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# The study of the relationship between aberrant expression of hot shock protein 70 (HSP70) and spontaneous abortion

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**Abstract. – OBJECTIVE: The present study is aimed to explore the relationship between aberrant expression of heat shock protein 70 (HSP) and spontaneous abortion.**

**PATIENTS AND METHODS: 50 patients with spontaneous abortion and 50 patients with induced abortion were continuously selected based on the nearest matching principle, and the proportion of age and gestational age was 1:1. The decidual tissues were obtained, and the cell apoptosis was determined by TUNEL assay. Further, the expression of HSP70 was assayed by immune-histochemical staining, and the expression of HSP70 mRNA was detected by the RT-PCR approach.**

**RESULTS: Apoptosis rate, HSP70 expression and HSP70 mRNA expression in the observation group were significantly higher than the control group.**

**CONCLUSIONS: HSP70 might induce apoptosis so as to cause spontaneous abortion.**

Key Words:
Heat shock protein 70 (HSP70), Spontaneous abortion, Apoptosis.

## Introduction

Spontaneous abortion is a common and frequently occurring disease in women of childbearing age. Recent studies have noticed that apoptosis plays an important role in mammalian reproduction[1]. The maintenance of normal pregnancy depends on the balance between proliferation, apoptosis of placenta and decidual tissues. So, abnormal apoptosis caused by a variety of reasons might lead to failure in pregnancy or pathological pregnancy[2]. Heat shock proteins (HSP) are a set of conservative protein molecules that widely exist in prokaryotes and eukaryotes. They play important roles in the improvement of cell's resistance against adverse conditions. Further, maintenance of cell survival, function, collaborative immunity, and regulation function of cell apoptosis are also significantly influenced by HSPs[3]. The present study is aimed to analyze apoptosis during a natural abortion, and associated expression of HSP70. So, the above study stimulates future searches for intervention targets.

## Patients and Methods

### Patients

50 patients with spontaneous abortion were selected as observation group, and 50 patients with induced abortion as a control group. The patients with the following etiology were excluded: genetic defection; the anatomic abnormality in reproductive system; endocrine disorder; immune, infection, thrombosis, and environmental risk factors. The male reproductive system and sperm function of patients were normal, and there was no blood rejection between mother and child. In observation group, the average age was (26.3 ± 5.5) years old (range 25 to 36 years old), and the average age gestation was (9.0 ± 3.5) weeks (range 6 to 15 weeks). In control group, the ave-



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rage age was (24.8±4.6) years old (range 22 to 34 years old), and the average age of gestation was (8.6±3.7) weeks (range 5.5 to 14 weeks). The hospital Ethics Committee, patients, and their family members had agreed with the study.

### Sampling

The negative pressure aspirator performed uterus suction. Decidual tissue was extracted and was fixed in 10% of marine fluid. Routine paraffin embedding then followed it. Sections were obtained by slicing machine (Leica, Wetzlar, Germany), attached with a glass processed by the lysine. Sections were reserved in the oven with the temperature of 70°C for 3 h.

### TUNEL Method to Detect Apoptosis

Specimens were dewaxed to water, treated by freshly prepared 3% $H_2O_2$ for 10 min at room temperature, and washed in distilled water for 2 min x 3 times. Tunnel assay was then performed according to the instructions provided from the BioVision Inc. (Milpitas, CA, USA). Apoptosis cells with tan particles in components were regarded as positive, and 3 vision of each section was selected randomly (400 x, Olympus, Tokyo, Japan). Apoptosis rate (AR) was counted by TUNEL method, and the average was recorded.

### Immuno-histochemical Staining to Detect the Expression of HSP70

Specimens were dewaxed to water, treated by freshly prepared 3% $H_2O_2$ for 15 min, and put in distilled water for 3 min. Then, they were washed in 0.01 M phosphate buffered saline (PBS) for 5 min for 3 times. Further, specimens were immersed in 0.01 M citrate buffer salt and heated in a pressure cooker. Washings in PBS for 5 min were then given for 3 times after cooling. The excess liquid was removed, and a washing with 5% bovine serum albumin (BSA) sealing solution for 20 min was given at the room temperature. 50 µl monoclonal antibody (anti-rat HSP70, 1:200, Sigma-Aldrich, St. Louis, MO, USA) and PBS liquid were added as a negative control, and placed in a wet box (immunohistochemical kit from the Sigma-Aldrich, St. Louis, MO, USA) at 37°C for 2 h. It was washed again in PBS, and a second antibody (rabbit monoclonal antibody anti-rat 1:50, Sigma-Aldrich, St. Louis, MO, USA) was added. The specimens were then treated with SABC reagent, diaminobenzidine (DAB) color agent, wood grain, for development and were observed under a microscope. Analyses of the results were performed by the BI-2000 medical image analysis system (Olympus, Tokyo, Japan).

