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# Study on prevention effect of Zishen Yutai pill combined with progesterone for threatened abortion in rats

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## ARTICLE INFO

## ABSTRACT

**Objective:** To observe preventive and therapeutic effects and the mechanism of actions on Zishen Yutai pill combined with progesterone on threatened abortion in rats.
**Methods:** After pregnancy, 50 SPF female SD rats were selected and divided into control group, model group, progesterone group, Zishen Yutai pill group, and progesterone plus Zishen Yutai pill group (combination group), with 10 rats in each group. The rats of control group and abortion model group were lavaged with 2 mL/kg normal saline on pregnancy day 1 for continuous 10 days. Rats in Zishen Yutai pill group were given 1.575 g/kg/d of Zishen Yutai pill for intragastric administration for continuous 10 days. Rats in progesterone group were given intramuscular injection treatment of 0.1 mL/d progestin, continuous for 10 days. Rats in combination group were given injection therapy of aqueous solution of Zishen Yutai pill for continuous 10 days, and other treatments were the same as previous two groups. Abortion model were established then and live births, numbers of abortion and average rate of abortion were compared between the five groups. Peripheral blood was collected to detect the estradiol (E2) and progestational hormone (P), and obtain ratio of Th1/Th2 cytokines (IL-2, INF-$\gamma$, IL-4, IL-10).
**Results:** Significant more live births of rats were found in the control group compared with other four groups ($P < 0.05$) ($P < 0.05$). The numbers of live births of the rats in abortion model group were significantly less than that of progesterone group, Zishen Yutai pill group and joint group ($P < 0.05$). The numbers of live births of rats in joint group were significant more than that of progesterone group and Zishen Yutai pill group. The serum E2 level of P of rats in the control group, progesterone group, Zishen Yutai pill group and joint group were significant higher than that of abortion model group ($P < 0.05$). Serum levels of E2 and P of rats in the control group, progesterone group, Zishen Yutai pill group and joint group were not significant different ($P > 0.05$) but these levels in the control group and Zishen Yutai pill group were significant lower than that of progesterone group and joint group ($P < 0.05$). The maternal–fetal interface $IL-4/IL-2$, $IL-10/IL-2$ of model group were balanced deviating to Th1, while the IL-4/IL-2, IL-10/IL-2 of progesterone group, Zishen Yutai pill group and joint group were balanced deviating to Th2, and joint group' deviation was better than progesterone group and Zishen Yutai pill group ($P < 0.05$).
**Conclusions:** Zishen Yutai pill combined with progesterone has a significant control effect for threatened abortion, which can obviously increase contents of maternal serums E2 and P, and regulate the Th1/Th2 balance with a remarkable effect.

## 1. Introduction

Threatened abortion is a common complication during pregnancy, which refers to a small amount of vaginal bleeding during the 28 weeks of pregnancy, paroxysmal abdominal pain symptoms of abortion, but cervix is not open and fetal membrane is intact without the passage of tissues [1–3]. The pathogenesis of threatened abortion is complex, which related to heredity, immune, general infection and endocrine disorder. It will develop into inevitable

abortion or lead to slow development of embryonic, which may seriously affect pregnancy outcome or worse influences to the patients and their families if it is not treated in time [4]. Therefore, it is extremely significant to seek for a more effective prevention and control method to improve the threatened abortion pregnant outcome. Some scholars believe that threatened abortion is related closely to the unbalance of Th1/Th2 cytokines [5–8], for instance, to correct the imbalance of maternal Th1/Th2 cytokine can prevent threatened abortion. Other studies confirm that Th1/Th2 cytokines balance plays a vital role in the process of normal pregnancy, abortion, embryo damage, premature birth and pathologic pregnancy [9,10]. In order to observe the preventive and therapeutic effects and the mechanism of actions of Zishen Yutai pill combined with progesterone, SPF female SD rats were selected to establish threatened abortion model, and Zishen Yutai pill combined with progesterone was used for intervention treatment. The effect of the treatment on rate of abortion, E2, P and Th1/Th2 cytokines ratio was observed.

## 2. Materials and methods

### 2.1. Source of animal

Fifty healthy female SD rats (SPF, 12 weeks old, weighing 250–350 g) were selected and provided by Laboratory Animal Centre of Zhejiang Chinese Medical University (certification: SCXK201500 13). Rats were fed with water and food *ad libitum* under 12 h/12 h light–dark cycle and packing was changed twice each week. The experiment was operated in experimental center of Zhejiang Chinese Medical University. All experimental procedures involving animals were strictly conducted in accordance to Regulations for the Administration of Affairs Concerning Experimental Animals and approved by ethics committee of the university.

