DOI: 10.66069/ojspub.20542216

Relationship between Recurrent Spontaneous Abortion and Reproductive Tract Microbiota and Research Progress on the Role of Traditional Chinese Medicine

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Abstract: ***The pathogenesis of recurrent spontaneous abortion (RSA) has not been fully elucidated. Reproductive tract microecological imbalance is considered one of its important pathogenic mechanisms. Based on the theory of "habitual abortion" (huatai), Traditional Chinese Medicine (TCM), with tonifying kidney and strengthening spleen as the main therapeutic principles, has preliminarily demonstrated potential in regulating reproductive tract microecology, improving immune imbalance, and enhancing endometrial receptivity. This review aims to summarize the pathological pathways by which microecological imbalance mediates RSA and to reveal the unique advantages of TCM in multi-target and multi-pathway intervention of RSA, thereby providing a theoretical basis for clarifying the scientific connotation of TCM in improving the reproductive microenvironment.***

Keywords: Recurrent spontaneous abortion, Reproductive tract microbiota, Traditional Chinese Medicine, Immunomodulation, Endometrial receptivity**.**

1. Introduction

Recurrent spontaneous abortion (RSA) is a female pregnancy disorder with complex etiology and incompletely understood pathogenesis, and there is currently no unified international definition. According to the Chinese Expert Consensus on the Diagnosis and Treatment of Recurrent Spontaneous Abortion (2022), RSA is defined as the loss of two or more consecutive pregnancies with the same partner before 28 weeks of gestation [1]. Epidemiological surveys indicate that the incidence of RSA accounts for 1%–5% of women of childbearing age and is increasing annually [2]. This condition has become a common and challenging disorder in reproductive medicine, severely compromising women's physical and mental health. The etiology of RSA is complex, including endocrine factors, infectious factors, immunological factors, and pre-thrombotic states; nevertheless, 40%–50% of patients have no identifiable cause, a condition termed unexplained recurrent spontaneous abortion (URSA) [3]. One of the core pathological mechanisms of URSA is the imbalance of the pro-inflammatory/anti-inflammatory immune homeostasis at the maternal-fetal interface [4]. Current treatment for RSA is highly dependent on etiological diagnosis, and there remains a lack of standardized and effective interventions for URSA.

In TCM, RSA falls into the categories of "habitual abortion" (huatai) or "repeated miscarriage" (shu duotai). The disease is located in the kidney and involves the liver, heart, spleen, and other viscera. TCM has a long history of treating RSA, accumulating rich diagnostic and therapeutic experience. Faced with the dilemma in modern medicine of "identifiable causes but lack of effective treatment options," TCM demonstrates unique advantages. It can improve oocyte quality and the endometrial environment by tonifying kidney and strengthening spleen, and by regulating qi and blood. Furthermore, through multi-target regulation of endocrine and immune homeostasis, it provides a safe and effective treatment regimen for patients with recurrent pregnancy failure [5].

2. Association Mechanisms between Reproductive Tract Microbiota and RSA

2.1 Composition and Dynamic Characteristics of the Female Reproductive Tract Microbiota

In healthy women, the vaginal microbiome (VM) comprises >80% relative abundance of Lactobacillus species. Lactobacillus maintains the acidic environment of the vagina through its metabolite, lactic acid, thereby inhibiting the growth of potential pathogenic bacteria [6]. The VM also includes Prevotella, Gardnerella, Bacteroides, and other genera, collectively maintaining vaginal microecological balance. The healthy cervical microbiome (CaM) is likewise dominated by Lactobacillus as the predominant genus. As important probiotics in the cervical flora, lactobacilli maintain an acidic environment and inhibit harmful pathogens. Additionally, the CaM includes resident bacteria such as Prevotella, Gardnerella, and Pepto streptococcus, which are generally relatively stable and low in abundance. In recent years, the classical concept of a "sterile uterus" has been challenged; however, consensus on the core microbiota of the endometrium has not yet been reached. Most studies suggest that the endometrial microbiota (EnM) of healthy women is dominated by Lactobacillus [7]. A few studies have reported that Comamonas, Pseudomonas, and Acinetobacter may predominate [8].

Regarding dynamic characteristics, the female reproductive tract microbiota exhibits cyclical fluctuations under the regulation of sex hormones. Microbiota diversity is relatively

increased during the proliferative phase and relatively decreased in the endometrium during the secretory phase [9]. In summary, the female reproductive tract microbiota is a microecosystem dominated by Lactobacillus, with a spatial gradient of decreasing biomass from the lower to upper reproductive tract, and is influenced by hormonal fluctuations and physiological events [10].

