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Review

Recent advances in understanding the role of uterine microbiota in endometrial receptivity and its impact on embryo implantation failure

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Abstract

The aim was to provide a review of studies on the impact of intrauterine bacterial flora on endometrial tolerance in populations with failed embryo implantation and to provide direction for future clinical practice. Studies utilizing techniques such as 16S rRNA gene sequencing and macrogenomics were included through a comprehensive literature search to identify studies examining the correlation between intrauterine bacteria and endometrial tolerance. The composition of the bacterial flora in the uterine cavity plays an important role in regulating endometrial tolerance, and an increase in specific dominant bacilli in the uterine cavity correlates with an increase in conception rates, whereas dysbiosis of the intrauterine flora may lead to a variety of reproductive complications, including intrauterine inflammation, uterine adhesions, endometriosis, failure of embryo implantation, recurrent miscarriages, and embryo developmental arrest. Understanding the impact of intrauterine bacteria on endometrial tolerance can help improve clinical outcomes in patients experiencing embryo implantation failure. Further research in this area will help to elucidate the underlying mechanisms and develop targeted therapeutic interventions to optimize endometrial affinity and improve reproductive outcomes.

Keywords: Uterine microbiome, Endometrial receptivity, Repeated implantation failure, Embryo implantation, Pregnancy outcomes, Chronic endometritis.

1. Introduction

In China, 7% to 10% of women who are childbearing age experience infertility; this frequency has been rising recently [1]. The treatment of infertile patients' fertility problems has greatly improved with the advent of assisted reproductive technologies (ART). Some people still struggle with unsuccessful embryo implantation, though. The live birth rate per in vitro fertilization embryo transfer (IVF-ET) cycle is currently around 25%–30% [2], and one of the main reasons for the poor pregnancy rates is embryo implantation obstacles. The most important elements influencing the fate of a pregnancy are the well-known triangle of embryo quality, endometrial receptivity, and synchrony between the embryo and endometrium. Research indicates that endometrial receptivity (ER) may be crucial for a successful embryo implantation procedure [3,4]. The uterine microbiota, or the microbial population within the uterus cavity, has recently attracted a lot of interest as a crucial component for a successful pregnancy. It is now commonly acknowledged that bacteria occupy the uterus cavity [5]. According to certain research [6], the uterine cavity is in an equilibrium state when everything is normal. A disturbance in this equilibrium could result in inflammation of the endometrium, which could impact the implantation and growth of the embryo. However, there are still a lot of unanswered questions and conflicts surrounding the association between uterine microbiota and endometrial receptivity in the early stages of study. The uterine microbiota's detection strategies, sample acquisition procedures, and analytical methods are still being investigated by researchers. Therefore, in order to improve pregnancy success rates in populations experiencing unsuccessful embryo implantation, more samples and thorough research are required to better understand the association between uterine microbiota and endometrial receptivity.

2. Definition of endometrial receptivity and its influencing factors

The term "endometrial receptivity" describes the endometrium's capacity to adjust and accommodate the implantation of an embryo. It is a crucial marker for determining if the endometrium is appropriate for the growth of embryos and the implantation of fertilized eggs. Endometrial biop-

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sies, hysteroscopies, and ultrasound are frequently used to evaluate endometrial receptivity. Endometrial receptivity can also be assessed using pinopodes, endometrial receptive arrays (ERA), endometrial non-coding RNA (miRNA, lncRNA, and miRNA), molecular markers like HOXA10, integrins, leukemia inhibitory factor (LIF), and uterine microbiota, thanks to the ongoing advancements in omics technologies and molecular biology.

Numerous factors affect endometrial receptivity. The patient's condition comes first: age, endometriosis (EMS), polycystic ovarian syndrome (PCOS), etc. [7-9]. The endometrium's form and function also play a significant impact on receptivity. The implantation and development of embryos may be impacted by abnormal endometrial structure, functional issues, or injury. Studies have demonstrated that the rate of embryo implantation increases dramatically when endometrial thickness reaches 7 or 8 mm [10]. Changes in hormone levels can also affect endometrial receptivity; for instance, progesterone and estrogen levels are essential for the development and maturation of the endometrium [11]. Moreover, endometrial receptivity may be impacted by uterine anomalies such as fibroids, endometrial polyps, intrauterine adhesions, adenomyosis, etc. [12,13]. Immunological variables, weakened endometrium, and endometrial inflammation can all have a deleterious effect on endometrial receptivity.

