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Research progress of endometrial microecology in infertile women

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Abstract: *There are many causes of infertility, some of which are unknown, which bring difficulties to the diagnosis and treatment of infertility.With the further deepening of human understanding of microecology and the development of 16s rRNA gene sequencing and other technologies, it is gradually recognized that the endometrium is not a sterile environment, and there is a correlation between microecological imbalance and infertility. The endometrial microbiome may have a previously unrecognized role in uterine physiology and human reproduction. The analysis of endometrial microbiota is helpful to find the risk factors of reproductive tract diseases and provide guidance and help for the treatment of infertility. This article mainly reviews the research progress of female endometrial microecology in recent years, in order to improve the treatment methods and clinical outcomes of infertility.*

Keywords: *Infertility, Endometrial microecology, 16s rRNA, Chronic endometritis*

The human body is composed of a complex microbial community. There are a large number of microorganisms in the body and a wide variety [1]. Normal flora is in a symbiotic state with the human body and establishes a close relationship with the human body, which plays a very important role in promoting the improvement of human physiological functions. Human microbial colonization is mainly located in four sites (skin, vagina, oral cavity and intestine) [2]. With the continuous development of basic research, the microbiome of the female reproductive tract has attracted more and more attention. The female reproductive tract microecosystem is a unique ecosystem composed of a variety of microbiota. Under the influence of systemic immune status, endocrine, sexual life and other factors, the composition of female reproductive tract microecology is also changing, and the imbalance of bacterial flora may have an impact on fertility and reproductive health [3-4].

The uterus is an important place for life, and uterine microecological disorders can directly affect female reproductive ability [5]. In recent years, the number of infertility in China has been increasing year by year, and its pathogenesis is complex and its etiology is diverse. The imbalance of female reproductive tract flora and pathogenic microbial infection play an important role in the occurrence and development of infertility [6]. The risk factors of reproductive tract diseases can be identified by detecting and evaluating the endometrial microecology in healthy or disease states. This article reviews the correlation between endometrial microecology and infertility, so as to provide guidance and help for the treatment of infertility.

1. Female endometrial microbiome

Most of the early scholars believed that the healthy uterine cavity was a sterile environment. The cervical mucus plug acts as a barrier to prevent vaginal microorganisms from entering the uterine cavity. With the development of technology, people began to realize that the uterine cavity is not a sterile environment. In 2015, a study by the University of Washington [7] sampled the reproductive tract of 58 enrolled women (all of whom underwent hysterectomy for benign diseases: 34% for bleeding, 26% for fibroids, and 29% for pain) and performed quantitative PCR. The resulting data suggest that the endometrial cavity is not completely sterile in 95% of women undergoing hysterectomies for benign disease.Although the concentration of bacteria in the uterine cavity is significantly lower than that in the vagina, the presence of low levels of bacteria in the uterus appears to be common rather than pathologic. In 2016, Walther-Antonio et al. [8] performed hysterectomy in 17 patients with benign lesions (abnormal uterine bleeding, fibroids, uterine prolapse, pelvic pain), 4 patients with endometrial

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hyperplasia (with or without dysplasia), and 17 patients with endometrial cancer (endometrioid carcinoma, mucous, serous, squamous cell carcinoma, and sarcoma). The microbiome was examined in samples taken at different locations along the reproductive tract. The microbiome of all female reproductive organs was significantly correlated($p<0.001$), Structural microbiome shifts are present in cancer and hyperplasia cases.Porphyromonas combined with high vaginal pH(>4.5) were statistically associated with the presence of endometrial cancer. There are microbiota in the uterine cavity of both benign and malignant uterine diseases. In the future, it is necessary to continue to explore the endometrial microecology in healthy or diseased states.

Belgian researchers [9] studied the endometrial microbiome of 19 non-pregnant women by 16s rRNA gene sequencing. Of 183 unique 16s rRNA gene amplicons, 15 phylotypes were present in all samples. This reconfirms the presence of microbiota in the female upper reproductive tract system including the uterus, fallotubes, and ovaries, and the composition of the microbiota is quite similar in about 90% of the included women, characterized by a limited number of specific phylotypes consistently present in quite similar distributions, which are assumed to be the uterine core microbiome. Bacteroides may play a role in the uterine endometrial flora of non-pregnant women. In 2017, Chen et al. [10] sampled the microbiota from the reproductive tract of 110 women of childbearing age, and studied the nature of colonization by 16s rRNA gene amplicon sequencing and culture. Culture and 16s rRNA gene sequencing revealed the existence of distinct bacterial communities throughout the female reproductive tract, forming a continuous change of microbial communities from vagina to ovary, and being associated with adenomyosis, uterine fibroids, endometriosis, fallopian tube obstruction and other gynecological diseases. Studies have shown the importance of endometrial microecology in clinical diagnosis and treatment.