### 2.2. Main reagents

Mifepristone was produced by Zhejiang Xianju Pharmaceutical Co.,Ltd., (specification:25 mg/tablet); Progesterone Injection was produced by Zhejiang Xianju Pharmaceutical Co., Ltd. (specification: 20 mg/mL); Zishen Yutai pill was produced by Guangzhou Baiyunshan Zhongyi Pharmaceutical Co. Ltd. (Approved: Z44020008, specification: 60 g/pill). Drugs dispensing: 25 mg mifepristone fine grinding was fully dissolved in 138 ml of distilled water, then the formulating concentration of 0.18 mg/mL was obtained for further use. Then progesterone injection was put into 2.75 mL of sterilization salad oil and formulating the concentration of 5.3 mg/mL for further use; the Zishen Yutai pill was changed into powder, then put into distilled water till it was fully dissolved and formulating concentration of 0.5 g/mL for further use; ELISA reagent boxes were all purchased from MultiSciences Company, and the operation was strictly conform to product specifications.

### 2.3. Experimental method

### 2.3.1. Pregnancy method

Vaginal washing fluid was used for microscopic examination and observation to ensure the selected rats' estrous cycle, and the estrus female and male rats were put in one cage according to the 2:1 ratio over night, and tested whether there was pessary *in vivo* of female rats on the next morning. If the pessary was discovered, the day could be identified as the first day of pregnancy after the test. If the pessary *in vivo* of female rats was not discovered, vaginal washing fluid could be taken for microscopic examination. The day can be identified as the first day of pregnancy if living sperms were discovered in the vaginal washing fluid; the rest rats without pregnancy were waiting for next estrous cycle.

### 2.3.2. Establishment of abortion model and intervention methods

50 pregnant rats were randomly divided into 5 groups, which were control group, abortion model group, progesterone group, Zishen Yutai pill group and joint group respectively, with 10 rats each group. Abortion model was not established in the control group, while the rest pregnant rats in 4 groups were lavaged with 3.75 mg/kg of mifepristone on the 10th days of pregnancy and established abortion model. The rats in control group and abortion model group were lavaged with 2 mL/kg of body mass normal saline during pregnancy day for continuous 10 days. Progesterone group rats were given intramuscular injection treatment of 0.1 mL/d of progesterone for continuous 10 days. The joint group rats were given injection therapy of aqueous solution of Zishen Yutai pill for continuous 10 days, the therapy method was the same to the previous two groups.

### 2.4. Observation index

After pregnancy of 1, 5 and 9 days (T1, T2 and T3), body weight, fur color and diet condition of the rats were observed. Rats were executed by ether anesthesia after establishing the model, and the blood serum in peripheral was separated for inspection. The estradiol (E2);level and progesterone (P) level were tested by adopting ELISA method. The content ratio of Th1/Th2 cytokines (IL-2, INF-γ, IL-4, IL-10) was tested, calculated and compared. Then rats in five groups were dissected, the numbers of live births and abortion were compared, and the average rate of abortion was calculated. Criterion: The embryo was intact, uterus was normal, the normal color was pink or reddish with no extravasated blood; partly abortion: the embryo was incomplete with extravasated blood in uterine cavity. Completely abortion: it was clearly visible of embryonic death, extravasated blood or embryo implantation site in uterus, with change of bamboo-structured in uterus, obvious decrease of body weight or vaginal bleeding before.

The formula of abortion rate: the abortion embryo numbers/(the abortion embryo numbers + normal embryo numbers) = rate of abortion.

### 2.5. Statistical analysis

The measurement data were expressed as mean ± SD, and analyzed by One-way ANOVA and *t*-test. SPSS19.0 statistical analysis software was used for data processing. $P < 0.05$ was considered statistically difference.

## 3. Results

### 3.1. Comparison of general status among rats in five groups

Pregnant rats in five groups all had smooth color, flexible reaction, and good appetite. During the period of drug delivery, Zishen Yutai pill was given with occasional loose stool which was not treated. No other adverse reactions were found. In control group, dissection of uterus was bright pink and nodular enlargement with uniformity of embryo, well development, clear boundaries between embryos and intact issue. The nodules in embryo of pregnant rats in the rest 4 groups were a little smaller than that of the normal group, but no significant difference in the number of embryos were found. There were clear blood traces in the embryo, and embryo deficiency with only embryo implantation site of pregnant rats in abortion model group. The degree of uterine bleeding of the progesterone group, Zishen Yutai pill group and joint group were obviously lighter than that of the abortion model group.

### 3.2. Body mass changes in different time points of the rats in 5 groups

After pregnancy, the body mass of rats in five groups was significantly increased in T2 and T3 compared with that in T1 ($P < 0.05$), and the comparisons of body mass of rats in five groups at different time points had no statistical difference. The results are shown in Table 1.

