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# Recent Advances in Treatment of Recurrent Spontaneous Abortion

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**Importance:** Recurrent spontaneous abortion (RSA) is a distressing condition experienced by approximately 1% of women trying to conceive. However, the treatment of RSA is a challenge both for clinicians and patients.

**Objective:** The aim of this review is to discuss the medical and surgical approach to the management of RSA, including those caused by anatomical, genetic, male, infectious, endocrine, and immune factors.

**Evidence Acquisition:** A literature search using MeSH terms for each topic was undertaken using PubMed, supplemented by hand searching for additional references. Retrieved articles were reviewed, synthesized, and summarized.

**Results:** Available treatments target hypothetical risk factors for RSA, although the effectiveness of many treatment options is controversial. Intervention should depend on the benefit-to-risk ratio of the proposed treatment.

**Conclusions and Relevance:** The etiology of RSA is heterogeneous, and patients often lack specific clinical manifestations, which has hindered the progress in predicting and preventing RSA to some extent. Despite intensive workup, at least 50% of couples do not have a clear underlying pathology. In addition, an evidence-based treatment is not available in most patients even if abnormal test results are present. Many new treatment directions are also still actively exploring; empirical and combined multiple treatments are still the main methods.

**Target Audience:** Obstetricians and gynecologists, family physicians.

**Learning Objectives:** After completing this activity, the learner should be better able to describe common risk factors for RSA; formulate individualized treatment plans to improve pregnancy outcomes; and propose supportive treatment recommendations for patients with unclear causes.

Recurrent spontaneous abortion (RSA) is defined as 3 or more consecutive pregnancy losses within 28 weeks of gestation with the same sexual partner. However, it is currently believed that patients who have 2 consecutive miscarriages have similar risk of recurrent miscarriage to those with 3 consecutive miscarriages; the European Society of Human Reproduction and Embryology manifests that 2 or more miscarriages should be considered for diagnosis of RSA,[1] and the American Society for Reproductive Medicine defines RSA as 2 or more pregnancy losses prior to the 20th week of gestation.[2] The incidence of RSA is 1% to 2% in all couples trying to conceive.[1] In approximately 50% of RSAs, the underlying causes remain unknown, and 80% of the unexplained abortions are closely related to immune factors.[3] Among the known causes, maternal immunological factors (including autoimmunity and allogeneic immunity), thrombus-prone factors (including genetic and acquired thrombus-proneness), anatomic anomalies of the uterus, and endocrine abnormalities are the 4 most important

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causes. Embryonic chromosomal abnormalities are still a common cause of RSA; however, parental chromosomal abnormalities accounted for only a small part of the etiology of RSA. In addition, the association of infectious factors and male factors with RSA is controversial. This article mainly focuses on the latest related research, combined with relevant guidelines, to give a review of the treatment progress of RSA from the aspects of anatomic, genetic, male, endocrine, infection, and immune factors. The focus of this review is on RSA, but many mechanisms underlying RSA also affect fertility. In such instances, we include discussions of infertility.

## ANATOMICAL FACTORS

Anatomical abnormalities of the uterus are thought to be related to recurrent miscarriages in the first and second trimesters.[4] Relevant uterine congenital abnormalities patients accounted for 8.4% to 12.6% in RSA patients,[5] including unicornuate, didelphys, bicornuate, arcuate, septate uterus, and so on, among which the septate uterus is the most common, accounting for 44.3% of all uterine malformations.[5] Acquired uterine abnormalities include Asherman syndrome, cervical insufficiency, uterine fibroids, and so on. Here we review the treatment of septate uterus, Asherman syndrome, thin endometrium, and cervical insufficiency.

### Septate Uterus

Septate uterus is caused by insufficient resorption of the müllerian ducts during intrauterine development, and the mechanism leading to RSA may be related to the reduction of the uterine cavity volume and insufficient blood supply.[4] Hysteroscopic surgery is the traditional surgical approach to remove the uterine septum, and hysteroscopic septum resection is currently considered the first-line treatment. However, there is still controversy regarding whether the correction of anatomical abnormalities can improve the pregnancy outcome of RSA. Furthermore, hysteroscopic septum resection may also cause complications such as bleeding, uterine perforation, postoperative intrauterine adhesions (IUAs), and subsequent uterine rupture.

Although no evidence from randomized controlled trial (RCT) supports the surgical procedure at present, data from the Cochrane Database of Systematic Reviews demonstrate that various retrospective research reports have shown that the live birth rate of women with septate uterus after hysteroscopic septum resection has been significantly improved.[6] Septate uterus is also the most common congenital uterine malformation in infertile women; a meta-analysis of reproductive outcomes after hysteroscopic metroplasty showed that the overall pregnancy rate was 63.5% (95% confidence interval [CI], 56.6–69.9), and the live birth rate was 50.2% (95% CI, 43.4–57.1) in women with miscarriage or infertility.[7] Furthermore, in terms of postoperative complications, a multivariate analysis of a cohort study showed that age is an independent risk factor for persistent infertility after surgery in women with RSA.[8] Therefore, surgery performed as early as possible can reduce the risk of secondary infertility. According to data from previous research we reviewed, for RSA women with septate uterus, hysteroscopic septum resection or recently launched hysteroscopic metroplasty should be considered, although there is a risk of complications.