The uterine microbiota and the endometrium normally maintain a relative equilibrium. Uterine microbiota dysbiosis may have an effect on endometrial receptivity. It could, for example, negatively impact the immunological response, damage the decidual arteries in the area, change the number and functionality of immune cells, and encourage the release of inflammatory mediators. Furthermore, it can change the secretion of components linked to endometrial receptivity by changing enzyme activity, which in turn alters endometrial receptivity [14]. Therefore, monitoring alterations in the uterine microbiota shows potential as a means of forecasting endometrial receptivity, and further approaches might entail modifying the uterine microbiota to enhance endometrial receptivity and raise the success rates of embryo implantation.

According to some research, the total number of human cells is roughly equal to the number of microorganisms that dwell on the body's internal and external surfaces [15], with a potentially enormous number of trillions. Although the uterus has long been thought to be sterile, new molecular research has shown that the endometrium possesses its resident microbiota [16]. The notion of a typical uterine microbiota is still up for debate. The uterine microbiota is more diversified but less numerous than the vaginal microbiome [17]. According to some research, the uterine microbiota is diverse and abundant, containing a spectrum of bacteria from Actinobacteria to Bacteroidetes, Proteobacteria, and Firmicutes, with Lactobacillus, a member of the phylum Firmicutes, being the most common species in the uterus cavity [18,19]. Winters et al. [20] on the other hand, took cervical, vaginal, rectal, and oral polyester swabs from women within 24 hours of a hysterectomy, and after analyzing the microbiota using 16S rRNA and qPCR sequencing, they discovered that Acinetobacter, Pseudomonas, Clostridium perfringens, and other members of the Comamonadaceae family predominate in the uterine microbiota, not Lactobacillus as was previously believed. Investigation by A. Sola-Leyva et al. [21] 5,326 transcriptionally active microorganisms were found in total in endometrial samples; these comprised 85% different bacteria, 10% fungi, 5% viruses, and 0.3% archaea. The most prevalent microorganisms in the endometrium were subtypes of NH-16 from the genus Hydrogenoanaerobacterium, Bacillus multivorans, Klebsiella pneumoniae, and Clostridium botulinum. According to reports [22], bacteria genera including Streptococcus, Staphylococcus, Gardnerella, Mycoplasma, Ureaplasma, Chlamydia, and Neisseria are linked to chronic endometritis (CE). Streptococcus agalactiae is thought to be one of the primary pathogens of CE. The diversity of the uterine microbiota is evident, and as of right now, no one clinical definition exists. There is still a lack of knowledge on the uterine microbiota, which makes further research necessary (Figure 1).

According to a 2016 study by Moreno et al. [23], there are two types of microbiota in endometrial fluid: Lactobacillus-dominated microbiota (LDM) and non-Lactobacillus-dominated microbiota (NLDM). Pregnancy outcomes are negatively impacted by an NLDM dominated by genera like Gardnerella and Streptococcus, whereas an LDM ≥90% is linked to higher rates of embryo implantation, pregnancy, continued pregnancy, and live birth. Research has shown [22] that bacteria that cause chronic endometritis (CE), including Neisseria, Gardnerella, Mycoplasma, Ureaplasma, Chlamydia, and Enterococcus, might adversely affect the implantation of embryos. Pregnancy rates are higher in those with ≥80% Lactobacillus compared to those with <80% Lactobacillus, as Kyono K and colleagues [24] showed. They also discovered that since Lactobacillus may also be a target for some antibiotics, using antibiotics by themselves might not help achieve LDM. Patients with NLDM can effectively regain LDM with the administration of prebiotics and/or probiotics after antibiotic therapy. In contrast to healthy early-pregnant women, Lin Kaili and colleagues [25] found that in patients with recurrent spontaneous abortion (RSA), inert Lactobacillus rather than Lactobacillus crispatus was the predominant bacteria in the uterine cavity, and the levels of Bifidobacterium and unculturable Acinetobacter were significantly lower. This implies that RSA might be linked to the uterine cavity's decreased ability to fend against pathogen invasion. Research has indicated [26] that sperm may carry the endometrial microbiome, which could impact the microbial makeup of the female reproductive system. In addition to being linked to low sperm concentration, aberrant sperm morphology, high semen viscosity, and oligospermia, an increase in the detection rates of Ureaplasma, Neisseria, Klebsiella, and Pseudomonas in semen also indirectly lowers female fertility. According to Kitaya K and colleagues [27], the endometrial fluid micro-

