The effect of letrozole with misoprostol for medical termination of pregnancy on the expression of steroid receptors in the placenta

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STUDY QUESTION: What is the effect of letrozole on the expression of steroid receptors in the placentae in cases of termination of pregnancies?

SUMMARY ANSWER: The expression of estrogen receptor-α (ERα) and progesterone receptor (PR) transcripts, as well as ERα protein, in placentae was suppressed by letrozole pretreatment in second trimester termination of pregnancy.

WHAT IS KNOWN ALREADY: There have been no data in the literature on the effect of letrozole in termination of human pregnancies.

STUDY DESIGN, SIZE, DURATION: This study is part of a clinical randomized trial in which 50 subjects were recruited and 44 placentae were collected.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Women (n = 50) requesting second trimester abortion between 12 and 20 gestational weeks were randomized to receive either letrozole or placebo pretreatment for 3 days before administration of vaginal misoprostol. Placentae were collected from both groups of women after the abortion. Total RNA from the frozen placenta samples was extracted and subjected to real-time RT–PCR analysis of ERα and estrogen receptor-β (ERβ), PR and glucocorticoid receptor (GR) transcripts. Immunohistochemical studies of ERα, ERβ, PR and GR expression, as well as Ki67 and PCNA staining for proliferation, were performed. TUNEL assays were performed to determine the extent of apoptosis.

MAIN RESULTS AND THE ROLE OF CHANCE: Real-time RT–PCR demonstrated that the median ERα {3.900 [95% confidence interval (CI): 2.0.643–8.443]} in the letrozole group versus 4.714 (95% CI: 1.776–7.652) in the control group; \( P = 0.005 \}) and the median PR [0.701 (95% CI: 0.333–1.069) in the letrozole group versus 1.774 (95% CI: 1.07–2.478) in the control group; \( P = 0.003 \}) were significantly lower in the letrozole group compared with the control group. Furthermore, ERα protein levels, in both syncytiotrophoblasts and cytotrophoblasts but not in villous stromal cells, were significantly reduced [H-score of 113 (95% CI: 103–119) in the letrozole group versus 217 (95% CI: 214–290) in the control group, in syncytiotrophoblasts; 100 (95% CI: 98–105) in the letrozole group versus 210 (95% CI: 200–286) in the control group, in cytotrophoblasts; \( P = 0.004 \}], while the expression levels of ERβ, PR, GR, PCNA, Ki67 and TUNEL were not significantly different between the two groups.

LIMITATIONS, REASONS FOR CAUTION: Only the placentae from the second trimester termination of pregnancy were collected in this study. Information from first trimester terminations is still lacking.

WIDER IMPLICATIONS OF THE FINDINGS: The results shed some light on the mechanism of action of letrozole pretreatment in termination of pregnancies.

STUDY FUNDING/COMPETING INTEREST(S): This study was funded by the GRF/RGC and CRCG grants of the University of Hong Kong.

TRIAL REGISTRATION NUMBER: HKClinicalTrials.com with trial number HKCTR-695.

Key words: letrozole pretreatment / misoprostol / second trimester / termination of pregnancy / steroid receptors
Introduction

Progesterone is pivotal in the maintenance of pregnancy and the use of progesterone receptor (PR) antagonist, mifepristone, during pregnancy facilitates the abortion process (Gemzell-Danielsson et al., 2006). In the second trimester of pregnancy, the combined regimen of mifepristone followed by misoprostol produces a high abortion rate of 97–100% within 24 h, with an induction-to-abortion interval of ~5–10 h (Kapp et al., 2007; Chai et al., 2009). However, mifepristone is expensive and not registered in many countries. The abortion rate of a misoprostol alone regimen is only 37–86% in 15–24 h depending on the regimen, route of administration and dosage used (Von Hertzen et al., 2009; Ngoc et al., 2011). The exploration of new regimens to achieve a safe second trimester abortion is important, especially in developing countries.