### Uterine Adhesions

Intrauterine adhesion, also known as Asherman syndrome, often occurs when the basal layer of the endometrium is damaged by infection, surgery, genetics, and other factors, resulting in uterine cavity partial or complete obliteration due to fibrous adhesions. Intrauterine adhesion usually leads to repeated miscarriages, infertility, and menstrual abnormalities. At present, hysteroscopic adhesiolysis is the main means of treating IUA; however, almost two-thirds of patients with severe adhesions will relapse after simple adhesiolysis.[9] Therefore, intrauterine devices, intrauterine balloon stent, Foley catheter, hyaluronic acid, and other antiadhesion barriers have been used to prevent recurrent adhesion through separating the anterior and posterior uterine walls, and hormones have been used to promote endometrial regeneration. However, the effect of estrogen on IUA is not definitive, and long-term, high-dose use of estrogen will increase the risk of breast and endometrial tumors.[10] In the past decade, some research on repairing damaged endometrium by infusion of different types of stem cells and bone marrow derivatives has been carried out, and gratifying results have been achieved in animal models and humans.[11] A recent experiment showed that eupatilin can markedly reduce the fibrotic activity of Ishikawa cell endometrial fibrosis induced by transforming growth factor β in vitro.[12]

### Thin Endometrium

The definition and cutoff for thin endometrium differ between studies, but most studies define thin endometrium as the thickness of the endometrium less than 7 or 8 mm on the day of human chorionic gonadotropin administration.[13] The cause of thin endometrium is similar to that of IUA, and the treatment also has a lot in common with IUA postoperative infusion therapy. At present, more attention is paid to the studies of platelet-rich plasma (PRP), granulocyte-macrophage colony-stimulating

factor (G-CSF), and stem cell infusion therapy, and significant advances have been made in the restoration and regeneration of the endometrium. Among them, G-CSF and mesenchymal stem cells have been confirmed in recent years that their mechanism of treating and preventing RSA not only includes repairing damaged endometrium and promoting angiogenesis, but also regulating the immune balance of the maternal-fetal interface, so they also play a role in immune-related RSA. Santamaria et al[14] transplanted bone marrow–derived stem cells into uterine spiral artery by catheter. After treatment, the endometrium of 16 patients with IUA and endometrial atrophy was thickened, and 15 patients resumed menstruation, among which 2 patients successfully gave birth to full-term infants. For G-CSF, there are few studies using it to treat RSA patients with thin endometrium, and most studies focus on the treatment of infertility caused by thin endometrium and unexplained abortion. Clinical trials of stem cell therapy are mainly focused on its efficiency in IUA. Platelet-rich plasma is often used in women with thin endometrium in assisted reproduction. A meta-analysis using a random-effects model (N = 625) showed that compared with the control group, the endometrial thickness in the PRP group increased significantly after intervention (standard mean difference, 1.79; 95% CI, 1.13–2.44; $P < 0.001$, $I^2 = 64\%$), and the probability of chemical pregnancy and clinical pregnancy and implantation rate were significantly higher. However, there was no difference between the PRP group and the control group regarding miscarriage (relative risk [RR], 0.72; 95% CI, 0.27–1.93; $P = 0.51$, $I^2 = 0\%$).[15] Therefore, more clinical trials are needed to identify whether they can increase the live birth rate for RSA patients with thin endometrium.

### Cervical Insufficiency

Cervical insufficiency often manifests as painless uterine orifice dilation, leading to the delivery of the fetus in middle and third trimesters without contractions or initiation of labor. Congenital cervical insufficiency is rare, and risk factors for acquired cervical insufficiency include mechanical cervical injury, cervical conization, extensive cervical resection, and so on. At present, most guidelines agree that transvaginal cervical cerclage should be administered in patients diagnosed as cervical insufficiency, and transabdominal cerclage can be considered for those who fail. Patients with RSA who are at risk of cervical insufficiency should undergo regular cervical monitoring from the second trimester. For those with painless cervical dilatation during pregnancy, emergency cervical cerclage should be performed to maximize the gestational week as much as possible. Meanwhile, for patients with advanced cervical dilation (>4 cm) and membrane protrusion in early second trimester, Uzun Cilingir et al[16] show that emergency cervical cerclage is not a reasonable choice because of its short extension time and high incidence of extreme prematurity and chorioamnionitis. Although cervical cerclage has been used for many years, the benefits of different groups of people are still controversial, and some scholars questioned the over-operation of cervical cerclage. A meta-analysis (N = 769) showed that in women with a singleton gestation, previous spontaneous preterm birth, and a second-trimester sonographic short cervix, there was no statistically significant difference between vaginal progesterone and cerclage in reducing preterm birth and improving perinatal outcomes.[17] Therefore, for RSA patients with cervical insufficiency, it is necessary for clinicians to formulate individualized treatment plans according to the specific conditions of the patients.