2.2 Pathological Association between Microbiota Dysbiosis and RSA

2.2.1 Diversity Changes

Current conclusions regarding the diversity characteristics of the reproductive tract microbiota in RSA patients remain inconsistent, possibly due to differences in population, sampling, and sequencing methods. One study reported that compared with healthy controls, Lactobacillus remained the dominant genus in the RSA group; however, the abundances of Bacteroides, Actinobacteria, and Firmicutes differed significantly. The RSA group had higher abundances of Streptococcus, Prevotella, and Proteus mirabilis, whereas the healthy control group was dominated by Gardnerella and Lactobacillus [11]. Vomstein found that compared with healthy women, RSA patients had increased Lactobacillus richness but decreased α-diversity [12]. However, Liu reported no significant difference in VM α-diversity between RSA patients and healthy women but a significant difference in β-diversity, with increased abundance of Atopobium and decreased abundance of Bifidobacterium in the RSA group [13]. Zhao found that although Lactobacillus remained the dominant genus in RSA patients compared with healthy women, the richness of Leptotrichia and Megasphaera increased, and VM richness was significantly reduced [14]. Another study found that Gardnerella was more abundant in the VM of RSA patients than in healthy women, and fungi were detected in the vagina of 36% of RSA patients, predominantly Candida [15]. These discrepancies may be attributed to differences in population race/ethnicity, geographic region, sampling time, anatomical site, and sequencing bioinformatics methods across studies.

2.2.2 Immune and Inflammatory Mechanisms

In healthy women, the reproductive tract microbiota is generally in a dynamic equilibrium, which is conducive to maintaining reproductive safety. In RSA patients, when harmful pathogens enter the female reproductive tract or when the reproductive tract microecology is imbalanced, infection or immune imbalance may occur, ultimately leading to miscarriage. One study found that certain microbial metabolites, such as 1-methylimidazole-4-acetic acid and imidazole propionic acid, were positively correlated with changes in Th1/Th17 cell levels in the miscarriage group [15]. Bai found that the microbiota could influence immune responses through the NOD-like receptor signaling pathway and disrupt the pregnancy-related immune balance of T helper 1 (Th1)/Th2 cells, increasing the risk of adverse pregnancy outcomes such as miscarriage [16]. In summary, there may be links among reproductive tract microbiota, altered immune parameters, and miscarriage.

2.2.3 Endometrial Receptivity

There is a certain relationship among EnM, uterine cavity microbiota, and endometrial receptivity. Kong found that in pregnant women, the endometrium was dominated by Lactobacillus, and patients with a Lactobacillus proportion >90% had higher embryo implantation and pregnancy rates compared with those with a lower Lactobacillus proportion, indicating that Lactobacillus is beneficial for maintaining endometrial receptivity [17]. Another study found that a non-Lactobacillus-dominant EnM adversely affected embryo implantation and clinical pregnancy [18]. Klebsiella, Neisseria, and Pseudomonas (phylum Proteobacteria) are closely related to follicular quality and endometrial receptivity, and their increased abundance adversely affects pregnancy outcomes. Dysbiosis of the EnM may alter endometrial receptivity, affect the endometrial immune environment, cause endometrial lesions, and lead to secondary infertility. Dysbiosis of the uterine cavity microbiota also adversely affects embryo implantation and endometrial receptivity. Moreno found that the presence of specific pathogenic bacteria in the uterine cavity together with depletion of Lactobacillus impaired reproductive outcomes [18]. Based on the above analysis, a balanced uterine cavity microbiota and EnM are beneficial for maintaining endometrial receptivity and improving pregnancy rates, whereas dysbiosis or increased pathogenic bacteria compromise pregnancy outcomes.

2.3 Impact of Microbiota on Assisted Reproductive Technology (ART)

Recent studies have found that reproductive tract microbiota dysbiosis is associated with adverse pregnancy outcomes during ART. Related research has identified that one of the risk factors for early pregnancy loss in patients undergoing ART is VM imbalance, and regulating the vaginal microecology can improve embryo implantation success rates [19]. Studies have shown that women with a Lactobacillus-dominant VM before embryo transfer (ET) have better pregnancy rates [19]. However, Thor demonstrated that vaginal microbiota represented by Atopobium and Gardnerella reduced the clinical pregnancy rate in women undergoing in vitro fertilization-embryo transfer (IVF-ET) [20]. Moreno also found that among infertile patients treated with IVF-ET, women with a non-Lactobacillus-dominant microbiota had significantly lower embryo implantation and live birth rates [7]. In summary, reproductive tract microecological homeostasis is an important foundation for successful ART outcomes.