2. Endometrial microecology of infertile women

The uterine cavity of women is not sterile, and there is a microbiome in the uterine cavity. What are the characteristics of the uterine microbiota in infertile women? Inflammation in the vagina may vertically infect the upper reproductive tract, causing uterine inflammation and leading to infertility. In 2017, a comparison of vaginal flora and the incidence of asymptomatic vaginopathy between healthy women and infertile women of reproductive age in India was conducted. The results of a study [11] showed that the destruction of vaginal ecosystem would change the flora of healthy vagina, change the pH value, and easily cause lower reproductive tract infection. The change of female reproductive tract flora by pathogenic organisms can rise from the vagina to the upper part of the reproductive tract and cause infertility. Compared with healthy women, women with infertility problems had a relatively lower content of lactic acid bacteria in the vaginal flora. Candida and bacterial vaginosis associated bacteria are significantly elevated in the vagina of women with infertility problems. Asymptomatic vaginosis is more common in women with infertility problems than in healthy women.

In 2018, a study [12] studied the reproductive tract microbiota of women with a history of childbearing and women with a history of infertility, collected vaginal, cervical and endometrial samples, and detected the microbiota by 16s rRNA gene amplification and quantitative reverse transcription polymerase chain reaction. The results showed that the vaginal and cervical microbiota were basically the same, but the endometrial microbiota was significantly different and the proportion was different. More Mycoplasma were detected in the vagina and Gardnerella were detected in the cervix in infertile women. It is speculated that the endometrial microbiome may be an important factor in unexplained infertility or repeated IVF cycle failure.

3. Endometrial microecology and assisted reproductive technology (ART)

The success rate of embryo transfer (ET) is affected by many factors, including age, embryo quality, endometrial receptivity, reproductive tract microecology, and transfer technology. Abnormalities in the vaginal microbiome may reduce pregnancy rates in patients undergoing in vitro fertilization (IVF). The study of reproductive tract microecology can provide new ideas for the diagnosis and treatment of infertility and recurrent ART failure.

In the process of in vitro fertilization-embryo transfer (IVF-ET), the embryo needs to be implanted into the uterine cavity using a catheter, and the catheter needs to pass through the cervix. The microorganisms in the cervix will be contaminated through the tip of the embryo transfer catheter, so there is the possibility of bacterial contamination during embryo transfer. There is increasing evidence

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that bacterial contamination of the uterine cavity after transcervical embryo transfer negatively affects implantation rates and pregnancy outcomes. Genital infections, particularly those caused by sexually transmitted microorganisms, are the most common cause of infertility. Fanchin et al. [13] studied the influence of cervical microbial flora on the outcome of in vitro fertilization pregnancy during embryo transfer. Among 279 patients, 143 bacterial cultures were positive, mainly Escherichia coli and Streptococcus. The comparison found that the implantation rate, clinical pregnancy rate and ongoing pregnancy rate of the positive bacterial culture group were significantly lower than those of the negative group, indicating that the cervical bacteria of the graft can affect the clinical outcome. Selman et al. [14] also conducted a similar study in 2007 to determine whether bacterial contamination during embryo transfer affected the pregnancy outcome of assisted reproductive technology. During embryo transfer, samples were collected from the vaginal floor, cervix, embryo culture medium before and after embryo transfer, catheter tip, and outer piece sites for microbiological examination. Of the 152 study patients, 133 (87.5%) tested positive for one or more organisms, and the remaining 19 (12.5%) tested completely negative for bacterial contamination. Although pregnancy rates were similar in the two groups, there was a significant difference in implantation rates between patients positive for one or more bacteria and those completely negative ($p \leq 0.001$)(12.4% vs 14%). The miscarriage rate was also higher in positive patients compared with complete negative patients. In vitro fertilization-embryo transfer(IVF)women positive for endocervical Enterobacteriaceae and staphylococci have reduced pregnancy rates and increased miscarriage rates. It is suggested that bacterial contamination during embryo transfer may affect pregnancy outcome.

Is a completely sterile environment more conducive to pregnancy? In 2005, some scholars [15] conducted an experiment to compare the pregnancy rate of germ-free mice after embryo transfer in a germ-free environment with that of conventional-bred mice. The results showed that the pregnancy rate of germ-free mice was lower, suggesting that the endometrial microbiota had a certain role in embryo implantation. A 2016 study [16] included a total of 33 patients, all of whom underwent embryo transfer (18 were pregnant and 15 were not). The most distal 5-mm portion of the graft catheter was aseptically placed in a DNA PCR tube. Sequencing of bacteria-specific 16s rRNA revealed that the endometrial microbiome was dominated by Lactobacillus, with variable proportions, regardless of pregnancy. An important aspect of embryo implantation may be influenced by the vaginal and uterine microbiota, namely the immune and cytokine milieu during conception. Several cytokines are involved in endometrial receptivity and embryonic development and are affected by infection and inflammation of microecological abnormalities.