The use of letrozole pretreatment followed by vaginal misoprostol in the first trimester abortion is more effective than misoprostol alone (Lee et al., 2011a, b). The use of letrozole in second trimester abortions seems to be promising with an abortion rate of 100% within 24 h, with a median induction-to-abortion interval of 11 h (range 6.1–19.3 h) (Lee et al., 2011c). We hypothesize that letrozole may facilitate medical abortion by affecting the corpus luteum and/or the placenta. In the second trimester, the effects on the corpus luteum, if any, are unlikely to be important factors in facilitating medical abortion. Therefore, we hypothesized that letrozole might work through steroid receptor expression in the placenta during this gestational period. The present study aims to address this possibility.

Materials and Methods

Study population

Women attending the Department of Obstetrics and Gynecology, the University of Hong Kong, for medical abortion at the gestational age between 12 and 20 weeks were recruited by a designated research nurse. Gestational ages were confirmed by ultrasound examination. Women were included if they were healthy; older than the age of legal consent (i.e. > 18 years old); had a live fetus corresponding to 12–20 gestational weeks as confirmed by ultrasound scanning on Day 1 of the study (i.e. day of letrozole administration); and had hemoglobin levels >10 g/l. We excluded women on the basis of: a history or evidence of adrenal pathlogy, steroid-dependent cancer; porphyria, diastolic pressure over 95 mmHg, bronchial asthma or arterial hypertension; regular use of prescription drugs before admission to the study; heavy smoking of >20 cigarettes per day; a history or evidence of thromboembolism, severe or recurrent liver disease or pruritus of pregnancy; an intrauterine device; or any abnormal values in pretreatment blood tests.

The study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. The study was registered on HKClinicalTrials.com with the trial number HKCTR-695. Written informed consent was obtained if the woman was eligible and agreed to participate in the study.

Randomization and treatment regimen

Women were recruited as described in a previous study (Lee et al., 2011a). In brief, the randomization schedule was prepared by the hospital pharmacist according to computer-generated random numbers. The packages of letrozole (10 mg daily for 3 days) and placebo tablets were prepared and numbered accordingly. Until the completion of the study, both patients and clinicians were blinded to the assigned group.

Women who were eligible for and willing to participate in the study completed a detailed history and physical examination before undergoing an ultrasound examination in order to confirm the gestational age. Women in the letrozole group received 10 mg letrozole on Days 1 and 2, whereas women in the placebo group received placebo tablets, which were of the same appearance as letrozole tablets. A designated research nurse supervised the subjects in taking the first dose of letrozole or placebo tablets on Day 1 and the subjects took the second dose herself on Day 2. The third dose of letrozole or placebo was given on admission to our hospital on Day 3, followed by 400 μg vaginal misoprostol soaked with normal saline every 3 h up to a maximum of 5 doses. The administration of misoprostol was withheld if the woman had strong uterine contractions. After the termination, the products of gestation were examined to see whether the abortion was complete. If necessary, exploration and evacuation of the uterus was performed under general anaesthesia.

Placentae were collected after abortion from the 50 women except those requiring surgical removal or those whose placenta was removed in piece-meal. The samples were first divided into two portions. One part was snap-frozen in liquid nitrogen and stored at ~80°C for RNA and protein extraction, and the other part was fixed in 4% paraformaldehyde for paraffin-embedding for immunohistochemical (IHC) analysis. The tissue blocks for IHC were cut into 6 μm thick sections, mounted on 3-amino-propyl-triethoxysilane-coated slides and dried overnight in an oven at 37°C. Sections were stained with hematoxylin and eosin and examined histologically.

Quantitative PCR

Total RNAs from placental tissues were extracted by the Absolutely RNA MicroPrep Kit (Stratagene) according to the manufacturer’s protocol. RNA samples were reverse-transcribed into cDNA using the TaqMan Reverse Transcription Kit (PE Applied Biosystems). Real-time PCR analysis of ERα (Hs00172183_m1), ERβ (Hs00230957_m1), PR (Hs00172183_m1) and glucocorticoid receptor (GR) (Hs00230818_m1) mRNA were performed using the TaqMan Universal PCR Master Mix and the ABI 7500 Sequence Detector (PE Applied Biosystems). Each sample was run in duplicate with the amplification conditions set as follows: 50°C for 2 min to optimize the AmpErase UNG enzyme activity, followed by 95°C for 10 min to activate the AmpliTaq Gold DNA polymerase, and then 40 cycles of 95°C for 1 s and 60°C for 1 min for the PCR. The resulting gene expression profile was determined using 18S as an endogenous standard. The threshold cycles (Ct) for each reaction were calculated and used for quantifying the amount of starting template in the reaction. The proportion of the gene of interest in a sample was presented as the difference in Ct values (∆Ct) between the respective gene and that of 18S. The relative gene expression among samples was calculated by the 2-ΔΔCt method as described elsewhere (Livak and Schmittgen, 2001).