## GENETIC FACTORS

Genetic factors include chromosomal abnormalities in couples and embryos. Embryonic chromosome abnormality is a common cause in early RSA, which usually increases with maternal age, and the detection rate of embryonic abnormal chromosome in women older than 35 years is as high as 78%.[18] Analysis of miscarriage specimens has revealed that 55.1% of early miscarriages are due to chromosomal abnormalities, including 40% with aneuploidy, 7.5% with polyploidy, 3.6% with partial aneuploidy, 2.2% with pathogenic microdeletion/microduplication, and 1.9% with uniparental isodisomy, and 2.8% obtained variants of uncertain significance.[19]

Aneuploidy is the main chromosomal abnormality; therefore, for RSA patients with previous embryonic chromosomal abnormalities, preimplantation genetic testing for aneuploidy (PGT-A) technology is often used for assisted reproduction. Lee et al[20] reported that PGT-A can improve live birth rates in in vitro fertilization cycles for high prevalence of aneuploidy patients with advanced maternal age, repeated implantation failure, recurrent miscarriage, and oocyte donors. For the recurrent miscarriage group, a significant increase in live birth rate was found in the PGT-A group compared with the non–PGT-A group (55.9% vs 32.8%). Whereas previous studies on PGT-A were confined to RSA couples with advanced maternal age, some clinical studies argued that PGT-A could not improve pregnancy outcomes in patients with RSA. Currently, a multicenter RCT of the clinical benefits of comprehensive chromosome screening in the treatment of RSA patients is underway[21]; the experimental results will help us to further understand the subpopulation that would most benefit from PGT-A.

In addition, using trophoblastic ectoderm (TE) biopsy for PGT-A has potential safety hazards, the range of embryos suitable for biopsy is limited, and its economic effectiveness in increasing live births of RSA patients has also been controversial. In recent years, noninvasive PGT-A (niPGT-A) based on sequencing DNA released from TE and inner cell mass into the culture medium may provide solutions to these problems. Moreover, a recent study showed that the positive predictive value and specificity of niPGT-A were much higher than TE biopsy PGT-A. The consistency of embryo ploidy and chromosome copy number of niPGT-A were higher than those of TE biopsy PGT-A, too.[22] This suggests that niPGT-A and cost-effective PGT-A may be a better test in clinical in vitro fertilization, but its reliability and sensitivity in RSA patients with chromosomal abnormalities still need to be confirmed by a larger sample size study.

## MALE FACTORS

The data show that 50% of recurrent miscarriage causes still cannot be shown, among which the male factor is an important aspect. Current studies have made it clearer that the main causes of male factors include severe sperm deformity, sperm DNA damage, and men's own chromosome abnormality.[23] Although some patients are still unable to determine the cause after a series of tests, studies suggest that avoiding certain adverse factors can benefit some patients, access to fertility. The direct causes of these sperm quality problems are varicocele, sperm membrane lipid peroxidation, and abnormal oxidative stress.

### Varicocele

It is generally believed that sperm motility is significantly reduced after DNA damage in the sperm nucleus, and the sperm is still fertile, but it is prone to recurrent miscarriage, placental abnormalities, and other adverse outcomes. However, approximately 20% of patients with varicocele have poor sperm quality, so the choice of treatment for varicose veins needs to be individualized. At the moment, the treatment of varicocele is focused on surgical interventions, whereas pharmacotherapy is limited to specific conditions or as an adjuvant therapy.

Surgical intervention for varicocele is mostly varicocelectomy, but the treatment effect of the operation is controversial. As far as the operative methods are concerned, they are divided into the traditional open operation, laparoscopic varicocele ligation, and the microscopic subinguinal approach. Compared with laparoscopic high ligation of spermatic cord, the recurrence rate and hydrocele formation rate of spermatic cord after microsurgical ligation of spermatic vein are lower; pregnancy rate and sperm quality were better improved[24]; both improved hormone levels and erectile function equally, but laparoscopic high ligation of spermatic cord lasted less time and was better for children or patients with low surgical tolerance. Interventional and Leonardo's robot procedures are often used as an alternative surgical treatment because of their short duration and low recurrence rate.

In these major surgical procedures, the increased use of auxiliary means is also beneficial to improve the surgical effect. The use of vascular Doppler ultrasound as an adjunct to surgery shortens the duration of surgery, increases the number of remaining testicular arteries, and optimizes sperm motility to a certain extent after ligation.[25] The research results of Esposito et al[26] indicate that after high ligation of testicular veins and lymph nodes was performed after isosulfonic blue staining, the blocking rate of testicular effusion and pain was higher than that without isosulfonic blue, and preoperative isosulfonic blue injection also can completely eliminate the formation of postoperative hydrocele. Postoperative follow-up is important, and the absence of varicocele and continuous spermatic venous reflux detected by testicular ultrasound can be used as an objective factor for semen parameter improvement.[27] Current studies do not demonstrate a direct link between hormone levels and fertility, although previous studies have shown that patients with high preoperative inhibin B levels have a more significant improvement in fertility than those with low levels, but there is no significant change in follicle-stimulating hormone and testosterone levels after surgery, so the direct relationship between the 2 is still controversial.[28,29] In patients with obstructive azoospermia or severe oligozoospermia who had multiple failed varicose veins, varicocele repair and testicular sperm extraction significantly improved fertility.

Drug therapy is less effective in improving fertility, but the cost-effectiveness value is higher in the early stage of treatment, and it can be used as an auxiliary therapy after operation. Studies have shown that the polysaccharide of *Morinda officinalis* was helpful for the improvement of sperm quality. Levocarnitine can also improve sperm quality and increase conception rates.[30]

In general, it is recommended that adult patients who meet the criteria for surgery should be treated with a gently dissected, artery-preserving varicocele ligation for the first time[31]; laparoscopic high ligation of the spermatic vein with preservation of the testicular arteries and lymph nodes is recommended in children who meet the criteria for laparoscopic high varicocele ligation after the first failure. If conditional, Leonardo's robot operation and vascular Doppler ultrasound-assisted operation should be chosen, and regular postoperative testicular ultrasound should be used to prevent recurrence.