### 3.3. The comparison of live births numbers, abortion numbers and average abortion rate of rats in five groups

The number of live births of rats in the control group were significantly higher than that of the other four groups ($P < 0.05$), but the numbers of abortion and average rate of abortion were significantly lower than that of the other four groups ($P < 0.05$); The number of live births of the rats in abortion model group were significantly lower than that of progesterone group, Zishen Yutai pill group and joint group, while the number of abortion and average rate of abortion were significantly higher than that of the three groups ($P < 0.05$). The numbers of live births of rats in joint group were distinctly higher than that of progesterone group and Zishen Yutai pill group, but the abortion rate and average rate of abortion were lower than that of the previous two groups (Table 2).

**Table 1**
Body mass changes in different time points of the rats in 5 groups ($n = 10$).

| Groups | T1 | T2 | T3 |
|---|---|---|---|
| Control group | 275.79 ± 15.71 | 284.31 ± 15.22 | 293.31 ± 21.42 |
| Abortion model group | 274.39 ± 19.91 | 287.19 ± 19.63 | 293.09 ± 22.51 |
| Progesterone group | 275.14 ± 21.90 | 284.14 ± 20.84 | 293.77 ± 14.48 |
| ZishenYutai pill group | 274.77 ± 14.05 | 285.39 ± 14.92 | 294.27 ± 9.32 |
| Joint group | 274.25 ± 18.91 | 284.53 ± 22.42 | 297.07 ± 24.48 |

**Table 2**
The comparison of live births numbers, abortion numbers and average abortion rate of rats in five groups ($n = 10$).

| Groups | Live births | Abortion | Average abortion rate (%) |
|---|---|---|---|
| Control group | 12.56 ± 2.29 | 0.55 ± 0.54 | 4.18 ± 4.04 |
| Abortion model group | 2.67 ± 1.90[a] | 9.66 ± 1.67[a] | 79.09 ± 13.61[a] |
| Progesterone group | 7.65 ± 1.86[a,b] | 5.12 ± 3.92[a,b] | 36.08 ± 25.33[a,b] |
| ZishenYutai pill group | 6.79 ± 1.90[a,b] | 5.39 ± 3.24[a,b] | 40.38 ± 19.71[a,b] |
| Joint group | 10.39 ± 1.54[a,b,c] | 2.59 ± 1.85[a,b,c] | 18.25 ± 11.44[a,b,c] |

[a] Compared to the control group, $P < 0.05$. [b] Compared to the abortion model group, $P < 0.05$. [c] Compared to progesterone group and ZishenYutai pill group, $P < 0.05$.

### 3.4. The comparison of serum levels of E2 and P of the rats in five groups

The serum levels of E2 and P of the control group, progesterone group, ZishenYutai pill group and joint group were obviously higher than that of the abortion model group ($P < 0.05$).

The serum levels of E2 and P in the control group, progesterone group, ZishenYutai pill group and joint group had no statistic differences ($P > 0.05$). Serum level of P in control group and ZishenYutai pill group were significantly lower than progesterone group and joint group ($P < 0.05$) (Table 3).

### 3.5. The comparisons of Th1/Th2 cytokines levels of rats in five groups

The results showed that the maternal–fetal interface IL – 4/IL – 2, IL – 10/IL – 2 of model group were balanced deviating to Th1, while the IL-4/IL-2, IL-10/IL-2 of progesterone group, ZishenYutai pill group and joint group were balanced deviating

**Table 3**
The comparison of serum levels of E2 and P in rats of the five groups ($n = 10$).

| Groups | E2 (pg/mL) | P (ng/mL) |
|---|---|---|
| Control group | 262.30 ± 9.84[a] | 14.52 ± 7.64[a] |
| Abortion model group | 232.49 ± 11.88 | 7.35 ± 5.81 |
| Progesterone group | 259.42 ± 13.24[a] | 16.55 ± 8.18[a,b] |
| ZishenYutai pill group | 245.73 ± 25.15[a] | 13.75 ± 3.21[a] |
| Joint group | 257.09 ± 33.98[a] | 16.71 ± 7.75[a,b] |

[a] Compared to abortion model group, $P < 0.05$. [b] Compared to ZishenYutai pill group, $P < 0.05$.

