

ORIGINAL RESEARCH ARTICLE

Study on the possible mechanism of Shoutai pill in the treatment of unexplained recurrent abortion

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Abstract

This study investigates the therapeutic mechanism of Shoutai Pill in treating unexplained recurrent spontaneous abortion. Clinical samples from affected individuals and healthy pregnant women were analyzed, alongside animal models and primary cell cultures. The results demonstrated that Shoutai Pill reduced pro-inflammatory cytokines such as tumor necrosis factor alpha and interferons alpha and gamma, while enhancing the anti-inflammatory cytokine interleukin-10 and antiviral proteins including interferon-induced protein 44-like, radical S-adenosyl methionine domain-containing protein 2, and bone marrow stromal cell antigen 2. In vitro, serum containing Shoutai Pill increased decidual cell viability and significantly decreased apoptosis. Transcriptome analysis identified key regulatory genes—proline dehydrogenase, serum and glucocorticoid-regulated kinase 2, and neurogenic locus notch homolog protein 4—implicating the PI3K-Akt and Notch signaling pathways. These findings suggest that Shoutai Pill exerts a protective effect by modulating immune responses, enhancing antiviral defense, and maintaining decidual cell stability. This multi-tiered approach provides mechanistic evidence supporting the clinical potential of Shoutai Pill as an adjunct therapy for unexplained recurrent abortion. (*Afr J Reprod Health* 2025; 29 [9]: 105-112).

Keywords: Shoutai Pill, Recurrent Abortion, Apoptosis, Mechanism

Résumé

Cette étude explore le mécanisme thérapeutique de la pilule Shoutai dans le traitement des fausses couches spontanées récurrentes inexplicables. Des échantillons cliniques provenant de patientes concernées et de femmes enceintes en bonne santé ont été analysés, parallèlement à des modèles animaux et à des cultures primaires de cellules. Les résultats ont démontré que la pilule Shoutai réduit les cytokines pro-inflammatoires telles que le facteur de nécrose tumorale alpha et les interférons alpha et gamma, tout en augmentant la cytokine anti-inflammatoire interleukine-10 et des protéines antivirales, notamment la protéine 44-like induite par les interférons, la protéine contenant un domaine radical S-adenosyl méthionine 2, et l'antigène 2 des cellules stromales de la moelle osseuse. In vitro, le sérum contenant la pilule Shoutai a amélioré la viabilité des cellules déciduales et réduit de manière significative l'apoptose. L'analyse du transcriptome a identifié des gènes clés de régulation — la proline déshydrogénase, la kinase 2 régulée par les sérums et les glucocorticoïdes, et la protéine homologue 4 du locus neurogénique Notch — impliquant les voies de signalisation PI3K-Akt et Notch. Ces résultats suggèrent que la pilule Shoutai exerce un effet protecteur en modulant les réponses immunitaires, en renforçant la défense antivirale et en maintenant la stabilité des cellules déciduales. Cette approche multi-niveaux fournit des preuves mécanistiques appuyant le potentiel clinique de la pilule Shoutai en tant que thérapie adjuvante dans le traitement des fausses couches récurrentes inexplicables. (*Afr J Reprod Health* 2025; 29 [9]: 105-112).

Mots-clés: Pilule Shoutai, fausse couche récurrente, apoptose, mécanisme

Introduction

Unexplained recurrent abortion refers to spontaneous termination of pregnancy for more than three times without a clearly obvious and definite cause¹. The causes of recurrent abortion may include chromosome abnormalities, uterine structural abnormalities, endocrine disorders, immune system

abnormalities, infection and environmental factors. However, about 50% of recurrent abortions have no obvious identifiable cause, which is often referred to as unexplained recurrent abortion². The incidence is about 3-5% among women of childbearing age. The risk factors of the disease include increasing maternal age, abnormal structure of reproductive system, abnormal immune function, endocrine

abnormality, and other factors. Unexplained recurrent abortion can cause increased mental stress, mood swings and other problems, as well as higher maternal mortality³. A large sample cohort showed that advanced age (≥ 35 years old) was an independent risk factor, with the risk of abortion increased by 12% for each additional year.⁴