### Oxidative Damage

Abnormal peroxidation not only damages sperm DNA and causes lipid oxidation deformity of the sperm plasma membrane, but also impairs the ability of sperm to absorb stereoscopic structure, accelerates apoptosis, and reduces sperm quality.[32] Research on varicocele patients were also found overproducing reactive oxygen species (ROS), inducing abnormal oxidative stress.[33]

Studies have shown that the body has its own oxidation-antioxidation mechanism, including catalase, glutathione, and other substances that contribute to peroxide. Smoking reduces these antioxidants, and moderate exercise increases their production in the body. Vitamin E supplementation in vitro helps to eliminate ROS, increase the number of sperm, reduce the rate of deformity, and post a protective effect on the sperm. α-Lipoic acid has been demonstrated to reduce the adverse effects of increased testicular temperature and oxidative stress in varicocele rats.[34] In addition, some Chinese herbs containing the antioxidant-polysaccharides can inhibit lipid oxidation and remove ROS; phenylethanoid glycosides have also been shown to repair damaged DNA.[35]

Furthermore, gene expression studies have shown that SOD1, Casp9, ATG7, Casp3, and SIRT1 are enriched in cells that regulate oxidative stress–induced cell death (GO: 1,903,201) and amyotrophic lateral sclerosis (Kegg: 05,014). It can be reasoned that hypoxia plays an important role in the development of varicocele, and the abnormal expression of autophagy-induced and apoptosis-related proteins may be involved in the development of varicocele-related infertility. SOD1, Casp9, ATG7, Casp3, SIRT1, and their modules can be considered as the pivotal genes of varicocele, which provides a new therapeutic target for varicocele.[36]

### Preimplantation Genetic Diagnoses

In men, chromosome abnormality can affect the development of the embryo, leading to RSA. Traditional preimplantation genetic diagnosis (including PGT-A, monogenic/single gene, and structural chromosome rearrangement) can effectively reduce the rate of abortion (odds ratio [OR], 21.79; 95% CI, 1.93–245.52).[37] A new study proposes direct point mutation detection, quantitative analysis of gene copy number, combined with linkage analysis assisted by single-nucleotide polymorphism information from single sperm (or polar bodies), establishing an all-in-one protocol for single embryonic cell preimplantation diagnosis for 2 coexisting genetic conditions (monogenic disease and chromosomal abnormality) on the next-generation sequencing–based platform, which could effectively screen for normal fertilized eggs of couples who also have or carry chromosomal translocation, reducing the risk of miscarriage.[38]

## INFECTIOUS FACTORS

Clinical studies have shown that ureaplasma urealyticum (UU), *Mycoplasma hominis* (MH), and *Chlamydia trachomatis* (CT) are closely related to RSA. *Mycoplasma hominis* and UU can cause maternal and fetal infection, even endometrial inflammation. They act on the embryo, increasing the toxicity to the embryo and resulting in damage to its development. It may also interfere with the regulatory mechanisms of the maternal immune system for the protection of embryos, leading to miscarriage.[39] With the increase in estrogen and progesterone during pregnancy, the female nonpregnant period of infection will make pathogenic changes, resulting in the occurrence of adverse pregnancy outcomes. Rose et al[40] showed that the positive rates of antisperm antibody and antiendometrial antibody were higher in patients infected with CT, MH, and *Neisseria gonorrhoeae*. Also, the pathological changes induced by pathogen infection will destroy the vagina acidic environment and change the balance of vaginal flora, further affecting the outcome of pregnancy.

Most international guidelines currently recommend azithromycin as a first-line treatment, but azithromycin alone can easily damage the liver and cause a series of headaches, nausea, and other adverse effects. Quinolones are recommended as second-line therapeutic drugs, but they are susceptible to produce drug resistance when used alone for a long time. Clinical studies have shown that azithromycin combined with doxycycline is more effective than azithromycin alone, and there was no significant difference in the incidence of adverse reactions between the 2 usages.[41] Sitafloxacin (STFX) is an alternative drug for the treatment of azithromycin-resistant *Mycoplasma genitalium*. In a clinical trial, the eradication rates of STFX on CT, MG, MH, UU, and *Ureaplasma parvum* were 96.1%, 71.4%, 100%, 85.7%, and 100%, respectively; STFX was shown to be useful not only for respiratory therapy but also for urinary tract infection, although its drug resistance required further study and observation.[42]

As a broad-spectrum antiviral drug, interferon can improve inflammation and body resistance, and it does not change the status of vaginal microenvironment, so interferon can be widely used for gynecological vaginal infections.[43] This suggests that a combination of azithromycin and interferon should be considered in the prevention of azithromycin resistance. In another clinical study, 50% of patients treated with metronidazole had a community state type IV (CST-IV) to CST-III, and

the CST was generally unaffected by azithromycin or tetracycline; in another study, after azithromycin treatment, *Lactobacillus iners*–dominated communities were most common, and the presence of the majority of lactic acid bacteria was associated with an increased risk of trachomatis infection, suggesting that the effect of antibiotic treatment on vaginal microbiota may be related to reinfection.[44,45] These experiments explain the need for strategies to protect and restore the best vaginal flora, in combination with the potential interference effects of antibiotics on vaginal flora, when treating *Chlamydia* and *Mycoplasma*, to reduce the impact on the body and the recurrence of infection.

