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Original Article

Factors related to completeness of medical abortion with mifepristone and misoprostol

Wen-Xiao Jiang, Fang-Fang He, Qi Shen, Xue-Jiao Tao, Chu-Chu Zhao, Zhao-Jun Shen, Xue-Qiong Zhu*

Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Wenzhou Medical University, Zhejiang, China

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Abstract

Background: Medical abortion that occurs in early pregnancy is generally safe and successful, but incomplete medical abortion can result in complications. This study aimed to examine factors related to completeness of medical abortion with mifepristone and misoprostol, and then to provide a new direction for research into establishing complete abortion with mifepristone and misoprostol.

Methods: Sixty-three patients with early pregnancy requesting medical abortion with mifepristone and misoprostol were selected. Immunohistochemistry was used to detect the expression and location of progesterone receptor, estrogen receptor, insulin-like growth factor-1, and vascular endothelial growth factor in chorionic villi among these women. Reverse transcriptase polymerase chain reaction was then used to determine the expression of insulin-like growth factor-1 and vascular endothelial growth factor mRNA.

Results: According to the outcome of medical abortion, the women were divided into either the incomplete medical abortion group ($n = 34$) or the complete medical abortion group ($n = 29$). Immunohistochemical analysis showed that progesterone receptor and estrogen receptor protein expression was not detected in chorionic villi in the two groups. However, compared with the complete abortion group, there was a marked decrease in the expression of insulin-like growth factor-1 and a significant increase in the expression of vascular endothelial growth factor ($p < 0.05$) in the incomplete abortion group. There was no significant difference in mRNA expression between the incomplete and complete abortion groups.

Conclusion: The expression of insulin-like growth factor 1 protein and vascular endothelial growth factor protein in chorionic villi may be related to the outcome of medical abortion with mifepristone and misoprostol.

Keywords: estrogen receptor; insulin-like growth factor 1; medical abortion; progesterone receptor; vascular endothelial growth factor

1. Introduction

Recently, medical termination has been the first choice for early pregnancy termination, and > 50% of the 53 million women with unwanted pregnancy worldwide requesting termination preferred medical abortion.[1] Mifepristone was the first approved medication for medical abortion. Actually, mifepristone in combination with misoprostol is safe and effective, has been used for more than 2 decades, and is available in 35 countries.[2] Mifepristone and misoprostol, in combination, generally demonstrate a high complete abortion rate of 92–99%.[3] However, the commonest causes of failure of this method are incomplete abortion (5%), excessive bleeding (3%), and ongoing pregnancy (1%).[4,5] Incomplete abortion commonly causes hemorrhage, infection, and abdominal pain, and has the potential for long-term emotional consequences. Furthermore, the risk of vaginal bleeding during medical abortion with mifepristone and misoprostol is increased in the early period of pregnancy.[6] Thus, it is important to find parameters to predict the

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Xue-Qiong Zhu, Department of Obstetrics and Gynecology, The Second Affiliated Hospital of Wenzhou Medical University, 109, Xueyuan Xi Road, Wenzhou, Zhejiang 325027, China.

E-mail address: zjwzzxq@163.com (X.-Q. Zhu).

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success of medical treatment. Various clinical methods have been suggested for the evaluation of the outcome of medical abortion, such as endometrial thickness determined by ultrasound[7] and serum beta-human chorionic gonadotropin measurement.[8] However, none of these clinical factors are highly accurate or reliable.[8−11] At a molecular level, prolonged bleeding after medical abortion with mifepristone and misoprostol is associated with elevated expression levels of matrix metalloproteinase-9 (MMP-9) in villi and of tissue inhibitors of metalloproteinases-2 (TIMP-2) in the decidua.[12] However, no research has been carried out focusing on villi to study the success of medical abortion. Hence, we decided to explore factors in chorionic villi to relate the success of medical abortion with mifepristone and misoprostol.

Mifepristone functions as an antiprogesterone by competing with progesterone in the endometrium and decidua for receptor binding, thus terminating early pregnancy.[13] An array of novel progesterone receptor (PR)-regulated gene pathways and high expression of PR were found in the decidua of pregnant mice treated with mifepristone,[14] indicating that progesterone may play important roles in the regulation of endometrial decidualization, which can be blocked by mifepristone. However, only limited information is available about the effect of mifepristone in early gestational chorionic villi. One study reported that mifepristone impaired the production of human placental lactogen and progesterone in cultured syncytiotrophoblasts,[15] but there have been no published reports studying the correlation between PR in human chorionic villi and the outcome of medical abortion with mifepristone and misoprostol.