Shoutai Pill is a traditional Chinese medicine composed of dodder seed, teasel root, taxillus taxilli and donkey-hide gelatin, which can regulate menstruation and promote blood circulation⁵. Studies have shown that Shoutai Pill has therapeutic effects on unexplained recurrent abortion, which can effectively regulate the level of reproductive hormones, adjust immune function, improve the endometrial environment and other measures⁶. At the same time, studies have confirmed that Shoutai Pill normalizes HIF-1 α -driven glycolysis at the maternal-fetal interface and restores trophoblastic cell vitality.⁷ It can be seen that Shoutai Pills may play a role through immune regulation, coagulation regulation, endometrial receptivity and mitochondrial protection⁸. However, its mechanism of action remains unclear. The possible reason is that the traditional Chinese medicine often has the characteristics of multi-components and multi-targets, which may affect the relevant signal pathways, regulate the activity of immune cells and reduce the overreaction of immune reaction. Thus, it is expected to play an active role in the treatment of unexplained recurrent abortion. This study used a human-animal-cell parallel-mutual evidence-design to verify whether STP can restore immune balance and inhibit decidua apoptosis

Methods

General information

Thirty-seven patients with unexplained recurrent abortion in Jiangxi Province Maternal and Child Health Hospital from June 2022 to September 2023 were recruited into the study. The average age was (31.5 ± 5.2) years; the mean duration of pregnancies was (57.6 ± 8.6) days; and the average number of previous miscarriages was (3.2 ± 1.1). Another 15 pregnant women with normal early pregnancy in the

same period without past miscarriages were recruited as controls. The control group had an average age of (30.6 ± 4.2) years and a mean pregnancy duration of (56.3 ± 7.5) days. There was no significant difference in age and pregnancy duration between the two groups ($P > 0.05$). It's comparable.

The inclusion criteria were: (1) women aged between 20 and 45 years; (2) having a history of unexplained recurrent abortion with at least two miscarriages; (3) excluding organic causes after comprehensive physical and related examinations; and (4) agreeing to participate in the study and signing an informed consent form.

The exclusion criteria were: 1) patients with obvious organic diseases, such as uterine deformities and endocrine disorders; 2) having been treated with immunomodulatory drugs; 3) having serious complications such as severe cardiovascular disease, liver, and kidney insufficiency; and 4) patients who were involved in other clinical studies.

Experimental animals and groups

Twenty adult Sprague Dawley (SD) female rats were randomly divided into two groups: Shoutai pill group ($n = 10$) and a control group ($n = 10$). Preparation of Shoutai Pills: Four traditional Chinese medicines, including dodder seed, teasel root, taxilli and donkey-hide gelatin, were mixed and boiled at a ratio of 2:1:1, and boiled until the crude drug content was 1g/mL. Rats were gavage at 2ml/100g (dose administered = commonly used clinically * animal equivalent area coefficient * 5). Preparation of Shoutai Pills: Four traditional Chinese medicines, including dodder seed, teasel root, taxilli and donkey-hide gelatin, were mixed and boiled at a ratio of 2:1:1, and boiled until the crude drug content was 1g/mL. Rats were gavage at 2ml/100g (dose administered = commonly used clinically * animal equivalent area coefficient * 5).

The control group was given the same amount of normal saline twice a day for one week. Aseptic blood was collected 1 hour after the last dose. After standing, the serum was collected by centrifugation and inactivated. After filtration and sterilization, the serum was stored at -80°C for later use. Figure 1



Figure 1: Picture of animal drug modeling.