In addition, because of the resistance of *Mycoplasma* and *Chlamydia* to azithromycin and moxifloxacin, some new drugs and treatments are constantly being studied and proposed. Gepotidacin is a new type of triazoleacenaphthene local isomerase inhibitor. It is a broad-spectrum active drug that can block the DNA replication of bacteria. In vitro studies have found that it has a strong anti–*M.* genitalium effect; there was no difference in minimum inhibitory concentration among sensitive strains, macrolide-resistant strains, quinolone-resistant strains, and multidrug resistant isolates, indicating that it had bactericidal effect, and the activity of gepotidacin was not affected by resistance to fluoroquinolones or macrolide. However, further clinical studies are needed to determine the development of drug resistance and potential protective effects of gepotidacin in combination with doxycycline.[46] SiRNA knockout showed that Erk12 and RSK1 were important pathways for *Chlamydia* infection, and the first-class Erk12 inhibitor BVD-523, currently undergoing phase II clinical trials, inhibited *Chlamydia* infection in both cell cultures and mouse models; in other words, kinases in the Erk, RSK pathway may act as host-directed therapy CT.[47] A study[48] pointed out that systemic enzyme therapy can reduce the $T_H2$ cells, thus reducing the imbalance of local inflammatory processes and the formation of immune disorders, improving the effectiveness of antibiotic therapy and reducing the occurrence of adverse effects. The results showed that the clinical and microbiological response rates in CT infection patients were 97.6% > 78.6% with doxycycline and root enzyme combination therapy, compared with monotherapy (doxycycline), which were statistically significant (OR, 11.2; 95% CI, 1.3–247.9; $P = 0.007$). Current immunopathological studies of trachoma pathogens have shown that $T_H1$ cell production and mediated protective immunity in interferon-mouse models can prevent infection transmission. There is a new way to develop a *Chlamydia* vaccine based on endogenous dendritic cell. Although clinical studies are limited, a human C. trachomatis vaccine trial using these or new adjuvants and available candidate antigens that could commence in the foreseeable future is feasible.[49]

## ENDOCRINE FACTORS

### Hyperprolactinemia

Hyperprolactinemia can affect the function of the hypothalamus-pituitary-ovary axis, resulting in the changes of follicle-stimulating hormone, luteinizing hormone (LH), prolactin, estradiol, progesterone, testosterone, and their receptors, which will lead to luteal dysfunction. Low progesterone secretion and endometrial dysplasia associated with luteal dysfunction may cause early abortion. The cause of hyperprolactinemia is not clear; however, the pathological hyperprolactinemia caused by pituitary prolactinoma is clear. The criterion standard of hyperprolactinemia treatment is dopamine agonist (DA), which reverses hyperprolactinemia and hypogonadism and, in most cases, promotes tumor shrinkage. Bromocriptine (BRC) and cabergoline (CAB) are commonly used drugs, although their exact effect is still controversial. The study of the drug sensitivity of 2 kinds of prolactinoma cell lines to CAB and BRC found that BRC induced the apoptosis of prolactinoma cells by ERK/EGR1 signaling pathway, and CAB induced autophagy death by inhibiting AKT/mTOR signaling pathway. The difference in the sensitivity and mechanism of inhibiting tumor cells provides a theoretical basis for the correct treatment of prolactinoma.[50] The results of a single retrospective study showed that for hyperprolactinemia the rate of prolactin normalization (87.4% "vs 41.4%, $P = 0.029$) and tumor volume reduction (79.8% ± 39.1% vs 54.1% ± 55.3%, $P = 0.015$) in the CAB group were significantly higher than those in BRC group, but the cure rate was similar, and patients taking BRC had more adverse effects (29.1% vs 5.3%, $P < 0.001$). Overall, CAB was more effective than BRC in controlling symptoms, hormone levels, and reducing prolactinoma, with fewer adverse effects.[51] In addition, another study found that invasive giant prolactinoma was resistant to BRC, whereas CAB was highly effective, and no recurrence was observed during an average follow-up of 63.47 months (range, 30–145 months).[52] However, the potential risks of pregnancy induced by DA include fetal exposure and symptomatic tumor growth. Once pregnancy is confirmed in microprolactinoma and macroprolactinoma (MAC) patients, DA therapy should be discontinued. For dilated/invasive MAC, DA maintenance should be considered, and clinical evaluation should be conducted regularly during pregnancy. The results of another study showed that the incidence of missed abortion, stillbirth, and low-birthweight infants in MAC women who continued to use CAB during pregnancy were not different compared

with the control group, and it did not improve the post-pregnancy recurrence of hyperprolactinemia or tumor remission.[53,54]