### RT-PCR Method to Detect HSP70 mRNA Expression

The total RNA was extracted rapidly according to the specification of EASYspin tissue/cell RNA rapid extraction kit (Beijing Yuanpinghao Biotechnology Co., Ltd., China). RT reaction was performed according to the specification of Revert Aid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Canada). Reverse transcription of cDNA was used as a template. 50 ml reaction system was applied to perform PCR amplification. The conditions of PCR reaction were pre-degeneration in 94°C for 5 min, degeneration in 94°C for 30 s, annealing in 58°C for 30 s and extension at 72°C for 1 min. A total of 35 cycles were performed and an extension at 72°C for 10 min. PCR products were analyzed by 2% agarose gel electrophoresis and GGDS-8000 gel imaging identification system. The experiment results were in gray scale and were scanned by Glyko BandScan5.0.

### Statistics Analysis

Data were analyzed by using SPSS20.0 statistical software (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed by (x±s), and the differences between groups were compared by *t*-test. $p < 0.05$ was statistically significant.

## Results

### Apoptosis Results

The apoptosis rate of observation group was significantly higher than those of control group ($p<0.05$) (Figure 1).

### Expression of HSP70

HSP70 positive cells mainly expressed in the decidual cell cytoplasm, and the detection of gray value was inversely proportional to the rate of positive. The gray value of observation group was significantly less than those of control group. The difference was statistically significant ($p<0.05$) (Figure 2).

### Expression of HSP70 mRNA

HSP70 mRNA expression of the observation group was significantly higher than that of the control group.

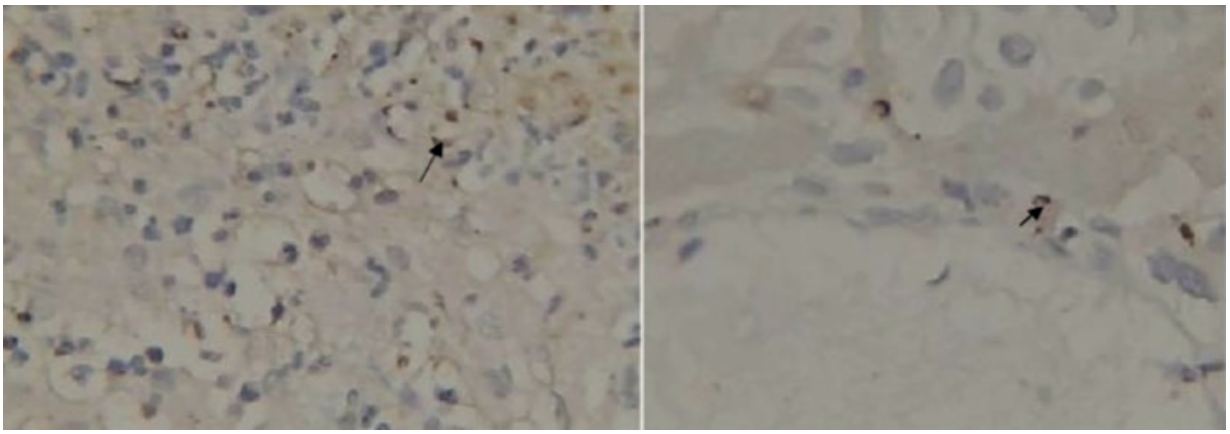

**Figure 1.** Tunel method to detect apoptosis situation (400 x, the arrow is apoptotic cells, the left is the observation group, the right is the control group).

## Discussion

A total of 21 kinds of proteins forms a HSP70 family. Further, four types are mainly focused in research studies viz. HSP70, heat stress homologous protein 70 (structure HSP70), glucose regulation protein 78 (found in the lumen of endoplasmic reticulum), and glucose regulatory proteins 75 (mainly located in the mitochondria). HSP70 family genes have multiple structures as well as multi-functions[4]. Further, Chen et al[5] confirmed that induced HSP70 has the ability to promote or inhibit apoptosis in variable conditions. Further, its mechanism to mediate apoptosis could be performed by the mitochondrial pathway or the JNK signaling pathway[6].

HSP is expressed in many human reproductive organizations, such as endometrium, decidua, and fallopian tubes[7]. HSP70 mainly distribute in the endometrial glandular cell, and in secretory period is significantly higher than in hyperplasia period. It is mainly located in the cytoplasm, and a small amount is located in the nucleus[8]. HSP expression is also noticed in human placenta. Immunohistochemical staining showed the HSP expression was weak in mesenchymal cells. The earlier studies also observed HSP70 in trophoblastic cells, endothelial cells, nucleus and cytoplasm during the processing of entire pregnancy[9].