**Table 4**
The comparisons of Th1/Th2 cytokines levels ($n = 10$).

| Groups | IL-4/INF-γ | IL-4/IL-2 | IL-10/INF-γ | IL-10/IL-2 |
|---|---|---|---|---|
| Control group | 1.28 ± 0.08 | 4.40 ± 1.28 | 0.95 ± 0.08 | 3.20 ± 1.16 |
| Abortion model group | 0.92 ± 0.07 | 2.63 ± 0.14 | 0.71 ± 0.14 | 2.02 ± 0.32 |
| Progesterone group | 1.15 ± 0.10 | 3.72 ± 0.26 | 0.82 ± 0.13 | 2.67 ± 3.33 |
| ZishenYutai pill group | 1.17 ± 0.07 | 4.31 ± 0.54 | 0.78 ± 0.14 | 2.78 ± 1.28 |
| Joint group | 1.25 ± 0.08 | 8.27 ± 3.87 | 0.82 ± 0.98 | 5.53 ± 2.92 |

to Th2, and the degree of deviation of joint group was better than the simply progesterone group and ZishenYutai pill group ($P < 0.05$), which indicated the ZishenYutai pill combined with progesterone could regulate maternal–fetal interface cytokines to a balance and normal state, and make it develop to a good direction, and reduce the abortion rate (Table 4).

## 4. Discussion

Threatened abortion refers to a small amount of vaginal bleeding during pregnancy with abortion symptoms of abdominal pain, backache and abdominal distension, which is a common complication during pregnancy. According to statistics, the spontaneous abortion rate during pregnancy is about 3.6%–16.0%, which is increased gradually [11–13]. Some scholars believe that the threatened abortion is related closely to the factors, such as heredity, immune, cryptorrhea and endometrial abnormality [14,15]. The treatments can start from oophoron and corpus luteum to ameliorate the growth of endometrium, improve embryo implantation and restrain uterine contractions to reach the prevention and cure effects; however, the commonly used drugs cannot achieve the ideal therapeutic effect at present. Therefore, it is extremely significant to seek for a more effective prevention and control method to improve the threatened abortion pregnant outcome.

Some studies have confirmed that the serum levels of E2 and P of threatened abortion model rats were obviously lower than normal pregnant rats [16–20]. Th1/Th2 was deviated from balance to reminder their morbidity was related closely to the insufficiency of maternal luteal function and the imbalance of Th1/Th2 cytokine. Progesterone, a clinical common used drugs for the treatment of threatened abortion, can effectively improve the development of the puerpera endometrium of threatened abortion, restrain uterine contractions, and increase incidence of parturition, which has a nice curative effect [21,22].

ZishenYutai pill consists of semen cuscutae, fructus amomi, ginseng, loranthus parasiticus, prepared rehmannia root, colla corii asini (fried), folium artemisiae argyi, Morinda officinalis, fleece-flower root, Atractylodes, dangshen, the fruit of Chinese wolfberry, teasel root, Cornu cervi degelatinatum, and tu-chung, which benefit the Qi and cultivates the Yuan, tonify kidney and spleen, strengthen body and nourish the blood and prevent abortion. It is often used to treat the habitual abortion and threatened abortion that result from asthenia of both the spleen and kidney and deficiency of thoroughfare and conception vessel [23,24]. In this study, the weight of rats in 5 groups at each time point had no significant difference ($P > 0.05$), indicating that ZishenYutai pill combined progesterone had no adverse influence to the nourishment state of threatened abortion rats; The serum levels of E2 and P of the control group, progesterone group, ZishenYutai pill group and joint group were obviously higher than that of the abortion model group ($P < 0.05$), which indicated combined two medicines would be more effective to strengthen hypophysis - ovarian lutropin function, restrain the uterine activity, and promote the estrogen activity, and be benefit for the grow and development of uterus. Some studies have shown that Th1/Th2 type cytokines would present the state of physiological imbalance in the process of normal pregnancy, and Th2 type cytokines occupy a certain advantage in matrix to inhibit type Th1 cytokines, for example, trophoblast cells of placenta and fetal injury may appear to lead the occurrence of abortion when the content level of Th1/Th2 type cytokines lose balance and deviate to Th1 type cytokines [25,26]. In the study, The live births of joint group was obviously higher than that of progesterone group, ZishenYutai pill group and control group ($P < 0.05$); abortion numbers and average abortion rate was significantly lower than that of the above three groups ($P < 0.05$), which showed that ZishenYutai pill combined with progesterone had obvious prevention and cure effect to the threatened abortion. In addition, the maternal–fetal interface IL – 4/IL – 2, IL – 10/IL-2 of model group were balanced deviating to Th1/IL – 2, the IL-4/IL-2, IL-10/IL-2 of progesterone group, ZishenYutai pill group and joint group are balanced deviating to Th2, and joint group's deviation is better than the simply progesterone group and ZishenYutai pill group ($P < 0.05$), which proved the ZishenYutai pill combined with progesterone could adjust maternal–fetal interface cytokines to a balance and normal state, and make it develop to a good direction, and reduce the abortion rate.

The study results indicated that ZishenYutai pill combined progesterone had significant prevention and cure effect for threatened abortion, and can significantly increase maternal serum levels of E2 and P and regulate the balance of Th1/Th2.

## Conflict of interest statement

We declare that we have no conflict of interest.

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