### Hyperthyroidism

The mechanism of RSA induced by hyperthyroidism is that systemic metabolic disorder in pregnant women leads to placenta dysfunction, which leads to abortion. Because of the teratogenic effect of thyroid drugs, it is necessary to use them carefully during pregnancy. For low-risk women with a history of hyperthyroidism who are taking antithyroid agent for pregnancy, discontinuation of the medication is recommended prior to the primary teratogenic period (6–10 weeks of gestation).[55] Treatment of hyperthyroidism during pregnancy should be done with a minimal dose of antithyroid agent to improve the patient's symptoms and laboratory thyroid function and to monitor adverse effects during treatment. Common antithyroid agent options during pregnancy include propylthiouracil and methimazole. High-risk patients are treated with propylthiouracil in the first trimester of pregnancy and then may transition to methimazole.[56] To keep normal free thyroxine 4 level or not higher than one-third of the upper limit of normal level by drug intervention is the ideal dosage. Radioactive iodine is banned during pregnancy, and in principle, no surgery is performed to treat hyperthyroidism. If a thyroidectomy is indeed needed, the best time for surgery is in the second trimester.

### Hypothyroidism

Hypothyroidism is mainly caused by autoimmune thyroid diseases. Thyroglobulin antibody in thyroid antibodies affects embryo implantation. Elevated thyroid-stimulating hormone (TSH) receptor antibodies lead to luteal insufficiency and maternal immune tolerance imbalance in pregnancy, which are associated with abortion. Therefore, nonpregnant hypothyroidism patients should be actively treated with drugs until their serum TSH level is within the reference range and then get ready for pregnancy.[57] For hypothyroidism during pregnancy, the effect of levothyroxine 4 (LT4) has been positive, but there are no clear guidelines to adjust the maternal LT4 dose during pregnancy. In 1 study, patients with gestational hypothyroidism were randomly divided into 2 groups: group 1 received an empirical 2-pill-per-week dose increase followed by weekly dose adjustment; in group 2, based on current TSH levels and LT4 doses, the LT4 dose was adjusted using a continuous method at microgram per day. In group 2, the TSH was monitored every 2 weeks for 1 and 2 months, and every 4 weeks for 3 months. The results showed that for women with hypothyroidism during pregnancy, continuous dose adjustment is as effective as an empirical dose increase.[58]

The iodine deficiency in pregnant women should be paid attention to in the course of treatment. The World Health Organization recommends the iodine intake of pregnant women at 150 to 249 μg/L. For pregnant women with subclinical hypothyroidism and who are thyroid peroxidase antibody (TPOAb) negative, LT4 therapy has been shown to have no benefit in preterm labor in TSH cutoff point subclinical hypothyroidism–TPOAb women, but LT4 can reduce the complications of preterm labor precisely by the newly recommended cutoff point ≥4.0 mIU/L.[59]

### Autoimmune Thyroid Disease

Autoimmune diseases of the thyroid include toxic diffuse goiter, Hashimoto thyroiditis and lymph nodes, and cellular thyroiditis, which lead to RSA mainly due to the imbalance of maternal and fetal immune tolerance and the dysfunction of T cells. Because the specific mechanism is not clear, whether and how to treat it are still a hot point of debate. Clinical studies have shown that LT4 treatment reduces the risk of preterm birth in pregnant women with autoimmune thyroid disease but without significant thyroid dysfunction.[60] Another group of studies showed that LT4 did not improve pregnancy outcomes in TPOAb-positive women with normal thyroid function.[61] As there is insufficient evidence of efficacy or harm, existing guidelines do not recommend or oppose intervention. Some recent studies suggest that intravenous immunoglobulin (IVIG) can be used to increase the level of anti-idiotypic antibodies, which are absent or undersecreted from the mother to reduce the risk of miscarriage in patients. Supplementation of selenium or vitamin $D_3$ can also be used to prevent miscarriage.[62] However, evidence-based medicine is still lacking, and more large samples and high-quality studies are needed to confirm their effectiveness.

### Polycystic Ovary Syndrome

Polycystic ovary syndrome (PCOS) is associated with many endocrine problems, such as insulin resistance/hyperinsulinemia, hyperandrogenemia, obesity, and so on. Women with or without PCOS who had RSA and normal pregestational glucose metabolism had greater insulin resistance in the first-trimester pregnancy than women in the healthy control group, which suggests that insulin resistance may be a direct cause of recurrent miscarriages.[63] Similarly, an increase in body mass index can be an independent factor in recurrent miscarriage.[64] A review article examined the efficacy of combined oral contraceptives with different ingredients and found that

combined oral contraceptives containing cyproterone (Diane-35) appear to be more effective in suppressing gonadotropin and estradiol levels in patients with PCOS.[65] In a comparative study of the efficacy of insulin-sensitizing drugs, the effect of clomiphene citrate (CC) and metformin alone on live birth was uncertain and varied according to body mass index. In obese women with PCOS, CC improves ovulation and clinical pregnancy more than metformin, whereas the combination of metformin and CC is better than CC alone.[66] An individual participant data meta-analysis showed that, compared with CC, letrozole improved live birth rates (3 RCTs, 1043 women [RR, 1.43; 95% CI, 1.17–1.75; moderate-certainty evidence]) and clinical pregnancy rates (6 RCTs, 1284 women [RR, 1.45; 95% CI, 1.23–1.70; moderate-certainty evidence]) and reduced time to pregnancy.[67] In addition, another study also showed that among unselected women with PCOS, letrozole was associated with a higher live birth rate than CC, whereas CC was better than metformin or placebo. In women with CC-resistant PCOS, the live birth rate of gonadotropin was higher than CC plus metformin, which was better than laparoscopic ovarian drilling.[68]

