium expresses subunit mRNA and produces hCG (Alexander et al., 1998) which was later confirmed by Reimer et al. (2000). The decidua, as a highly differentiated endometrium found during pregnancy, can also produce hCG. hCG effects appear to be triggered by binding of hCG to known non-gonadal hCG/LH receptors (Reshef et al., 1990).

The question arises whether hCG production in the decidua has a biological function or if it is a epiphenomenon. We think that the decidual hCG functions as an autocrine and paracrine hormone that
may play an important role in implantation and early pregnancy. The human decidua forms other proteins and cytokines, which supply prerequisites for adequate morphological and functional transformation during implantation and early pregnancy, and for innate immunodefence or embryonic allogene immunomodulation (Krasnow et al., 1996; Rier and Yeaman, 1997; Hammer et al., 1999; King et al., 1999). It was also demonstrated, using the in-vivo uterine flushing method, that patients with an adequate luteal phase

Table I. Immunohistochemical and clinical data of patients with tubal pregnancy

<table>
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<th>Patients</th>
<th>Proliferation</th>
<th>Secretory transformation</th>
<th>Stromal differentiation</th>
<th>Immunohistochemical hCG</th>
<th>Haematosalpinx</th>
<th>Uterine bleeding</th>
<th>Gestation week</th>
<th>Serum hCG (mU/ml)</th>
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<td>High</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>5.1</td>
<td>117</td>
</tr>
<tr>
<td>6</td>
<td>Inferior</td>
<td>Early</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>6.3</td>
<td>514</td>
</tr>
<tr>
<td>7</td>
<td>Inferior</td>
<td>Without</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td>5.1</td>
<td>199</td>
</tr>
<tr>
<td>8</td>
<td>Inferior</td>
<td>Without</td>
<td>–</td>
<td>–</td>
<td></td>
<td>+</td>
<td>5.1</td>
<td>915</td>
</tr>
</tbody>
</table>

Figure 6. SDS–PAGE of hCG and Western blotting. The blots were visualized using polyclonal (A, B) and monoclonal (C, D) hCG primary antibody for hCG controls and decidual specimens. Molecular weight markers (not demonstrated) and hCG control products (lane 1, left, Serono; lane 2, left, and lane 1, right, Sigma; lane 3, left, Biotrend; lane 5, left, own preparation) were run together with lysates of placental (lane 4, left) and several patient specimens obtained from tubal pregnancy decidua (lanes 2–6, right). The bands in A and B were stained with a polyclonal antibody (A0231, Dako) and in C and D with monoclonal antibody (INN22, Serotec), followed by biotinylated second antibody, ABC complex and the DAB procedure according to the described method. The decidual β-HCG shows a predominant band of ~30 kDa with a faint 33 kDa band in comparison to the 33 kDa main product of hCG controls.
endometrium demonstrate secretion of hCG into the uterine cavity (Alexander et al., 1998; Licht et al., 1998). It seems that during pregnancy the endometrium continues to secrete hCG. The local function of hCG in this tissue is not yet clear, but it can be assumed that hCG stimulates specific hCG/LH receptors in epithelial, stromal and endothelial cells of endometrium and decidua (Eta et al., 1994). Therefore, hCG could locally prepare the endometrium for implantation and prime the decidua for an ongoing pregnancy via paracrine action. Local hCG effects on the induction or inhibition of uterine cytokine formation were described by several authors. hCG is known to amplify the interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFα) secretion in the endometrial cells (Schäfer et al., 1992; Uzumcu et al., 1998). In-vivo administration of small quantities of hCG into the uterine cavity raises vascular endothelial growth factor (VEGF) and insulin-like growth factor binding protein 1 (IGF-BP1) and reduces the macrophage colony-stimulating factor (M-CSF) secretion of tissue into the uterine perfusate (Licht et al., 1998).

Further, hCG-stimulated prostaglandin E2 (PGE2) induces a switch from the pregnancy-threatening endometrial TH1 cytokine environment characterized by IL-12 to an IL-10-dominated and pregnancy-promoting TH2 cytokine profile (Kraan et al., 1995; Kelly et al., 1997). Interestingly, hCG may be characterized as a chemo-attractant for peripheral mononuclear cells (Reinisch et al., 1994). Moreover, hCG directly restrains activation of local sessile macrophages capable of expressing inflammatory cytokines (Song et al., 2000). Additionally, hCG shows potent vasodilatory characteristics in the vascular endothelium and vascular smooth muscle of the uterine spiral arteries (Hermsmeier et al., 1999). hCG also supports maintenance of uterine quiescence caused by suppression of oxytocin-induced myometrial contraction (Kurtzman et al., 1999). Thus, hCG seems to be involved in important immunomodulating cytokine effects on decidual differentiation.

Moreover, it was reported that hCG induction of cyclooxygenase-2 (COX-2) enzyme gene expression in stromal fibroblast cells promotes morphological and functional differentiation into decidual cells (Han et al., 1996). In contrast to humans, non-human primate endometrium stromal decidualization begins only after implantation and embryonic choriongonadotrophin secretion. However, it has been recently shown that the transformation of baboon endometrial stromal cells to epithelioid-like decidual cells can also take place in vivo if hCG is infused into the normal cyclic uterus (Fazleabas et al., 1999; Han et al., 1999). The physiological regulation of decidualization includes similar or comparable metabolic pathways. Both human and non-human primate endometrial transformation are connected with the disruption of the actin skeleton and the formation of decidual α-smooth muscle actin, directly induced through hCG and terminated by rising decidual IGF-BP1 levels (Kim et al., 1998, 1999). hCG could be regarded as a growth hormone-like protein when compared with other cysteine-knot hormones (Linthorn et al., 1994).

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References


HCG mRNAs and protein in uterine decidua during tubal pregnancy


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