Progesterone, estrogen, and their receptors can be modulated by each other, and are responsible for physiological changes of the endometrium and also essential for the maintenance of pregnancy and development of embryo. As early as 1975, Hsueh et al[16] showed that progesterone can decrease the sensitivity of tissue to estrogen by acting on the cytoplasmic estrogen receptor (ER) to decrease the quantity of uterine ER. Decreased ER alpha, ER beta, and PR-B levels in the human endometrium may be related to prolonged uterine bleeding after medical abortion by mifepristone accompanied by misoprostol.[17] Therefore, some investigators proposed that downregulation of ER was also related to the outcome of medical abortion; such a proposition, however, completely focuses on the endometrium.

Many other factors such as insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF),[18,19] are thought to contribute to the development of human embryos and to be related to the outcome of medical abortion. Antiestrogen tamoxifen may inhibit the growth of leiomyoma by interrupting an IGF-1 autocrine loop in the leiomyoma cell lines.[20] Animal experiments have established that the VEGF mRNA and protein can be upregulated by ER and PR, contributing to vasculogenesis and embryo development.[18] Studies about IGF-1 and VEGF are mostly carried out on leiomyoma and endometriosis, and there are few reports evaluating the roles of IGF-1 and VEGF in chorionic villi in medical abortion with mifepristone and misoprostol. Hence, IGF-1 and VEGF, which were hypothesized to be related factors for prediction of completeness of medical abortion, were also investigated using immunohistochemistry and reverse transcriptase polymerase chain reaction (RT-PCR) in chorionic villi tissues from women undergoing abortion.

In the present study, the relationship between ER, PR, IGF-1, and VEGF expression in chorionic villi and the outcome of medical abortion with mifepristone and misoprostol were studied. In addition, whether PR, ER, IGF-1, and VEGF in chorionic villi were related to the outcome of medical abortion was also explored.

2. Methods

2.1. Patients

Between March 2009 and April 2011, 89 women with early pregnancy requesting medical abortion for termination of pregnancy at The Second Affiliated Hospital of Wenzhou Medical University were considered for recruitment in this prospective study. Of these 89 potential study participants, 68 provided consent for inclusion in this study (Fig. 1). All patients were initially assessed by their provided medical history, physical examination, urine pregnancy test, routine blood test, electrocardiogram, liver function test, and ultrasonography. The inclusion criteria were as follows: (1) age 18−30 years old; (2) healthy women requesting medical abortion; (3) gestational age 35−49 days (based on the onset of the last menstrual period, bimanual examination, and ultrasound) and a gravid 1 para 0 with a singleton intrauterine pregnancy, with the presence of fatal heart beat being confirmed by careful ultrasound examination; and (4) women being informed of the advantages and risks of medical abortion, and signing an agreement of consent. The exclusion criteria were as follows: (1) complication of pregnancy; (2) drug allergy to mifepristone or misoprostol; (3) current use of long-term systemic steroids; (4) a medical history of disease related to the cardiovascular system, respiratory system, liver, kidney, or adrenal gland; (5) uncontrolled hypertension, cardiovascular disease angina, or diabetes mellitus; (6) *in situ* intrauterine devices; (7) pelvic inflammatory disease; (8) hemoglobin <80 g/L; (9) breastfeeding; (10) addiction to alcohol or smoking; and (11) lost to follow-up. The study protocol was approved by the Research Ethical Committee of The Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, China (20090103) and met the standards of the Declaration of Helsinki. Written consent was obtained from patients before the collection of samples.