Culture and identification of decidual cells in vitro

The whole-component decidual cells were cultured. The decidual tissue of SD rats was taken under aseptic condition, washed repeatedly with ice Phosphate-Buffered Saline, shredded into paste in aseptic glass dish, fully mixed with 2 times the volume of compound digestive enzymes (2.5g/L trypsin, 2g/L hyaluronidase and 2g/L collagenase mixed at 2:1:2) and placed in the cell incubator for static 30min. To stop digestion, the digested tissue was passed through No. 23 needle four times, the supernatant was discarded from the eccentric, and the cell precipitation was collected, mixed twice in the same method, PBS was added, the cells were blown with a liquid transfer gun, and the cells were suspended, and the cells were collected and transferred to the culture bottle through a 40 μ m cell filter and 1200r/min centrifugation 5min.

After 24 hours, the liquid was changed to remove red blood cells and non-adherent cells. The expression of prolactin in placenta was detected by Immunocytochemistry, and its purity was determined. The isolated and cultured cells showed fibroblast morphology under the microscope, and the brown granules were observed to be positive under the Immunocytochemistry microscope, and the positive cells were > 90%. It was confirmed that rat decidual cells were successfully isolated and cultured with high purity

Experimental method

Cell Counting Kit-8: The cell suspension was inoculated in a 96-well plate and cultured in a 5%CO₂ incubator at 37 °C. 10 μ L CCK-8 solution was added to each well. After incubation in the

incubator for 1-4 h, the absorbance at 450nm was determined by enzyme labeling instrument. The cell activity was calculated according to the formula of cell viability (%) = [A (experiment)-A (blank)] / [A (control)-A (blank)] × 100.

Flow cytometry

The cultured cells were collected, washed with PBS buffer two times, Resuspend in Annexin V binding buffer (10 μ cells/mL) containing 2.5 mM CaCl₂, add Annexin V-FITC and PI and incubate in the dark for 15 minutes. A BDFACSCelesta™ flow cytometer was used to collect 10,000 events, debris was removed by FSC/SSC, adhesions were removed by a single cell gate, and apoptosis rates were finally calculated using Annexin V-FITC (FL1) and PI (FL3) dual-parameter scatter plots. Instruments: BD FACSCelesta™, 488 nm laser, FL1(530/30 nm, FITC), FL3(670/14 nm, PI). Probe: Annexin V-FITC(1:50, ebioscience, CAT # 11-8006-42); PI (1 μ g/mL). Compensation: Single dyeing control: Annexin V (without PI) and PI (without Annexin V), the software automatically compensates. Gating strategy: P1(FSC/SSC living cell) → P2(FSC-A/FSC-H single cell) → Q1-Q4 (Annex V vs PI). Data analysis: FlowJo v12.9 calculates the percentage of each quadrant, and GraphPad Prism 10 carries out ANOVA statistical analysis.

Western blot

In clinical practice, decidua tissues of normal and observation groups were removed, and cultured rat decidua cells were collected in cell test. The cell lysate was added and shaken to dissociate the cell membrane. It was then centrifuged to remove nuclei and fragments. The protein samples were separated

by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis gel electrophoresis, and then the proteins were transferred to Polyvinylidene Fluoride membrane.

The Polyvinylidene Fluoride membrane was dissolved with 5% fat emulsion powder in an unspecific binding site in the Tris-Buffered Saline with Tween 20 solution to prevent non-specific binding.

Immunoblotting reaction was performed with corresponding primary and secondary antibodies, and enhanced chemiluminescence was used to observe the expression level of proteins.

Screening differential genes by RNA-seq

Combined with the RNA-seq results of the first stage, the genes with the opposite trend of differential expression in stage 1 and stage 2 were clustered and focused on immune-related pathways.

Statistical method

The experimental data were analyzed by SPSS 22.0 software, and the experimental data were expressed by mean \pm standard deviation ($\bar{x} \pm s$). Single factor analysis of variance was used for comparison among groups, and LSD-t test was used for pairwise comparison between groups.

Ethical considerations

Ethical approval number Human body: 2022-MCH-032; Animal: IAUC-2022-041.

Results

Tumor Necrosis Factor α (TNF- α), Interleukin-10 (IL-10), Interferon α (IFN- α), and Interferon γ (IFN- γ) protein expression

The TNF- α , IL-10, IFN- α and IFN- γ in decidual tissue of group A and B were detected by WB. As shown in Figure 1, the TNF- α , IL-10, IFN- α and IFN- γ in group B were reduced as compared to group A.