Immunohistochemical detection

IHC staining was performed as described previously (Liu et al., 2010). In short, primary anti-PR (1:1000 dilution; Dakocytomation), anti-ERα (1:1000 dilution; Dakocytomation), anti-ERβ (1:1000 dilution, abcam), anti-GR (1:10 dilution; Leica Biosystems), anti-PCNA (1:1000 dilution; Dakocytomation) and anti-Ki67 (1:1000 dilution; Dakocytomation) antibodies in 10% rabbit serum were incubated with tissue sections at 4°C overnight. After thorough washing with phosphate-buffered saline, the tissue sections were incubated with biotinylated polyclonal rabbit–anti-mouse IgG at 1:600 dilution in 10% rabbit serum, followed by StrepABCComplex/HRP. A positive brown signal was visualized with 3,3’-diaminobenzidine as the substrate (Dakocytomation). The sections were then counter-stained in light hematoxylin, dehydrated, cleared and mounted. A known positive control of breast tissue was used for PR and ER, a known positive control...
of skin was used for GR while a known positive control of endometrium was used for PCNA and Ki67.

**In situ TUNEL assay**

Apoptotic cells were detected by the TUNEL assay with the in situ Cell Death Detection Kit, AP (Roche Diagnostics GmbH), following the manufacturer’s instructions. Briefly, deparaffinized placenta sections were permeabilized with 0.1% Triton X-100 diluted in 0.1% sodium citrate for 8 min on ice. The sections were then incubated in 50 μl staining solution, which contained fluorescein-labeled dUTPs and terminal deoxynucleotidyl transferase (TdT) enzyme to label DNA breaks. After 1 h TdT reaction at 37°C, the signal was amplified by incubation with the alkaline phosphatase (AP)-conjugated anti-fluorescein antibody for 30 min at 37°C, and was visualized with Fast Red (Roche Diagnostics GmbH). Positive controls were prepared by pretreatment of the sections with DNase I (USB Corporation, Cleveland, OH, USA) at 40 IU/ml for 10 min at 37°C before labeling. Sections for the negative control were incubated with the staining solution free of TdT.

**Outcome measures**

The primary outcome measure of this laboratory work was the PCR values of the steroid receptors. The secondary outcome measures were the IHC scoring of the receptors and the results of the TUNEL assay of apoptosis markers.

**Scoring and statistical analysis**

Slides were examined under the microscope at ×400 magnification. Immunoreactivities in deciduae and trophoblastic villi were assessed separately. They were judged semi-quantitatively using McCarty’s H-scoring system which was calculated by the equation: $H$-score = $\sum P_i(i + 1)$, where $i$ was the intensity of staining graded as 0, 1, 2 and 3 with 3 being the highest intensity, while $P_i$ was the percentage of tissue stained at each intensity (0 – 100%). This gave a total score that varied from 0 to 300. All the slides were scored by a single investigator who was blinded to the treatment group from which the sample was derived.

All data were analyzed using the SigmaPlot software (Jandel Scientific, San Rafael, CA, USA). Statistical analysis was performed by the Kruskal–Wallis or Mann–Whitney U-test as appropriate. The semi-quantitative IHC data were analyzed using the Mann–Whitney test. $P < 0.05$ was considered statistically significant.

**Results**

**Demographic data**

The flow of participants from recruitment to inclusion is presented in Fig. 1. Surgical removal of the placenta was required in 6 of the 50 women. As a result, a total of 21 and 23 placentae samples were collected in the control group and in the letrozole group, respectively. No significant differences were found in the women’s age, body mass index, number of previous terminations, number of previous deliveries, gestational weeks by ultrasound and induction-to-abortion interval.
between the two groups (Tables I and II). There were seven samples in the letrozole group and eight samples in the control group containing decidual tissues.