2.2. Administration and efficacy

The method and dose administration of medicine followed the recommendation of the study of Zhuang et al.[12] All applicants were treated with 50 mg mifepristone administered orally on their first visit, followed by 25 mg mifepristone administered orally every 12 hours (total 150 mg) at home. During a second visit 48 hours later, women received 600 mg oral misoprostol and remained in the hospital under observation for 4 hours. During this observation period, tissues discharged from the

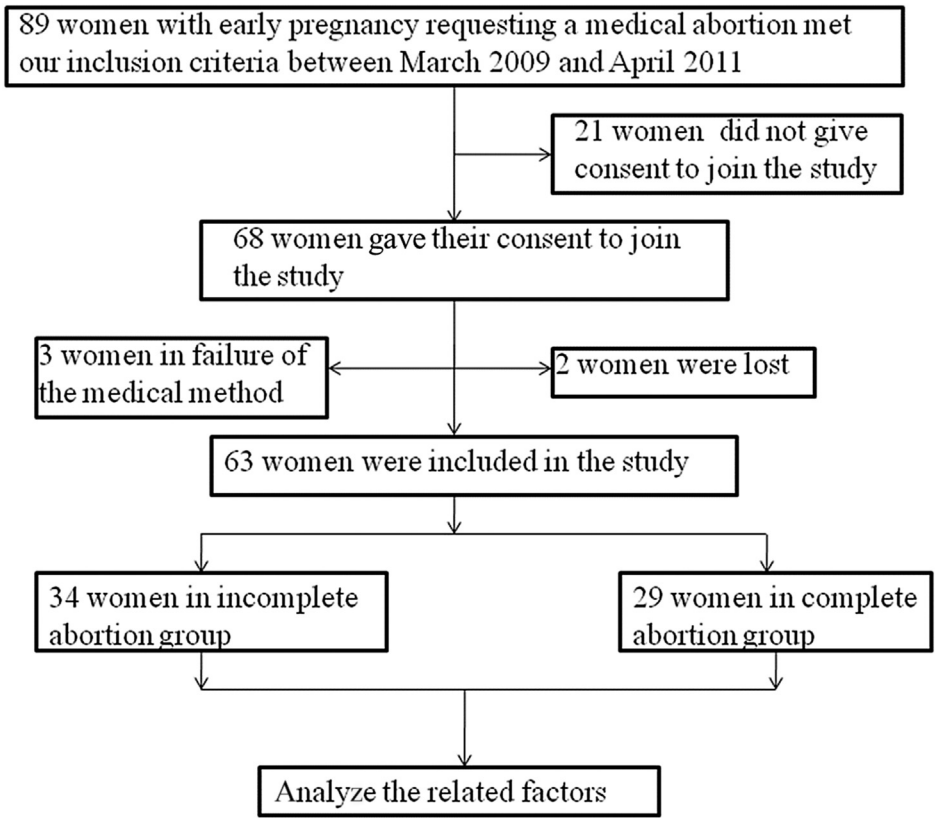

Fig. 1. Flow diagram representing the patient inclusion in the study.

vagina (including placental villi and decidua) were collected by doctors, then rinsed thoroughly in precooled phosphate-buffered saline solution to remove excess blood, and thereafter dissected. One-half of the tissues were fixed overnight in 4% buffered formalin, dehydrated, and embedded in paraffin for histological confirmation and immunohistochemistry. The other half was frozen in liquid nitrogen and then stored at −80°C until use. The observation was limited to 4 hours to ensure that all samples were collected within this timeframe; those patients who took longer to discharge tissue would therefore not be included in the successful abortion population in this study.

Follow-up visits were scheduled for 2 weeks after mifepristone administration to assess abortion status by means of clinical history and ultrasound. Complete abortion was considered as complete evacuation of the uterine contents without a uterine aspiration procedure at any point. Incomplete abortion was defined as contractions, pain, and vaginal bleeding during pregnancy; an open cervical os; and sometimes partial expulsion of products of conception.[21] Surgical evacuation was indicated for continuing pregnancy, excessive bleeding, or missed abortion, and these were considered as failures of the medical method.