Interferon Induced Protein 44 Like (IFI44L), Radical S-Adenosyl Methionine Domain Containing 2 (RSAD2), and Bone Marrow Stromal Cell Antigen 2 (BST2) protein expression

The IFI44L, RSAD2 and BST2 in decidual tissue of group A and B were detected by WB. The IFI44L, RSAD2 and BST2 in group B were increased as compared to group A. The difference was statistically significant (Figure 2).

Determination of serum concentration of Shoutai pills by CCK8

The effect of serum addition of Shoutai pill on the activity of mouse decidual cells was detected by CCK8. It was found that the activity of decidual cells in the group B was higher than that in group A, under the condition of 20%ShoutaiPill serum, the activity of mouse decidual cells increased by 28% compared with the blank serum., (Figure 3).

Effect of Shoutai Pill on protein expression of TNF- α , IL-10, IFN- α and IFN- γ in Mouse decidual cells

When the amount of serum was 20%, WB detected the TNF- α , IL-10, IFN- α and IFN- γ in decidual cells of mice treated with Shoutai pill. The TNF- α protein in decidual cells in group B was reduced than group A, while the IL-10, IFN- α and IFN- γ in the group B were raised than group A (Figure 4).

Effect of Shoutai Pill on apoptosis of decidual cells in mice

Apoptosis was detected by flow cytometry. The apoptosis in group B was reduced than group A (Figure 5).

Bioinformatics analysis of possible targets of Shoutai pills in the treatment of recurrent abortion

The genes up-regulated in ES group vs S group and down-regulated in ST group vs Ctrl group are PRODH and SGK2, which are down-regulated in ES group vs S group and up-regulated in ST group vs Ctrl group, as shown in Table 1.

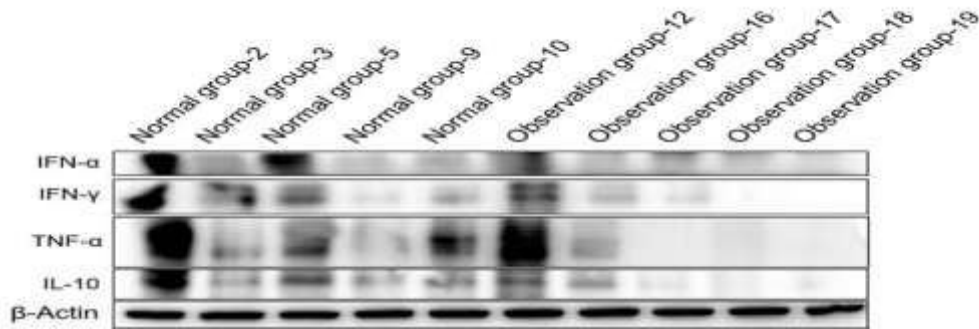


Figure 1: Detection of TNF- α, IL-10, IFN-a, and IFN- γ protein expression by WB.

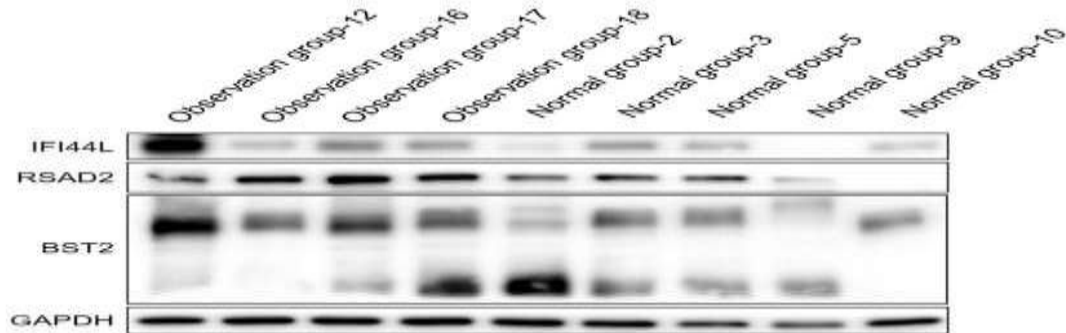


Figure 2: Detection of IFI44L, RSAD2, and BST2 protein expression by WB.