3. Mechanisms of TCM Intervention in RSA

3.1 TCM Pathogenesis Understanding of RSA

TCM has long recognized RSA and classifies it into the category of “habitual abortion” (huatai). The Golden Mirror of Medicine: Essential Teachings of Gynecology states, “Repeated miscarriage is called habitual abortion,” which is the earliest record of the term “habitual abortion” [21]. TCM considers RSA as a condition of root deficiency and branch excess. The disease location is the uterus, and the core pathogenesis can be summarized as “spleen-kidney deficiency” and “kidney deficiency with blood stasis” [22]. The Complete Works of Jingyue: Rules for Women states, “In

pregnancy, those who repeatedly miscarry must be due to deficiency and damage of qi and vessels... it is nothing but kidney deficiency and dual deficiency of qi and blood" [23]. The spleen and kidney are the roots of acquired and innate constitution, respectively. Spleen-kidney deficiency leads to insufficient generation of qi and blood, disharmony of the Chong and Ren meridians, and failure to nourish and secure the fetus. If accompanied by emotional stagnation, as described in Fu Qingzhu's Gynecology, "liver wood not soothing will invariably overact on spleen earth" [24], liver depression overacting on the spleen exacerbates qi and blood deficiency. Prolongation inevitably leads to stasis. Corrections of Errors in Medical Works points out that in women with consecutive miscarriages, "there is first blood stasis occupying the uterus" [25], and blood stasis blocking the uterus further aggravates fetal instability. This pathogenic theory of "deficiency leading to stasis, with intermingled deficiency and excess" is consistent with the pathological changes in modern medicine, such as abnormal uterine blood flow perfusion and microthrombosis in RSA patients [26].

3.2 Role of Chinese Herbal Medicine in Regulating Reproductive Tract Microbiota

Chinese herbal medicine regulates reproductive tract microecology with the characteristics of "combined internal and external treatment, holistic regulation." Oral formulas often target spleen-kidney deficiency and damp-heat, such as Wandai Decoction (Decoction for Ending Vaginal Discharge) to strengthen spleen and resolve dampness, and Yihuang Decoction (Decoction for Treating Yellow Vaginal Discharge) to clear heat and eliminate dampness. A clinical randomized controlled trial showed that Wandai Decoction improved vaginal pH and increased Lactobacillus count in patients with bacterial vaginosis [27]. External preparations act locally to directly inhibit bacteria, repair mucosa, and promote Lactobacillus colonization. According to related studies, Kougan (Sophora flavescens) gel does not significantly inhibit Lactobacillus and even partially promotes its proliferation, while improving vaginal pH and vaginal cleanliness [28]. Furthermore, compound sea buckthorn seed oil suppositories, in multiple multicenter RCTs, synergistically improve the vaginal acid-base environment and microbial community structure. Their mechanisms of action mainly include: selectively inhibiting pathogenic bacteria (including disrupting biofilms), regulating local immune and inflammatory pathways, promoting vaginal epithelial repair and mucus barrier reconstruction, and lowering vaginal pH without harming lactobacilli, thereby restoring the "Lactobacillus-dominant" microecological pattern [29].

3.3 Immune and Metabolic Regulation

According to relevant studies, TCM kidney-tonifying therapy can regulate systemic immunity by stimulating thymic proliferation. Kidney-tonifying and fetus-protecting formulas can correct Th1/Th2 imbalance, inhibit natural killer cell function, and induce maternal immune tolerance, thereby improving pregnancy outcomes [30]. As a representative formula, Shoutai Pill (Fetus-Longevity Pill) exhibits significant miscarriage-preventing effects. Its mechanism may be related to its active components increasing estrogen and progesterone levels, improving endometrial receptivity, and regulating coagulation function and immune - inflammatory status in RSA patients [31]. Moreover, addressing the pathogenesis of "deficiency leading to stasis," modern research indicates that kidney-tonifying Chinese herbal medicines combined with blood-activating and stasis-resolving herbs such as Panax notoginseng (Sanqi) and Angelica sinensis (Danggui) can optimize hemodynamic parameters, improve uterine and ovarian arterial blood flow, and enhance endometrial receptivity [32]. In summary, the TCM therapeutic approach of tonifying kidney combined with activating blood and resolving stasis aligns with modern medical mechanisms of regulating immune inflammation, improving endocrinology and hemodynamics, thereby improving pregnancy outcomes through multiple pathways.