2.3. Immunohistochemistry

Immunohistochemistry staining was carried out using a standard streptavidin peroxidase method,[22] on 4 mm-thick sections from 1 cm × 1 cm × 0.3 cm tissue samples that were formalin fixed and paraffin embedded. The specific primary antibody [antiestrogen monoclonal mouse antibody (Maixin, Fujian, China), antiprogesterone polyclonal rabbit antibodies (Zymed Corporation, San Francisco, CA, USA), and anti-IGF-1 polyclonal rabbit antibodies at a dilution of 1:50 and anti-VEGF monoclonal mouse antibody at a dilution of 1:200 (Santa Cruz Corporation, Dallas, TX, USA)] were used, and biotinylated goat antimouse/rabbit antibody was used as a secondary antibody, streptavidin peroxidase as a label, and diaminobenzidine as a chromogen. The sections were counterstained with Mayer hematoxylin to enhance nuclear detection. Appropriate positive and negative control slides were stained in parallel. The negative control was incubated with phosphate-buffered saline solution in the absence of the primary antibody. Human leiomyomas were taken as positive controls for PR, ER, IGF-1, and VEGF.

Under a microscope, the PR- or ER-positive cells showed yellowish brown staining in the nucleus, and IGF-1- or VEGF-positive cell showed yellowish brown staining in the cytoplasm. Counting was done by two observers (W.J. and F.H.), without any knowledge of the diagnosis and the results of the other observer's counts. All immunopositive cells were counted in at least 10 high-power fields (40× objective, 10× eyepiece) chosen at random. The numbers of positive cells in ER, PR, IGF-1, and VEGF were given as a percentage for each case. The percentages of positive cells examined were scored as 0 points (0−5%), 1 point (6−24%), 2 points (25−49%), 3 points (50−74%), and 4 points (75−100%). Staining intensity was graded as 0 points (negative), 1 point (weak), 2 points (moderate), and 3 points (strong). The extent and location of

immunohistochemical staining for PR, ER, IGF-1, and VEGF were assessed according to the immunoreactive score by multiplying the individual scores of extent by intensity.[23]

2.4. Real-time polymerase chain reaction

RT-PCR determined the expression of IGF-1 and VEGF mRNA. Total RNA was extracted using Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA), and 4 μg was subjected to RT-PCR using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Republic of Lithuania) and the following primers: IGF-1: forward 5-GCTGTGCCTGCTCACCTTC-3 and reverse 5-CCCTGTG GGCTTGTTGAAAT-3; VEGF: forward 5-TTGCCTTGCTGC TCTACCTC-3 and reverse 5-TGCATGGTGATGTTGGAC TC-3. The cycling parameters for IGF-1 (35 cycles) and VEGF (29 cycles) were as follows: denaturation (94°C, 30 seconds), annealing (62°C, 30 seconds), and extension (72°C, 30 seconds); and denaturation (94°C, 30 seconds), annealing (60°C, 30 seconds), and extension (72°C, 30 seconds), respectively. All experiments were performed in triplicate. The expression of β-actin in the same tissue was determined for the possibility of RNA degradation or RNA transcription default. The PCR products were electrophoresed and the sequence was analyzed.

2.5. Statistical analysis

Statistical analysis was performed using the SPSS version 16.0 package (Statistical Package for the Social Sciences Inc., Chicago, IL, USA). The data were expressed as mean ± standard deviation. Data were first subjected to the Kolmogorov–Smirnov test to confirm normal distribution and Levene's test for analysis of variance. Two-sample *t* test was used to compare age, gestational age, and IGF-1 and VEGF proteins in each tissue between the two groups, which displayed a normal (Gaussian) distribution and homogeneity of variance. The *t* test was used to evaluate any significant difference of IGF-1 and VEGF mRNA expression in each tissue between the two groups, which displayed a normal distribution and heterogeneity of variance. A p value < 0.05 was considered significant.

3. Results

3.1. Demographic characteristics

Of the 68 women first included in this study, three experienced failure of the medical abortion method and two were lost to follow-up. Therefore, this study ultimately included 63 women with early pregnancy who had requested medical abortion, including 34 with incomplete medical abortion and 29 with complete medical abortion (Fig. 1). Overall, there was no statistically significant difference in age, gestational age, serum beta-human chorionic gonadotropin level, and body mass index between the two groups (Table 1).

3.2. Immunohistochemical results

Decidual cells from early pregnancy showed the presence of ER with stained nuclei; however, chorionic villus cells did not express ER in either group. However, the negative control group showed negative expression, and positive control group leiomyoma and endometrium tissues strongly expressed ER in nuclei. The expression of PR was similar to that of ER with no expression of PR in villi in both the complete abortion group and the incomplete abortion group.