For obese PCOS patients, nondrug treatment is also usually recommended because of its effectiveness and safety. A study showed that nondrug interventions significantly increased the pregnancy rate compared with the control group (RR, 1.37; 95% CI, 1.04–1.81; $P = 0.03$), but whether they improved the live birth rate was unclear.[69]

To sum up, for RSA patients with PCOS, correcting the disorder of the body sex hormone secretion to ensure adequate estrogen and progesterone during pregnancy is a valid measure to achieve embryo protection. For example, oral metformin before pregnancy can lower the concentration of LH and androgen and the level of insulin, facilitate normal ovulation, and reduce the rate of abortion. Diane-35 can also reduce the secretion of LH and serum androgen levels.

## IMMUNE FACTORS

In recent years, reproductive immune studies have shown that more than 60% of RSA is caused by immune disorders.[3] Antiphospholipid antibodies (APLs) are considered to be one of the most relevant pathogenic factors in RSA caused by autoimmunity. Clinically, APLs can be detected in 5% to 20% of RSA patients.[2] Antiphospholipid antibody promotes decidual angiopathy and placental thrombosis at the maternal-fetal interface, participates in complement activation, and increases the release of inflammatory factors, eventually resulting in fetal death from ischemia and hypoxia. Alloimmune RSA is related to the imbalance of maternal immune tolerance, causing embryo being recognized by maternal immunity at the maternal-fetal interface and rejected as allogeneic.

### Antiphospholipid Antibody Syndrome

Antiphospholipid antibody syndrome (APS) is generally considered to be a noninflammatory autoimmune disease characterized by the production of a large amount of APLs, which is one of the most important and treatable causes of RSA. At present, obstetric treatment of RSA patients with APS is mainly a symptomatic treatment to prevent the recurrence of thrombosis and abortion. Commonly used anticoagulant drugs are aspirin and low-molecular-weight heparin (LMWH) according to the relevant guidelines. A meta-analysis of RCTs aimed at the efficacy of aspirin or heparin or both in the treatment of RSA in women with APS showed that aspirin plus LMWH or LMWH alone could significantly improve the live birth rate (RR,1.23 [95% CI, 1.12–1.36; $P < 0.0001$]; RR,1.18 [95% CI, 1.03–1.35; $P = 0.02$]). At the same time, LMWH alone was effective in the prevention of preterm delivery. However, there was no significant difference in live birth between aspirin and the placebo group.[70] In recent years, more and more studies have approved that APL-mediated RSA is not only a simple thrombotic disease, but also an immune inflammatory disease.[71] A study of anticoagulant plus anti-inflammatory (prednisone + hydroxychloroquine [HCQ] + low-dose aspirin [LDA] + LMWH) regimen and anticoagulant regimen (LDA + LMWH) in the treatment of APS-related RSA showed that the curative effect of the former is better with a lower rate of repeated pregnancy loss (11.11% vs 22.7%) and better pregnancy outcomes.[72] In terms of drug safety, the 2016 guidelines on drug safety during pregnancy issued by the British Society for Rheumatology show that it is safe for pregnant women to use prednisone, HCQ, LDA, and LMWH.[73] Therefore, combined use of LDA and LMWH is recommended for RSA patients with elevated APLs, and the addition of prednisone and HCQ can be considered to improve the embryo protection effect.

### Alloimmunization

Currently, alloimmune RSA is still in the research stage, often referred to as unexplained RSA (URSA), which is considered to be related to immune cell abnormality, lack of blocking antibody (BA), and high histocompatibility of human lymphocyte antigen.

Cyclosporine A (CsA) is an immunosuppressant. Animal studies have suggested that low-dose CsA can induce maternal-fetal immune tolerance and enhance trophoblast invasion. Fu's[74] research demonstrated that for refractory

RSA patients who failed to receive aspirin, prednisone, LMWH, husband leukocyte immunotherapy, and IVIG, the live birth rate was 76.92% when CsA was added before and after pregnancy (the concentration of CsA was maintained at 80–150 ng/mL during the treatment). In a prospective non-RCT of CsA alone, from the detection of pregnancy, in 2 groups of oral administration CsA 100 mg/d for 30 days and progesterone 20 mg/d, respectively, until 12 weeks, the results showed that the live birth rate in CsA group was apparently higher (62.4% vs 30%, $P < 0.001$), and there were no obvious adverse effects in pregnant women. No intrauterine growth retardation or birth defects were observed.[75] However, a recent meta-analysis of 4 RCTs, including 415 patients with immune recurrent abortion, showed that the use of CsA alone or CsA in combination with other drugs did not significantly improve the pregnancy success rate compared with conventional drugs and methods other than CsA (RR, 1.12; 95% CI, 0.93–1.35; $P > 0.05$).[76] In summary, current studies suggest that maintaining the lowest concentration of CsA at 80 to 150 mg/L during medication may be a method for the treatment of URSA, especially refractory URSA, but the larger samples of clinical studies are needed to prove its effectiveness.