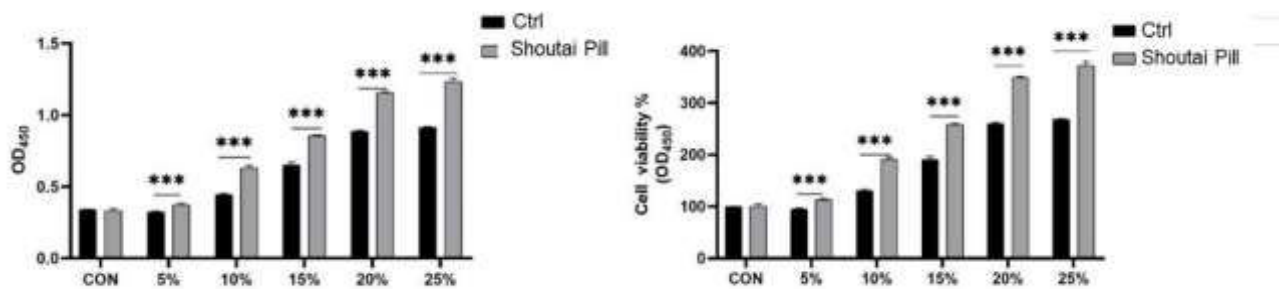


Figure 3: Determination of serum concentration of Shoutai pills by CCK8

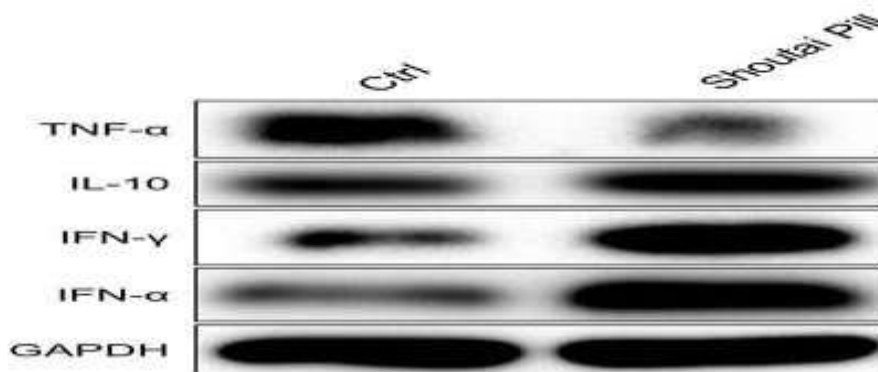


Figure 4: Detection of protein expression of TNF-α, IL-10, IFN-an and IFN-γ by WB.

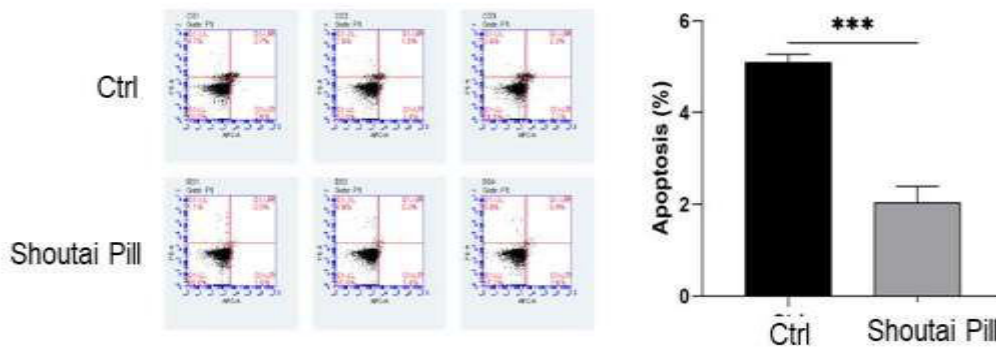


Figure 5: Detection of apoptosis in each group by flow cytometry.