Moreno et al. [17] conducted a study on the effect of endometrial microbiota on the success or failure of implantation. They sampled the uterine cavity and vagina of subjects and analyzed the microbiota. Different colonies were found, which could be classified into the microbial flora (>90% of Lactobacillus species) and non-Lactobacillus species (>90% of the Lactobacillus genus and >10% of other bacteria). The reproductive microbiome is not cyclically regulated by hormones, and the number of Lactobacillus is significantly associated with pregnancy outcomes, The increased number of non-Lactobacillus decreased the implantation rate (60.7% vs. 23.1%), pregnancy rate (70.6% vs. 33.3%), ongoing pregnancy rate (58.8% vs. 13.3%) and delivery rate (58.8% vs. 6.7%). The adverse effect of the number of non-lactobacilli in the endometrium on endometrial function is considered to be a possible cause of implantation failure and miscarriage. In 2018, Benner et al. [18] suggested that the uterine microbiome may influence the subsets of immune cells required for implantation, and the microbiome is also crucial in preventing uterine infection by competing with pathogens. The complex interactions of cells during healthy pregnancy are still poorly understood, and a healthy endometrial state, including the local immune environment, is critical not only for fertility but also for placenta formation, the initiation of which is highly dependent on interactions with immune cells. In the future, more in-depth studies are needed to consider the uterine microbiome as a related factor.

4. Endometrial inflammation and female infertility

Endometrial inflammation is a very common disease in reproductive system infectious diseases. chronic endometritis (CE) is the most prominent situation of infertility caused by endometrial microbial abnormalities. At present, itis believed that pathogen infection in the uterine cavity is the main pathogenic factor, and its pathogens mainly include: Streptococcus, Escherichia coli, Enterococcus, Staphylococcus, Mycoplasma, Proteus, Klebsiella pneumoniae, Pseudomonas aeruginosa, yeast, Gardnerella, etc. [19-20].

In 2005, some scholars [21] studied the implantation related cytokines and chemokines in the

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endometrium, and the results showed that cytokines were involved in the complex process of embryo implantation and placenta formation. Interference with the expression or action of these cytokines results in complete or partial failure of embryo implantation and abnormal placentation. Chemokines play a role in the apposition and adhesion stages of implantation. Cytokines and chemokines, their expression patterns in the endometrium are consistent with the potential role of implantation and the placenta. In 2013, Pietro et al. [22] compared the expression profiles of 25 genes encoding proteins related to the inflammatory response, proliferation and apoptosis of the endometrium at the implantation stage by high-throughput real-time RT-PCR in 16 women with chronic endometritis diagnosed by hysteroscopy and histology and 10 healthy women as controls. The results showed that the expression of some genes in the endometrium of CE patients was significantly altered. Among them, the expression of genes including interleukin-11 (IL-11), a multi-functional cytokine secreted by endometrial cells, can promote embryo implantation and have a positive significance in the maintenance of pregnancy. The decreased secretion of IL-11 may cause a series of signal transduction disorders, which may lead to the decreased invasion ability of trophoblast cells and induce embryo implantation failure.

Does antibiotic treatment of endometritis improve pregnancy outcomes in women with a history of recurrent implantation failure (RIF)? In 2015, Cicinelli et al. [23] conducted a retrospective study on 106 women with unexplained infertility and RIF history; all patients underwent hysteroscopy and endometrial sampling for histological and microbiological investigation. Women diagnosed with CE were treated with antibiotics and the effect of treatment was confirmed by hysteroscopy and biopsy. All women underwent further IVF attempts within 6 months of treatment. CE was diagnosed at hysteroscopy in 70 (66.0%) women. Sixty-one cases (57.5%) were confirmed as CE by histology, and 48 cases (45.0%) were confirmed as CE by culture. Common bacteria and mycoplasma were the most common pathogens. Of the 61 women diagnosed with CE by hysteroscopy and histology, 46 (75.4%) were normal on examination after control with appropriate antibiotic therapy, whereas 15 (24.6%) cases still had signs of CE. When IVF was attempted after treatment, results showed that the clinical pregnancy rate and live birth rate were significantly higher in the post-treatment normal group than in the persistent infection group. Studies have shown that antibiotic treatment of endometritis can significantly increase clinical pregnancy rates and improve pregnancy outcomes. In 2017, some scholars [24] studied the prevalence of chronic endometritis (CE)in infertile women with a history of repeated implantation failure (RIF) and determined whether oral antibiotic treatment could improve the live birth rate in embryo transfer (ET) cycles. The results showed that about one-third of women with RIF infertility were diagnosed with CE. The results also show that oral antibiotic treatment of CE is effective in improving reproductive outcomes in infertile women with a history of RIF.

5. Conclusion

With the development of the Human Microbiome Project and the development of scientific detection technology, the methods for studying microbiome are more and more advanced. 16S rRNA genome and metagenome based sequencing technology is a rapid, sensitive and specific method for microbial identification. The application of microbial identification, diversity analysis and microbiome related research will be more extensive [25]. The understanding of the microbial community of the female reproductive tract has been gradually deepened and paid more attention to, which is expected to bring more progress to the pathogenesis of infertility. However, at present, the research on endometrial microecology is still in its infancy, and the causal relationship between reproductive tract microbial imbalance and infertility has not been established. It is necessary to continue to study and explore the correlation between the changes of endometrial microecology and the pathogenesis of infertility and the specific pathogenesis, and to further study the relationship between the characteristics of endometrial microecology and infertility. It will ultimately help to improve the treatment of infertility and clinical pregnancy outcomes.

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