### Real-time PCR on steroid receptor transcripts

Total RNA was isolated from the placenta samples and subjected to real-time RT–PCR analysis. Real-time RT–PCR results demonstrated that the median ERα (3.900 [95% confidence interval (CI): −0.643 to 8.443] in the letrozole group versus 4.714 [95% CI: 1.776–7.652] in the control group; \( P = 0.005 \)) and the median PR (0.701 [95% CI: 0.333–1.069] in the letrozole group versus 1.774 [95% CI: 1.07–2.478]) in the control group; \( P = 0.003 \)) were significantly lower in the letrozole group compared with the control group. There were no significant differences in the placental expression of ERβ and of GR between the two groups (Fig. 2).

### Expression of steroid receptors

Moderate ERα immunostaining was observed in the cytotrophoblasts and syncytiotrophoblasts, while weak staining was observed in villous stromal cells in both groups (Fig. 3A). Weak or no staining was demonstrated in the samples of decidua. There was a significant decrease in ERα immunostaining in both cytotrophoblasts and syncytiotrophoblasts (Fig. 3A, Table III). Yet, there was no significant difference in GR immunostaining was observed in both the decidua and chorionic villi. GR was expressed in the cytotrophoblasts and villous stromal cells of the placenta (Fig. 3A, Table III). Yet, there was no significant difference in GR expression in either decidua or chorionic villi between the two groups. No GR immunoreactivity was detected in the syncytiotrophoblasts.

### Assays of proliferation and apoptosis

For the proliferative markers, strong PCNA immunostaining was observed in the nuclei of cytotrophoblasts, while weak immunostaining was observed in the villous stromal cells. There was also weak immunoreactivity of PCNA observed in the decidua but not in the syncytiotrophoblasts. Mild-to-moderate Ki67 immunostaining was demonstrated in the nuclei of villous stromal cells, but not in the syncytiotrophoblasts. No Ki67 immunostaining was found in the decidua and decidial glands (Fig. 3B). The immunoreactivities of both Ki67 and PCNA were not affected by the administration of letrozole (Fig. 3B, Table III). The percentage of TUNEL-positive cells remained low in both the letrozole and the control groups. No significant difference in the percentage of TUNEL-positive cells was found with or without the administration of letrozole (Fig. 3B, Table III).

### Discussion

In the present study, we found that letrozole pretreatment in second trimester suppressed the expression of ERα and PR transcripts, as well as the expression of ERα protein in placenta. There were no changes in PCNA or Ki67 expression (proliferative markers) or in TUNEL staining (apoptosis marker) after letrozole treatment for termination of pregnancy.

Estradiol and progesterone are the two most important steroid hormones produced by the human placenta. Progesterone is pivotal for pregnancy maintenance, but there is still controversy about the presence of PR in the human placenta (McCormick et al., 1981; Younes et al., 1981; Khan-Dawood and Dawood, 1984; Goldman and Shalev, 2006; Taylor et al., 2006). We demonstrated the presence of PR expression in the nuclei of human decidua together with the absence of PR in the chorionic villi, which is compatible with a previous report (Taylor et al., 2006). There was evidence that the use of mifepristone in the first trimester did not alter the expression of PR in the placental and decidual tissues.

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**Table I** Demographic data.

<table>
<thead>
<tr>
<th></th>
<th>Letrozole group (n = 23)</th>
<th>Control group (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.9 ± 6.6</td>
<td>27.3 ± 8.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 5.0</td>
<td>22.8 ± 5.0</td>
</tr>
<tr>
<td>No. of previous TOP</td>
<td>2 (1–2)</td>
<td>1 (1–2)</td>
</tr>
<tr>
<td>No. of previous deliveries</td>
<td>1 (0–3)</td>
<td>1 (0–4)</td>
</tr>
<tr>
<td>Gestational weeks by USG</td>
<td>15.6 ± 1.3</td>
<td>16.0 ± 1.4</td>
</tr>
<tr>
<td>Induction-to-abortion interval (h)</td>
<td>10 (6–83)</td>
<td>9 (4–42)</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard deviation or median (range). No significant differences were found between the two groups.