Table 1: Bioinformatics analysis of possible targets of Shoutai pills in the treatment of recurrent abortion.

Gene_id	Gene_name	ES_vs_S_l log2fc	ES_vs_S_p value	ES_vs_S_ padj	ST_vs_Ctrl_ log2fc	ST_vs_Ctrl_ pvalue	ST_vs_Ctrl_ padj
ENSG00000100033	PRODH	1.441	<0.001	<0.001	-2.637	0.001	0.156
ENSG00000101049	SGK2	1.143	<0.001	0.022	-1.897	0.019	0.568
ENSG00000204301	NOTCH4	-1.287	0.049	1	1.681	0.026	0.628

The above genes were annotated by KEGG Pathway database to further explore the possible pathways in which these genes may be involved. The results suggest that gene PRODH may be involved in immune-related pathways Arginine and proline metabolism, gene NOTCH4 may be involved in immune-related pathways Notch signaling pathway and Human papillomavirus infection, and gene SGK2 may be involved in immune-related pathways FoxO signaling pathway and PI3K-Akt signaling pathway.

Discussion

This study used a parallel and mutual evidence strategy of "clinical sample-animal-primary cells-multiple omics" to systematically clarify the multi-target intervention mechanism of ShoutaiPill (STP) in unexplained recurrent spontaneous abortion (URSA). The main findings can be summarized into four points: In decidua tissues of patients with URSA, STP significantly down-regulated the pro-inflammatory cytokines TNF- α , IFN- α , IFN- γ , and upregulated the anti-inflammatory factor IL-10;^② At the same time, it significantly increased the antiviral related proteins IFI44L, RSAD2, and BST2;^③ 20% drug-containing serum increased the vitality of primary mouse decidua cells by 28%, and

halved the overall apoptosis rate;^④ Transcriptome cross-analysis identified PRODH, SGK2, and NOTCH4 as the main common differential genes, suggesting that the PI3K-Akt and Notch signaling link may be the core pathway. The above results confirm each other and provide experimental basis for STP to "regulate immunity-anti-apoptosis-abortion prevention".

Inflammation and immune homeostasis

Typical immunological features of URSA are increased Th1/Th2 ratio and excessive activation of NK cells, of which TNF- α and IFN- γ are considered key molecules for embryotoxicity⁹. This study confirmed that STP can simultaneously inhibit TNF- α , IFN- α/γ , and significantly upregulate IL-10, suggesting that it can restore maternal and fetal immune tolerance by "reducing Th1-pro-inflammation and enhancing Th2-anti-inflammation". This result is consistent with the conclusion that dodder seed-taxillus prescriptions improve the Th1/Th2 imbalance in animal studies¹⁰.

Antiviral proteins and innate immunity

IFI44L, RSAD2 and BST2 belong to the interferon-stimulating gene family, which enhance antiviral innate defenses and inhibit excessive

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inflammation^{11,12}. Its increase suggests that STP may reduce the risk of abortion triggered by virus/pathogen, providing molecular annotations for TCM's concept of "strengthening the body and eliminating evil".

Apoptosis inhibition and PI3K-Akt/Notch axis

Excessive apoptosis of decidua cells can destroy the placenta-uterus connection and induce abortion. Drug-containing serum experiments showed that STP significantly inhibited the AnnexinV positive rate, which was consistent with previous reports that quercetin could inhibit LPS-induced apoptosis¹³. RNA-seq further identified PRODH, SGK2 (PI3K-Akt related) and NOTCH4 (Notch related) as inverse co-regulated genes, both of which have been confirmed to be involved in embryo implantation and decidualization¹⁴⁻¹⁶.

It is analyzed that STP may increase SGK2's activation of Akt phosphorylation and inhibit BAD/Caspase signaling; at the same time, it may regulate NOTCH4 to maintain the balance of differentiation in the uterine microenvironment, thereby achieving two-way regulation of "immune-apoptosis".