IGF-1 immunostaining was found in chorionic villi cells in the two groups, whose immunoreactivity was localized in the cytoplasm (Figs. 2A, 2B, S1A, and S1B). In the incomplete abortion group, IGF-1 expression was 4.27 ± 1.26, which significantly decreased from that in the complete abortion group (5.67 ± 2.28, $p < 0.05$; Fig. 2E).

Positive VEGF staining was detected in the cytoplasm of chorionic villi cells in the two groups. In the incomplete abortion group, VEGF immunostaining was strongly positive in chorionic villi cells (Figs. 2D and S1D), while in the complete abortion group, VEGF staining was weak (Figs. 2C and S1C). The difference of VEGF expression between the two groups was found to be statistically significant ($p < 0.05$; Fig. 2E).

3.3. RT-PCR results

IGF-1 mRNA expression and VEGF mRNA expression were observed among the two groups by RT-PCR. Lower IGF-1 mRNA expression, but higher VEGF mRNA expression, occurred in the incomplete abortion group compared with the complete abortion group (Table 2 and Fig. 2F); however, there was no statistically significant difference between the two groups either for IGF-1 mRNA expression or for VEGF expression.

4. Discussion

This study investigated whether the levels of PR, ER, VEGF, and IGF-1 in chorionic villi are related to completeness

Table 1
Demographic characteristics of women undergoing medical abortion in the two groups.

Group	*N*	Ages (y)	Serum beta-hCG level (IU/L)	Gestational age (d)	Body mass index
Complete abortion group	29	22.71 ± 2.24	5046 ± 1967	37.71 ± 4.10	21.8 ± 1.5
Incomplete abortion group	34	23.53 ± 3.07	5587 ± 2239	39.24 ± 4.82	22.1 ± 1.3

Values are shown as mean ± SD.
hCG = human chorionic gonadotropin; SD = standard deviation.