HSP70 expression was observed in late pregnancy placenta, and no change in the whole process of labor was noticed[10]. On the other

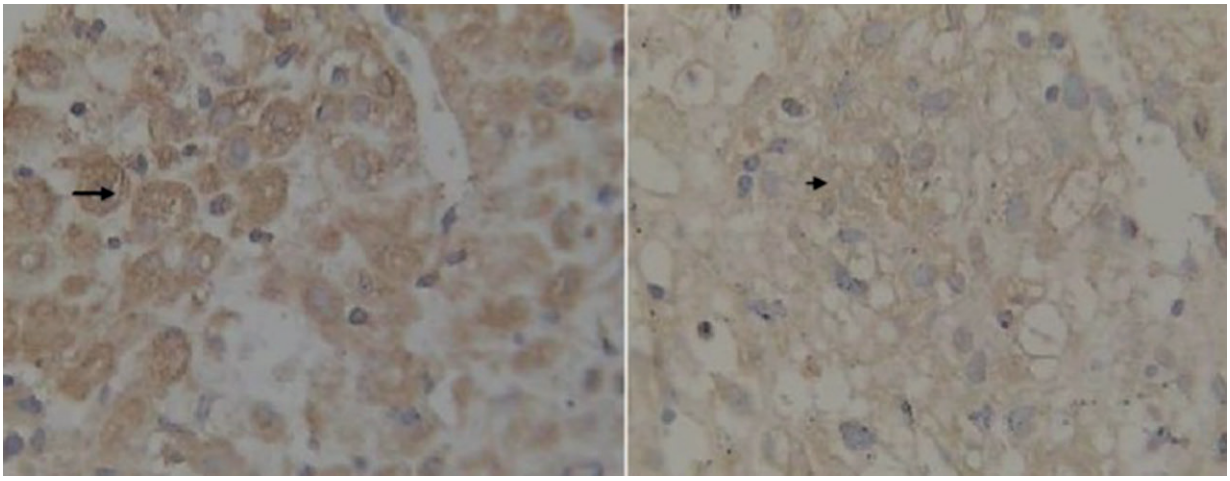

**Figure 2.** Immunohistochemical staining to detect the expression of HSP70 (400 x, the arrow ispositive cells, the left is the observation group, the right is the control group).

hand, another study noticed differences in HSP70 expression in the placenta during early abortion or normal labor[11]. In the present study, we have observed a significant elevation in HSP70 expression in the observation group. Further, excessive expression of HSP70 has been noticed to cause the ischemia of decidual tissue, restriction of embryo growth, stimulation of immune cells, and pregnancy failure[12]. Moreover, HSP in decidual tissue has been observed to cause the hormone disorder and enhancement of uterine sensitivity leading to abortion[13].

Ishihara et al[14] studied the apoptosis cells in the placenta in each period of normal pregnancy by using transmission electron microscopy and TUNEL method. The results showed that apoptotic nuclei distribute in trophoblast cells and form-fitting trophoblast cells. In 4-5 weeks of pregnancy, the nourish cells and form-fitting trophoblast cells are ascendant in the placenta. Further, the apoptosis cells significantly got reduced after seven weeks. Thus, significantly increasing the placental nourish trophoblast cells in late pregnancy reflects the aging or biological changes related to the delivery.

Kokawa et al[15] observed the apoptosis of villus and decidual tissue in patients with spontaneous abortion in comparison to patients with normal pregnancies So, the above observation is in sync with our results suggesting decidua tissue apoptosis might be one pathogenesis of spontaneous abortion. Shiraishi et al[16] used TUNEL method to detect placenta in the rat with spontaneous abortion after peritoneal injection of recombinant IL-22. He also found that extent of apoptosis of villus nourish cells was more in comparison to normal pregnancy rats. Further, Olivares et al[17] cultivated decidual lymphocytes from normal early pregnancy and spontaneous abortion respectively with fluff nourish cells JEG23 department. They found that apoptosis rate of spontaneous abortion was higher than that of normal pregnancy. The activating decidual lymphocytes were considered to be responsible for induction of apoptosis of trophoblast cells so as to cause spontaneous abortion.

## Conclusions

So, from above citations and our results, it could be inferred that HSP70 might be responsible for induction of apoptosis leading to spontaneous abortion.


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## Conflict of interest

The authors declare no conflicts of interest.

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