BMI, body mass index; TOP, termination of pregnancies; USG, ultrasonography.

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**Table II** Clinical data (hormonal concentration).

<table>
<thead>
<tr>
<th></th>
<th>Letrozole group (n = 23)</th>
<th>Control group (n = 21)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormonal concentrations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol (Day 0) (pmol/l)</td>
<td>18 867 (6674–70 931)</td>
<td>18 106 (7643–60 315)</td>
<td>0.925</td>
</tr>
<tr>
<td>Estradiol (Day 3) (pmol/l)</td>
<td>520 (173–17147)</td>
<td>20 282 (9260–54 791)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progesterone (Day 0) (nmol/l)</td>
<td>109 (63–290)</td>
<td>103 (53–868)</td>
<td>0.541</td>
</tr>
<tr>
<td>Progesterone (Day 3) (nmol/l)</td>
<td>112 (41–357)</td>
<td>105 (51–225)</td>
<td>0.074</td>
</tr>
</tbody>
</table>

Data presented as median (range) and compared by the Mann–Whitney U-test.
Our previous studies demonstrated that there was no change in the serum progesterone and cortisol concentrations after the use of letrozole in either first or second trimester abortions (Lee et al., 2011a,b,c). Yet, the level of serum estradiol level is significantly down-regulated in the letrozole group (Tables I and II). Estrogen is well known to stimulate ER and PR expression. In line with this, the present study revealed that the expression of both ERα and PR transcripts were suppressed by letrozole as shown by real-time RT−PCR. However, no significant change in PR protein expression due to letrozole was found.

The role of estradiol in the maintenance of pregnancy is less clear compared with that of progesterone. The depletion of estradiol by aromatase inhibitor in pregnant baboons induces abortion while with the use of an estrogen supplement together with aromatase inhibitor could maintain the pregnancies of baboons (Albrecht et al., 2000). We demonstrated that letrozole with misoprostol was more effective than misoprostol alone in first trimester medical abortion in humans (Lee et al., 2011a). The expression of GR in decidual stromal cells is suppressed by the use of mifepristone, which reflects its anti-glucocorticoid effect with down-regulation of GR expression (Chan et al., 2003). There was no observed effect of letrozole on adrenal steroidogenesis and, as shown by our previous study, there was no change in the cortisol concentrations after the administration of letrozole in early pregnancies (Lee et al., 2011b). There was also no alteration in the expression of GR in placentae after the use of letrozole in this study. Androgen receptor was not detected in all our samples with two different antibodies and therefore no quantification could be performed (unpublished data).

Figure 2  Effect of letrozole on the expression of steroid hormone receptors in human placenta. The mRNA expression levels of ERα, ERβ, PR and GR were determined by real-time PCR in human placenta with or without letrozole (Let) treatment followed by misoprostol (Miso). Data were presented as median with 10th, 25th, 75th, and 90th percentiles and outliers (*). Statistical analysis was performed by the Kruskal–Wallis test and a P-value of <0.05 was considered significant different between groups.
Two proliferative markers were used in this study. The Ki67 antigen is present during the active phase of the cell cycle and its location varies in different phases of the cell cycles (Scholzen and Gerdes, 2000). Our Ki67 staining results were comparable with those of previous studies which demonstrated its presence in the cytotrophoblasts and villous stromal cells with much weaker immunostaining in the decidual cells (Korgun et al., 2006; Meng et al., 2009). PCNA is another proliferation marker and it is expressed in the nuclei at G1 and S phases of the cell cycle (Takahashi and Caviness, 1993; Pan et al., 2011). The pattern of PCNA immunoreactivities in the placentae in the placebo group was similar to that reported in another study (Korgun et al., 2006). As syncytiotrophoblasts lack proliferative capacity, no immunoreactivity of Ki67 or PCNA were found in these cells. In sum, the expression of these two proliferation markers was not altered by the use of letrozole pretreatment in the second trimester pregnancies, indicating no effect on cell proliferation. With a similar percentage of TUNEL-positive cells between the letrozole and control groups, it was also concluded that letrozole pretreatment did not have any significant effects on apoptosis.