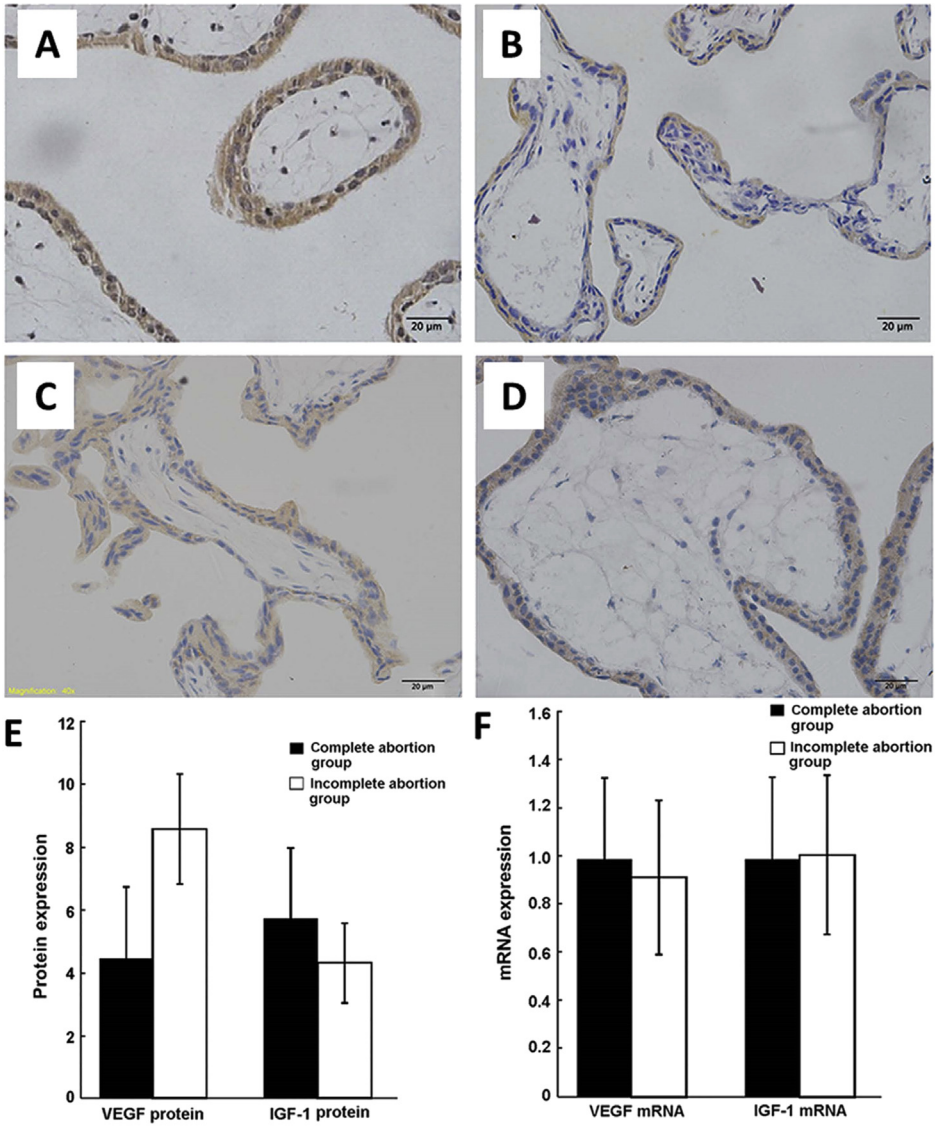

Fig. 2. IGF-1 and VEGF expression in chorionic villi in the complete and incomplete abortion groups after medical abortion with mifepristone and misoprostol. (A) The expression pattern of IGF-1 in the complete abortion group showed a high degree of brown staining in the cytoplasm of chorionic villi, suggesting high levels of IGF-1 (magnification 400×). (B) The expression pattern of IGF-1 in the incomplete abortion group showed some brown staining in the cytoplasm of chorionic villi, suggesting lower levels of IGF-1 in this group (magnification 400×). (C) The expression of pattern VEGF in the complete abortion group showed some brown staining in the cytoplasm of chorionic villi, suggesting low levels of VEGF (magnification 400×). (D) The expression pattern of VEGF in the incomplete abortion group showed a high degree of brown staining in the cytoplasm of chorionic villi, suggesting higher levels of VEGF in this group (magnification 400×). (E) Quantification of the protein expression of VEGF and IGF-1 in both groups. (F) Quantification of the mRNA expression of VEGF and IGF-1 showed no significant difference between the two groups. IGF-1 = insulin-like growth factor; VEGF = vascular endothelial growth factor.

Table 2
Expression of IGF-1 and VEGF mRNA in the complete and incomplete abortion groups.

	N	IGF-1	VEGF
Complete abortion	29	0.99 ± 0.34	0.99 ± 0.46
Incomplete abortion	34	1.01 ± 0.33	0.91 ± 0.27
p		0.652	0.363

Values are shown as mean ± SD.
IGF-1 = insulin-like growth factor; SD = standard deviation; VEGF = vascular endothelial growth factor.

of medical abortion. The results showed no expression of ER and PR, but IGF-1 decreased and VEGF factor significantly increased in the incomplete abortion group in comparison with the complete abortion group. However, some bias to results may be present as a low success rate of medical abortion (29/63) was found compared with previous studies. This may be because we selected those patients who had evacuated the gestational sac from the vagina within 4 hours after misoprostol administration in order to use fresh tissue samples; therefore, a proportion of the patients who needed longer than 4 hours for the discharge would not be evaluated in our study. We need to estimate the overall abortion rate after 2 weeks,

which we imagine would provide a higher value. Several other factors may also affect the successful abortion rate: gestational age, parity, previous termination of pregnancy, initial serum beta-human chorionic gonadotropin levels, different routes of administration of misoprostol, and the time interval between mifepristone and misoprostol administration.

Liu et al[24] found that mifepristone regulated many genes and pathways in chorionic villi in early pregnancy, using cDNA microarray. Meanwhile, Abd-Elnaeim et al[25] revealed that PR and ER were detected in trophoblast cells, chorionic villous stroma, microcaruncular epithelium, and microcaruncular stroma in equine placenta. Meng et al[26] found that ER and PR were present in the nuclei of human first-trimester chorionic villi exposed to postovulatory administration of 1.5 mg of levonorgestrel. Although inconsistent with the previous research, our results indicated that PR and ER were not in chorionic villi in either abortion group; however, without a comparison group of women who underwent surgical abortion without prior administration of mifepristone or misoprostol, we cannot categorically say that PR and ER were not present. This is different from what was expected. Gligorijević et al[27] reported negative immunohistochemical results in the detection of ER and PR in the acinar cells of the acrimal gland, postulating that the results are the consequence of the affinity of antibodies used in the immunohistochemical procedure. However, in the present study, the antibodies showed positive staining of ER and PR in decidual tissues and in the positive control uterine leiomyoma tissue. The postulated mechanism may be that, through binding to PR, mifepristone may change the construction of PR or inhibit the transformation of PR, resulting in its negative expression.