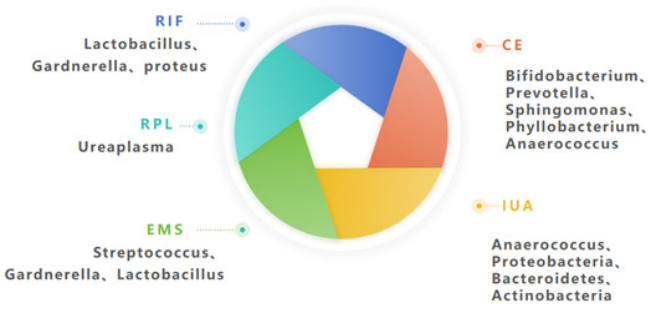

Fig. 1. The Effects of uterine microbiota on the results of pregnancy.

biome of women with recurrent implantation failure (RIF) and control groups is more diverse in terms of bacterial species and has a greater α-diversity than vaginal secretions. Lactobacillus dominated the uterine microbiota of the RIF group, but they also found higher concentrations of Burkholderia cepacia complex and Gardnerella than the control group. The uterine microbiota of RIF patients was found to be richer than that of the control group, according to research by Fu M and colleagues [28]. Of the 37 metabolites that showed significant differences between the two groups, 16 were significantly upregulated, and five others—benzopyrone, fatty alcohols, pyrimidine nucleosides, glycerophospholipids, and naphthopyranes—were significantly downregulated (Figure 2).

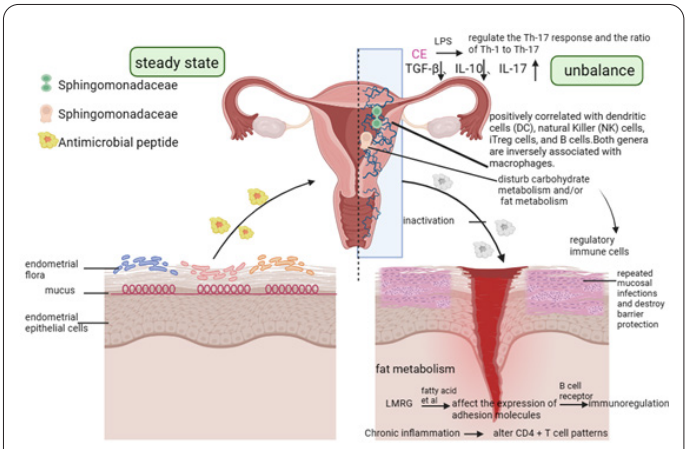

Fig. 2. Influence of uterine microbiota on endometrial receptivity.

3. Uterine microbiota's damage to the endometrial barrier

Together, the endometrial microbiota and endometrial epithelial cells develop a symbiotic relationship that protects the endometrium and releases a variety of antimicrobial peptides into the uterus. These peptides have the ability to alter how microbial membranes are metabolized, particularly by interfering with the ability of epithelial cells to defend against different proteolytic enzymes originating from pathogens [29]. Recurrent mucosal infections and breakdown of the barrier protecting the mucosa can result from inactivation of antimicrobial peptide function [30]. According to research, the uterine microbiota stimulates endometrial cells to secrete mucus, which strengthens the endometrial barrier's integrity and stabilizes the connections between endometrial cells [31]. The endometrial barrier can be shielded by Lactobacillus in the reproductive system, which keeps Neisseria gonorrhoeae from adhering to the endometrium, according to research by Li H and colleagues [26]. It is clear that disruption of the endometrial barrier might alter endometrial receptivity by making the endometrium more vulnerable to invasion.