Taken together, letrozole suppressed the expression of ERα and PR transcript and the expression of ERα protein in the placentae, while other steroid receptors, proliferative and apoptotic markers were not altered. It is plausible that letrozole facilitates the abortion induced by misoprostol, directly or indirectly, through its effects on ERα and PR target genes in the downstream signaling pathway. Since in a previous paper, the clinical outcomes were significantly improved with letrozole in first trimester termination of pregnancies, but not in second trimester terminations, we hypothesized the effect of letrozole may act through decidua (Lee et al., 2011c). Further laboratory work should be carried out to confirm these findings.

**Figure 3** IHC staining of steroid hormone receptors and proliferative and apoptotic markers in human placenta. (A) The expression of ERα, ERβ, PR and GR in human placenta were determined by IHC staining. The signal (brown, arrow) was mainly localized in the nuclei of the cytotrophoblast, as well as the decidua (inset), of the placenta. (B) The expression of proliferative markers (PCNA and Ki67) were comparable between the misoprostol and Let + Miso groups, and mainly localized at the cytotrophoblast and the stroma of the placenta. There were also no significant changes in the number of TUNEL (pink) positive cells between the two groups. The negative controls were performed when the primary antibody was replaced with isotypic control for the immunohistochemistry. Scale bar = 50 µm. IHC, immunohistochemical; Let, letrozole; Miso, misoprostol.
out with the conceptus products from the first trimester, as the present work with placentae from second trimester broadens the potential targets of letrozole in terminating pregnancy.

Acknowledgements

We would like to thank the hospital pharmacist and research nurses who took part in this study.

Authors’ roles

P.-C.H. and W.S.-B.Y. designed the study. E.H.-Y.N. and V.C.-Y.L. were responsible for the recruitment and clinical management of patients. W.S.-B.Y. and K.-F.L. were responsible for the collection and preparation of samples. V.C.-Y.L., J.G. and K.-F.L. were responsible for the laboratory work. V.C.-Y.L. was responsible for data analysis, data interpretation and drafting of the manuscript. All authors approved the final version of the manuscript.

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Conflict of interest

None declared.

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Table III  Immunostaining scores of steroid receptors and proliferation markers, and the proportion of apoptotic cells.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Letrozole group (n = 23)</th>
<th>Control group (n = 21)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid receptors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERα</td>
<td>113 (103–119)</td>
<td>217 (214–290)</td>
<td>0.004</td>
</tr>
<tr>
<td>CT</td>
<td>100 (98–105)</td>
<td>210 (200–286)</td>
<td>0.004</td>
</tr>
<tr>
<td>VS</td>
<td>92 (50–98)</td>
<td>74 (43–75)</td>
<td>0.106</td>
</tr>
<tr>
<td>ERβ</td>
<td>35 (3–107)</td>
<td>32 (7–60)</td>
<td>0.776</td>
</tr>
<tr>
<td>PR</td>
<td>58 (9–104)</td>
<td>32 (0–73)</td>
<td>0.234</td>
</tr>
<tr>
<td>GR</td>
<td>110 (56–171)</td>
<td>108 (94–150)</td>
<td>0.717</td>
</tr>
<tr>
<td>Proliferation markers</td>
<td></td>
<td></td>
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<tr>
<td>PCNA</td>
<td>123 (110–150)</td>
<td>120 (108–166)</td>
<td>0.581</td>
</tr>
<tr>
<td>Ki67</td>
<td>29 (14–77)</td>
<td>23 (10–58)</td>
<td>0.326</td>
</tr>
<tr>
<td>Apoptosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TUNEL positive (%)</td>
<td>1.9 (0–15.6)</td>
<td>1.8 (0–6.5)</td>
<td>0.617</td>
</tr>
</tbody>
</table>

Data presented as median (range). Compared by the Mann–Whitney U-test.

ST, syncytiotrophoblast; CT, cytotrophoblast; VS, villous stromal cells; D, Decidual cells. 

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