IGF-1 has been implicated as a potential regulatory factor for reproduction and participates in many aspects of modulating early term pregnancy. Our data revealed that IGF-1 was expressed in the cytoplasm of chorionic villi cells in the two groups, but had significantly higher expression in the incomplete abortion group. There are many cross-talk pathways between estrogen and IGF at the molecular level in breast cancer, and IGF-1 can upregulate ER.[28,29] ER in chorionic villi appears to be under the regulation of IGF-1, which can upregulate the production and activity of ER in chorionic villi. It is likely that the significantly low expression of IGF-1 in incomplete medical abortion with mifepristone makes less contribution to ER upregulation, which has the potential to profoundly influence uterine contraction and shedding of endometrial layers and chorionic villi, leading to incomplete abortion.

As a key regulator of angiogenesis, VEGF is essential in embryo development for regulating the extensive microvascular net and early hematopoiesis in pregnancy. It was proposed that cells with high expression of VEGF are comparatively insensitive to mifepristone.[30] Low doses of mifepristone (2 mg or 5 mg daily) reduced stromal VEGF protein expression, contributing to the development of amenorrhea,[31] suggesting that mifepristone-induced amenorrhea is associated with a decrease in stromal VEGF. In accordance with these studies, our results also indicated that the VEGF increases sharply in the incomplete abortion group compared with that in the complete abortion group, and this occurs almost exclusively in the cytoplasm of chorionic villi cells. Except for its antiprogesterone action, mifepristone also acts on endometrial blood vessels, causing vascular damage that further compromises the embryo.[32] Therefore, we postulate that upregulation of VEGF expression by mifepristone is associated with abnormal vascular formation, contributing to uterine contraction and shedding of decidua and chorionic villi. Thus, the increased expression of VEGF in chorionic villi suggests incomplete abortion with mifepristone.

The significant difference between IGF-1 and VEGF protein expression was not seen at the mRNA expression level. We presume that IGF-1 and VEGF activities can be regulated post-transcriptionally, but this mechanism needs further study.

As a preliminary study, we made every effort to avoid confounding factors that might have an impact on the results. This included restricting the inclusion criteria to patients younger than 30 years of age, and inclusion of only nonsmokers and primigravidae. However, owing to this restriction of the study population, the results have less generalizability to the overall population. Some of the patients did not agree to provide blood samples, so we could not evaluate the influence of hormones such as human chorionic gonadotropin, estrogen, and progesterone on these results. Previous research has shown an association of the success of medical abortion with factors such as serum beta-human chorionic gonadotropin, endometrial thickness, and body mass index,[7,8,33,34] but we did not compare these factors between the two groups. These measurements are needed to be included in future studies. In addition, the mean gestational age in days was higher in the incomplete abortion group than in the complete abortion group, which, although not statistically significant, might have an influence on the analysis of VEGF and IGF-1, and which might adjust with the gestational age; however, we consider this unlikely, and so we did not adjust for it. Nonetheless, this will need further investigation in the future.

In conclusion, our findings demonstrate that the expression of IGF-1 and VEGF protein in chorionic villi is associated with the outcome of medical abortion with mifepristone and misoprostol. Whereas PR and ER expression in chorionic villi is not related to the outcome of medical abortion, this suggests a new direction for further study on the mechanism of incomplete abortion with mifepristone and misoprostol, which may provide methods to achieve complete medical abortion.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcma.2016.03.011

S1A:IGF-1 immunostaining was localized in the cytoplasm in the complete abrtion group.

S1B:IGF-1 immunostaining was localized in the cytoplasm in the incomplete abrtion group.

S1C:VEGF immunostaining was localized in the cytoplasm in the complete abrtion group.

S1D:VEGF immunostaining was localized in the cytoplasm in the incomplete abrtion group.

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