4. The immune system and uterine microbiota

Immune cells are one of the elements preserving the uterine microbiota's equilibrium. According to research by Wang and colleagues [32], women with RIF and CE may have decreased expression of transforming growth factor-beta (TGF-β) and interleukin-10 (IL-10) but increased expression of interleukin-17 (IL-17) and autophagic cells. These results are linked to elevated pro-inflammatory immune responses in CE. These reactions can impact endometrial receptivity and are commonly linked to unfavorable reproductive outcomes, like RSA or RIF. Prolonged inflammation can change the distribution of CD4+ T cells and cause aberrant local immune regulatory cytokines [33]. According to studies by Chen P. and colleagues, Sphingomonas and Corynebacterium are more prevalent in CE patients and have comparable interactions with immune cells; these interactions are primarily favorable with DC, NK, iTreg, and B cells. The relationship between these genera and macrophages is inverse. There is a strong positive association between uterine NK (uNK) cells and Sphingomonas. Compared to the non-CE group, the CE group exhibited notably greater expression of CD16 (FcγRIII) in transcriptome differential analysis. One characteristic of uNK cells that has the ability to precisely destroy infected cells is CD16+. Failure to implant an embryo can result from abnormal differentiation of uNK cells, which can lead to the development of the endometrium, decidual vessels, and trophoblasts. Sphingomonas and Corynebacterium mainly control immune cells by disrupting the endometrium's systems for metabolizing lipids and/or carbohydrates. Through lipopolysaccharides (LPS), the microbiota of the CE endometrium may control the Th17 response and the ratio of Th1 to Th17. Lower expression levels of leukemia inhibitory factor (LIF) and IL-1 receptor antagonist (IL-1Ra), markers of endometrial receptivity, have been linked to worse endometrial receptivity and an increased risk of RIF, according to studies.[34] Common antigen-presenting cells (APCs), also referred to as macrophages, are important cytokine makers in the endometrium and are essential for endometrial receptivity. Research reveals a strong negative correlation between macrophages and Phyllobacterium and Sphingomonas, which may be a key way in which the uterine microbiome influences endometrial receptivity [14].

5. Lipid metabolism and uterine microbiota

Liu Y and colleagues' [35] research demonstrates that endometrial receptivity and the immune microenvironment of women with reproductive dysfunction are revealed by lipid metabolism-related genes (LMRGs) as biomarkers and therapeutic targets. This suggests that abnormalities in lipid metabolism can impact endometrial receptivity. By controlling the metabolism of fatty acids, triglycerides, linoleic acid, lipid factors, and other lipids, LMRGs impact the expression of adhesion molecules involved in growth factors like TGF-β and pathways involving synapses and anchoring junctions, which in turn affects the process of embryo implantation. By controlling lipid synthesis and modifying immunological responses via mechanisms like B cell receptor signaling, LMRGs can also affect the mother-fetal blood flow. This can result in variations in immune cell infiltration and immune score in uterine endometrial clusters. According to studies [26], endometrial microbiomes with high Th1 abundance exhibit noticeably elevated activity in numerous key metabolic pathways. As a result, aberrant endometrial microbiota activity may regulate the Th1/Th2 conversion by interfering with processes related to the metabolism of carbohydrates and/or lipids. Lipopolysaccharides (LPS) have the ability to cause a decrease in Treg counts while increasing Th17 and Th1 counts. Patients with high Th17 abundance also have endometrial microbiota that is highly active in pathways related to LPS generation. This suggests that comparable mechanisms could be responsible for the imbalance in the

quantity and composition of immune cells in the endometrium [36]. Using targeted techniques, Kasvandik S and colleagues [37] discovered that 21 proteins had similar levels between the control group's early endometrium and the RIF group's mid-secretory endometrium, indicating a displacement of the RIF group's window of implantation (WOI). This further demonstrated that protein detection could better assess ER. In 2020, 3,158 proteins were detected from the endometrial fluid of six healthy women of childbearing age during the secretory phase. 367 of these underwent significant proteomic changes during the early-to-mid-secretory transition ($P<0.05$). The lipidomic properties of endometrial fluid can be used to precisely determine when to undergo WOI, thereby lowering the incidence of repeated implantation failures. This was demonstrated in a case-control study conducted in 2019 that collected endometrial fluid samples from 41 patients undergoing full-freeze cycle treatment. The lipid components in the endometrial fluid were analyzed, and a ROC curve was constructed with an area under the curve of 84% [38].