Comparative and innovative with previous research

Clinically, STP combined with western medicine can improve the second pregnancy survival rate of URSA¹⁷, but the mechanism remains at the empirical level for a long time. This study obtained multiple layers of evidence of "tissue-serum-cell" in the same patient population, and used cross-validation of different genes to lock the target, overcoming the defect of insufficient extrapolation of a single model; at the same time, it reported for the first time the regulatory effect of STP on ISGs, expanding its "abortion" extension.

Advantages and limitations

Advantages: ① The design introduces the patient's real metabolic products (drug-containing serum) into in vitro experiments, which has higher biological relevance; ② Multi-platform verification (WB, flow, RNA-seq) ensures reliable results. Limitations: ① The sample size is limited, and the outcome of live births has not been followed up; ② The hidden causes such as endocrine and thrombotic tendencies have not been ruled out; ③ Only mouse

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decidual cells are used in cell experiments, and cross-species differences need to be vigilant.

In the follow-up, multi-center large-sample randomized controlled trials need to be carried out, combined with single cell sequencing to deeply analyze cell lineage changes.

Clinical and policy implications

STP is composed of common traditional Chinese medicines and has low preparation cost. If it is further proven to be effective, it can be used as an affordable adjuvant treatment for URSA, especially for resource-limited areas.

Drug supervision and guideline formulation agencies may consider including them in the "evidence-based level needs to be improved" catalog to encourage standardized research.

Write a conclusive statement here

ShoutaiPill down-regulates pro-inflammatory cytokines, upregulates IL-10 and ISGs, inhibits decidua cell apoptosis, and may reshape maternal-fetal immune tolerance and decidua stability through the PRODH/SGK2-PI3K-Akt and NOTCH4-Notch pathways, thus playing a multi-target comprehensive role in preventing and treating URSA. This study provides systematic evidence for the modern pharmacological mechanism of STP and lays a theoretical foundation for subsequent high-quality clinical trials

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Authors' contribution

Fang Fang and Xiaoyong Chen conceptualized manuscript contents and prepared the original draft. Lili Hu and Jia Xu participated in manuscript writing, reviewed and supervised manuscript writing. All authors contributed to the article and approve