3.4 TCM Treatment for RSA

TCM has rich theoretical and practical experience in treating RSA, often adhering to the principles of tonifying kidney and strengthening spleen, and regulating the Chong and Ren meridians. Prevention is emphasized before pregnancy and fetal protection after pregnancy, using internal TCM therapies such as decoctions and pills, and external TCM therapies such as acupuncture and moxibustion, achieving significant clinical efficacy. TCM treatment of RSA advocates "prevention before pregnancy, fetal protection after pregnancy," with tonifying kidney and strengthening spleen as the core. Shoutai Pill is a basic formula for preventing miscarriage. Meta-analysis shows that its combination with Western medicine increases the live birth rate of RSA by more than 1.8 times and significantly reduces the risk of early miscarriage [31]. Taishan Panshi San (Taishan Anticonception Powder) focuses on supplementing qi and strengthening spleen, increasing serum estrogen and progesterone levels, and correcting Th1/Th2 imbalance at the maternal-fetal interface [33]. For the branch excess of "deficiency leading to stasis," Guizhi Fuling Pill (Cinnamon and Poria Pill) may be used short-term during non-pregnancy or after miscarriage to resolve stasis, stop bleeding, and promote uterine involution; however, due to its blood-activating properties, it is strictly contraindicated during pregnancy [34]. External therapies often use acupoints Guanyuan (CV4) and Qihai (CV6) to reinforce the root and strengthen the original qi, and Sanyinjiao (SP6) to regulate the Chong and Ren meridians. Although standardized acupuncture intervention can synergistically improve the success rate of fetal protection, given the special nature of pregnancy, the stimulation intensity and manipulation techniques for abdominal and specific distal points must be strictly controlled to avoid inducing uterine contractions [35].

4. Challenges and Future Research Directions

Based on current research, there are many challenges regarding the association between RSA and reproductive tract microbiota and the role of TCM: no consensus has been reached on the composition of the reproductive tract microbiota in RSA patients; the specific pathogenic pathways and molecular mechanisms by which reproductive tract microbiota dysbiosis induces RSA have not been fully elucidated; research on TCM regulation of reproductive tract microbiota for RSA treatment is still in preliminary

exploration, mostly focusing on the antibacterial, anti-inflammatory, and immunomodulatory effects of single herbs or classic formulas, with a lack of studies on the targeted regulatory mechanisms of "Chinese medicine – reproductive tract microbiota – immunity – pregnancy outcome," and a lack of standardized, normalized clinical intervention protocols and efficacy evaluation systems.

Future research directions may include: First, conduct multi-center, large-scale longitudinal studies to define the characteristic maps of microbiota at different reproductive tract sites in Chinese RSA patients and establish microbial markers and risk prediction models for RSA. Second, deeply analyze the core molecular pathways through which reproductive tract microbiota and their metabolites regulate maternal-fetal interface immune tolerance and endometrial receptivity, revealing the microecology-immunity mechanism of RSA pathogenesis. Third, focus on the core TCM therapeutic principles of "tonifying kidney and strengthening spleen, supplementing qi and resolving stasis, regulating Chong and Ren meridians," explore active ingredients and compound formulas that target the promotion of beneficial bacterial proliferation and inhibition of pathogenic bacterial colonization in the reproductive tract, and establish integrated TCM and Western medicine intervention protocols combined with microecological preparations. Fourth, establish a clinical efficacy evaluation system for TCM combined with microecological regulation in the treatment of RSA, explore individualized and precise fetal protection strategies, and provide new theoretical bases and practical approaches for the prevention and treatment of unexplained recurrent spontaneous abortion.

5. Conclusion

The occurrence of recurrent spontaneous abortion is closely associated with reproductive tract microbiota dysbiosis. Microecological imbalances in the vagina, cervix, endometrium, and uterine cavity can disrupt pregnancy maintenance through pathways such as immune - inflammatory disorders, maternal-fetal interface tolerance imbalance, and reduced endometrial receptivity. TCM, with the core pathogenesis of "spleen-kidney deficiency and kidney deficiency with blood stasis," employs combined internal and external treatments to tonify kidney and strengthen spleen, and regulate qi and blood. It can multi-targetedly regulate reproductive tract microecology, restore immune tolerance, and improve endometrial receptivity. This review integrates the TCM pathogenesis of "deficiency leading to stasis" with the microscopic pathological mechanisms of abnormal uterine blood flow perfusion and micro thrombosis in modern medicine, revealing the micro-intervention mechanisms by which TCM reshapes the "Lactobacillus-dominant" microecological environment. Accordingly, TCM provides a safe and effective integrated treatment approach for RSA patients and those undergoing ART, compensating for the limitations of modern medicine in regulating reproductive tract microecology and local immune disorders, and offering new intervention strategies and theoretical support for improving pregnancy outcomes in RSA and ART.

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