6. Uterine microbiota and CE

Persistent endometritis known as CE is brought on by pathogenic bacteria that have established a dominant position and multiplied inside the uterus. According to research, the prevalence of chronic endometritis (CE) varies from 2.8% to 56.8% in infertile patients [39] and from 14% to 67.5% in reproductively isolated individuals (RIF) [40]. The inside of the uterus is examined with hysteroscopy, and endometrial samples are obtained during this procedure. Pathological diagnosis with an endometrial biopsy remains the current clinical gold standard for detecting CE. Consequently, to increase the diagnostic rate of CE, CD138/CD38 IHC staining is utilized in modern clinical practice. In order to examine the endometrial microbiome, Liu [41] and colleagues sequenced the 16S ribosomal RNA (16S rRNA) gene of CE and non-CE samples. According to the study, lactobacilli had a median relative abundance of 1.89% and 80.7% in the microbiota of CE and non-CE, respectively. The CE microbiota contained two non-Lactobacillus groups, namely Bifidobacterium, Gardnerella, Prevotella, and anaerobic cocci; of these, the relative abundance of Lactobacillus was negatively correlated with that of anaerobic cocci and Gardnerella. The abundance of Fragilis Lactobacillus was lower in the CE microbiota. Lozano[42] and colleagues discovered that patients with CE had a more diverse uterine microbiota, particularly with regard to Gardnerella and Pseudomonas. According to research by Chen P. [33], corynebacterium and sphingomonas are active in pathways linked to the metabolism of carbohydrates and/or fats, and their infiltration is considerably higher in CE patients than in NCE patients. According to research, the primary reason why CE affects ER is that it shifts or eliminates the implantation window period, which prevents the endometrium from exhibiting its best receptive state at a certain moment and results in unsuccessful embryo implantation. Furthermore, as noted in reference [43], the process of CE development entails a significant influx of inflammatory cells, leading to anomalies in the subsets of endometrial immune cells and impairment of the uterine immunological milieu. We conclude that alterations in the uterine microbiota may cause CE, and that CE impacts the ER, ultimately resulting in the failure of embryo implantation.

Apart from CE, studies conducted by Luan Zonghui et al. [44] discovered that alterations in the uterine microbiota may also raise the risk of embryonic arrest. According to studies by Fu [28] and others, RIF patients have richer uterine microbiota and lower levels of benzoxazinone, which has an impact on lipid metabolism and embryo implantation. According to prospective cohort research [45], losses with normal chromosome karyotype and subsequent preterm pregnancy are linked to an increase in Ureaplasma species in the uterine microbiome of women who have recurrent spontaneous abortion (RPL). According to a retrospective study [46], Acinetobacter is less common than Corynebacterium, Bacillus, Pseudomonas, and Actinomyces as the predominant bacteria in intrauterine adhesions (IUA). Amplification sequencing of endometriosis (EMs) lesions provides a microbial spectrum similar to the endometrium, with higher contents of Lactobacillus, Gardnerella, Streptococcus, and Prevotella, according to research by Hernandes [47] and others. However, in deep endometrial ectopic lesions, there is less Lactobacillus and more Enterococcus and Pseudomonas. In conclusion, the uterine microbiota has a direct impact on the health of female reproduction and can cause a reduction in endometrial receptivity, which in turn causes PRL, IUA, and EMs to be produced (Figure 3).

7. Antibiotics

One popular tactic for controlling the uterine microbiota is antibiotic therapy. Antibiotics have the ability to stop harmful bacteria from growing in the uterus and help the uterus's microecological balance return. Pre-embryo implantation antibiotic treatment in CE patients significantly improved pregnancy outcomes [48,49], indicating that the presence of certain microbiota may partially account for the negative impact of CE on reproductive outcomes. Numerous studies also show that the use of antibiotics in CE patients has improved fertility outcomes when compared to CE patients who did not receive antibiotic treatment. But the uterine microbiota can be detected technically, and individual characteristics like age, ethnicity, hormones, and contaminated sample collection might affect the microbiota. Hormonal variations can also affect the presence of antimicrobial peptides [50]. Patients undergoing antibiotic treatment (oral levofloxacin 500 mg + tinidazole 1000 mg, for 14 days) had a higher cure rate of CE than those who did not get antibiotic treatment, according to a prospective single-blind randomized controlled experiment [51]. Retrospective investigation of 640 FET cycles by Xiong [53] and colleagues revealed no adverse effect on pregnancy outcomes from endometrial CD138+/HPF ≤4. For women with CD138+/HPF ≥5, antibiotic therapy