References

1. Lejeune V. Fausses couches spontanées précoces répétées: quelle prise en charge proposer en 2006? [Early

- recurrent spontaneous abortion: How to take care in 2006?]. *Gynecol Obstet Fertil*. 2006 Oct;34(10):927-37. *French*. doi: 10.1016/j.gyobfe.2006.08.008.
2. Yu N, Kwak-Kim J and Bao S. Unexplained recurrent pregnancy loss: Novel causes and advanced treatment. *J Reprod Immunol*. 2023 Feb;155:103785. doi: 10.1016/j.jri.2022.103785.
 3. Jia Z, Ren Z, Ye D, Li J, Xu Y, Liu H, Meng Z, Yang C, Chen X, Mao X, Luo X, Yang Z, Ma L, Deng A, Li Y, Han B, Wei J, Huang C, Xiang Z, Chen G, Li P, Ouyang J, Chen P, Luo OJ, Gao Y and Yin Z. Immune-Ageing Evaluation of Peripheral T and NK Lymphocyte Subsets in Chinese Healthy Adults. *Phenomics* 3, 360–374 (2023).
 4. Du M, Li Y, Gu H, Gao M, Xu H, Zhong W, Liu X, Zhong X. Assessment of the risk of unexplained recurrent spontaneous abortion based on the proportion and correlation of NK cells and T cells in peripheral blood. *Technol Health Care*. 2023;31(S1):97-109. doi: 10.3233/THC-236010.
 5. Wang Q, Wang C, Wu L, Zhang H, Gao H, Li J, Chen F. [Visual analysis of influenza treated by traditional Chinese medicine based on CiteSpace]. *Zhonghua Wei Zhong Bing Ji Jiu Yi Xue*. 2020 Jul;32(7):779-784. Chinese.
 6. Song C, Zhang S, Gao X, Zhang H, Zuo S, Qin Y, Bi X and Chen H. Shoutai pills for threatened abortion: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)*. 2023 Mar 17;102(11):e33173. doi: 10.1097/MD.00000000000033173.
 7. Zhao L, Xu Y, Duo X, Tian X, Wang P. A probe into effect of Shoutai pill on endometrial receptivity. *Exp Ther Med*. 2018 Apr;15(4):3765-3768. doi: 10.3892/etm.2018.5874.
 8. Liu Z, Zhang J, Li L, Zhang T, Huang L, Yin Q. Shoutai Pill Enhances Endometrial Receptivity in Controlled Ovarian Hyperstimulation Mice by Improving the In-Vivo Immune Environment. *Comb Chem High Throughput Screen*. 2025;28(4):711-723. doi: 10.2174/0113862073274708231028185333.
 9. Kuroda K, Nakagawa K, Horikawa T, Moriyama A, Ojio Y, Takamizawa S, Ochiai A, Matsumura Y, Ikemoto Y, Yamaguchi K and Sugiyama R. Increasing number of implantation failures and pregnancy losses associated with elevated Th1/Th2 cell ratio. *Am J Reprod Immunol*. 2021 Sep;86(3):e13429. doi: 10.1111/aji.13429.
 10. Wang P, Jiang G, Ju W, Cai Y, Wang J and Wu F. Influence of Bushen Tiaochong Cycle Therapy on Th1/Th2 Deviation, Sex Hormone Level, and Pregnancy Outcome of Alloimmune Recurrent Spontaneous Abortion. *Evid Based Complement Alternat Med*. 2021 Sep 21;2021:8624414. doi: 10.1155/2021/8624414.
 11. Yang M, Luo J, Yang Q and Xu L. Research on the medication rules of Chinese herbal formulas on treatment of threatened abortion. *Complement Ther Clin Pract*. 2021 May;43:101371. doi: 10.1016/j.ctcp.2021.101371.
 12. Zeng L, Yang K, Liu L, Zhang T, Liu H, Tan Z and Lei L. Systematic biological and proteomics strategies to explore the regulation mechanism of Shoutai Wan on recurrent spontaneous Abortion's biological network. *J Ethnopharmacol*. 2020 Dec 5;263:113156. doi: 10.1016/j.jep.2020.113156.
 13. Zhang Y, Gao Z, Liu J and Xu Z. Protective effects of baicalin and quercetin on an iron-overloaded mouse: comparison of liver, kidney and heart tissues. *Nat Prod Res*. 2011 Jul;25(12):1150-60. doi: 10.1080/14786419.2010.495070.
 14. Saccone G, Schoen C, Franasiak JM, Scott RT Jr and Berghella V. Supplementation with progestogens in the first trimester of pregnancy to prevent miscarriage in women with unexplained recurrent miscarriage: a systematic review and meta-analysis of randomized, controlled trials. *Fertil Steril*. 2017 Feb;107(2):430-438.e3. doi: 10.1016/j.fertnstert.2016.10.031.
 15. Ismail AM, Abbas AM, Ali MK and Amin AF. Peri-conceptional progesterone treatment in women with unexplained recurrent miscarriage: a randomized double-blind placebo-controlled trial. *J Matern Fetal Neonatal Med*. 2018 Feb;31(3):388-394. doi: 10.1080/14767058.2017.1286315.
 16. Scarpellini F and Sbracia M. Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: A randomised controlled trial. *Hum. Reprod*. 24 (11), 2703–2708.
 17. Li HF, Shen QH, Li XQ, Feng ZF, Chen WM, Qian JH, Shen L, Yu LY and Yang Y. The Efficacy of Traditional Chinese Medicine Shoutai Pill Combined with Western Medicine in the First Trimester of Pregnancy in Women with Unexplained Recurrent Spontaneous Abortion: A Systematic Review and Meta-Analysis. *Biomed Res Int*. 2020 Aug 8;2020:7495161. doi: 10.1155/2020/7495161..