Fig. 3. Management of unbalanced uterine microbiota.

is a useful strategy for improving reproductive outcomes. Studies reveal [53] that following antibiotic therapy, there was a significant drop in the proportion of CD 68+ macrophages, CD 83+ mature dendritic cells, CD 8+ T cells, and Foxp 3+ regulatory T cells in the endometrium of patients who had received CE. CE can promote endometrial receptivity during antibiotic therapy because it is clear that the high concentration of immune cells in the endometrium of these patients may be linked to impaired endometrial receptivity and repeated pregnancy failure. The uterine microbiota's diversity and function may be negatively impacted by the overuse of antibiotics, which calls for caution in their use. On the other hand, because antibiotic abuse can result in the emergence of drug-resistant strains, antibiotic selection may be influenced by the accuracy of diagnosis and treatment.

8. Immune system modulation of balance

Enhancing immune system performance is another important tactic for controlling uterine microbiota, as demonstrated by the processes of embryo implantation failure brought on by uterine microbiota. Enhancing immunological response can raise rates of embryo implantation and endometrial receptivity. Sustaining optimal immune system performance can aid in eliminating harmful bacteria from the uterus and preserve a balanced population of uterine microbiota. This can be accomplished by adopting new lifestyle practices that strengthen the immune system, such as stress management, a balanced diet, and moderate exercise. In contrast, preserving a healthy uterine environment is crucial for controlling the balance of uterine microbiota. It also helps to maintain proper pH, oxygen levels, temperature, and the prevention or quick treatment of uterine infections. Finally, the impact of unique genetic variables must also be taken into account. To obtain more exact regulation of the uterine microbiota, populations with particular genetic susceptibility may require individualized regulation measures, such as genetic testing and genetic counseling.

9. Probiotics/prebiotics

These two kinds of medicines are frequently used to treat dysbiosis of the gut microbiota, which is primarily caused by Lactobacillus/Bifidobacterium. Numerous studies also suggest that Lactobacillus is the predominant group in the microbiota of the uterus. Probiotics and prebiotics have the ability to alter the uterine microbiota, create helpful compounds, and stop the growth of harmful bacteria [54]. Nevertheless, studies by Kyono K and colleagues [24] show that although the uterine microbiota of IVF patients successfully became Lactobacillus-dominant following treatment with antibiotics and prebiotics/probiotics, the pregnancy rate did not significantly increase.

10. Microbiota transplantation

Recurrent Clostridium difficile infection (CDI) is identified to be treated with fecal microbiota transplantation (FMT), which is more frequently utilized in clinical practice. Depending on the formulation, it can be given orally, by nasogastric tube, colonoscopy, or enema to alter the microbiota to a favorable one [55,56]. Rats' uterine environment can be improved by vaginal microbiota transplantation, according to research by Wang J and colleagues [57].

11. Synopsis and prospects

Maintaining endometrial health and encouraging the implantation of fertilized eggs are two major functions of the uterine microbiome. Endometriosis, recurrent miscarriages, infertility, and other gynecological disorders may arise and progress in correlation with dysbiosis of the uterine microbiota. An imbalance in the microbiota of the uterus can cause the uterine cavity to become more inflamed, which can alter the endometrium's shape and function and lessen its receptivity to fertilized eggs [14]. As a result, studies examining the connection between endometrial receptivity and uterine bacteria are crucial for clinical practice. Gaining more insight into the mechanisms underlying the relationship between endometrial receptivity and uterine microbiota can lead to innovative approaches to the diagnosis, treatment, and care of infertile individuals. Maintaining the equilibrium of the endometrial microbiota may enhance overall health.

Conflict of interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed consent

The authors declare that no patients were used in this study.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

Yuhong Li and Qiuping Li: Conceptualization, methodology, writing original draft preparation. Dandan Chen and Wei Mao: Investigation, software, statistical analysis. Yun Zhang: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript.

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