Pregnancy is considered to be a model of semiallogeneic transplantation. The tolerable immune environment at the maternal-fetal interface produces BA by recognizing the paternal antigens expressed in the embryo, which obviously protects the embryo from overexuberant immune activation.[77] Lymphocyte immunotherapy (LIT) is an active immunotherapy that induces BA production by injecting lymphocytes from the husband or a third party into the woman's body. Research has found that most BAs become positive after LIT treatment, and the maternal BA conversion rate increases with the increase in active immunization times (>4 times, $P < 0.05$). Furthermore, the pregnancy rate and live birth rate were improved after LIT, whether BAs turned positive or not.[78] A meta-analysis that included 18 RCTs with LIT showed that the live birth rate in the treatment group was significantly higher (OR, 3.74; 95% CI, 3.07–4.57), the administration before and during pregnancy was superior than that only before pregnancy (OR, 4.67; 95% CI, 3.70–5.90), and the effect of lower dose per treatment (less than $100 \times 10^6$ lymphocytes or 100 ml peripheral blood) is better.[79] Chen et al[80] also showed that active immunotherapy could significantly improve the pregnancy outcome of patients with RSA. However, the efficacy and safety of LIT remain controversial. The meta-analysis by Wong et al[81] (N = 641) considered that LIT had no significant effect on live birth (OR, 1.22; 95% CI, 0. 89–1.69). The guidelines for the diagnosis and treatment of recurrent abortion issued by the Royal College of Obstetricians and Gynecologists also do not recommend BA tests and LIT treatment. Cavalcante et al[82] compared the reasons for the success and failure of treatment and indicated that the effectiveness of LIT treatment may be related to the immune imbalance of the patient and the use method of LIT. Through the above studies and related guidelines, we conclude that multiple intradermal injections of fresh low-dose lymphocytes ($<1 \times 10^8$ per treatment) before and during pregnancy in specific URSA patients can be used as an individualized therapy.

Tumor necrosis factor α (TNF-α) is an inflammatory factor mainly secreted by mononuclear macrophages, natural killer (NK) cells, and T lymphocytes.[83] Many studies have shown that the expression of TNF-α in peripheral blood and decidual tissues in patients with URSA was significantly increased, which eventually leads to trophoblast apoptosis, oxidative stress upregulation, and blood coagulation system activation, resulting in placental vascular thrombosis and adverse pregnancy outcomes.[84,85] In an RCT, TNF-α inhibitor (enalapril) was used in URSA women with increased serum TNF-α or NK cell activity during pregnancy, and the final results showed that the enalapril group (N = 95) had a higher live birth rate than the placebo group (N = 93; 89.47% vs 72.04%, $P = 0.01$; OR, 3.30; 95% CI, 1.49–7.32). In addition, the incidence of pregnancy complications in the enalapril group was lower, and there was no significant difference in the occurrence of neonatal congenital malformations and therapeutic adverse effects between the 2 groups.[86] However, another prospective multicenter cohort study (N = 2027) showed that although patients with autoimmune diseases treated with TNF-α inhibitors had the same live birth rate and fetal weight as normal pregnant women, there was a higher risk of birth defects in the exposed group (5.0% vs 1.5%; adjusted OR, 2.2; 95% CI, 1.0–4.8).[87] Therefore, although TNF-α inhibitors are a promising strategy for the treatment of immune RSA patients, the safety and efficacy need to be further confirmed, and one should remain cautious about its use.

Intralipid is often used for parenteral nutrition. Some studies have found that its active components can lower the activity of $T_H1$ cytokines and NK cells and also promote the invasiveness of trophoblast cells.[88,89] A prospective RCT showed that both intralipid and IVIG could reduce the concentration of NK cells and increase successful pregnancy rates. In addition, there was no significant difference in the therapeutic effects between the 2 treatments ($P > 0.05$).[88] Therefore, intralipid can be considered as an alternative therapy for IVIG, which is expensive and has many adverse effects in the treatment of URSA. Interestingly, a double-blind RCT with intralipid and saline showed that intralipid supplementation

did not increase the frequency of chemical pregnancy.[89] All in all, intralipid needs more data to support its effectiveness in improving successful pregnancy rates, and the results of studies related to persistent pregnancy and live births should be further investigated.

## CONCLUSIONS

In summary, there are currently many treatment options for RSA caused by various etiologies; available treatment targets hypothetical risk factors for pregnancy loss, although the effectiveness of many medical interventions is controversial. Intervention should depend on the benefit-to-risk ratio of the proposed treatment. Antiphospholipid antibody syndrome, uterine structural abnormalities, and chromosome structural abnormalities are the parental pathologies most closely tied to RSA. The benefit of combined LMWH and LDA in APS is supported by robust prospective evidence. Further investigation is also required to prove the efficacy of its combination with anti-inflammatory drugs. Uterine structural abnormalities are usually corrected by hysteroscopic surgery; in order to prevent complications and recurrence, it is necessary to choose individual treatment options, prevent postoperative adhesion, and promote restoration and regeneration of the endometrium. The timing of surgery is also important. Clinicians should continue to recognize the contribution from embryonic aneuploidy. Chromosome analysis of abortion specimens can provide an explanation in many cases. For RSA patients with previous embryonic chromosomal abnormalities, traditional preimplantation genetic diagnosis or new detection techniques can effectively screen to reduce abortion rate. In terms of endocrine factors, the existing literature supports a strong correlation between RSA and positive TPOAb.[90] It is recommended to use LT4 in patients with TSH >4 mIU/L. If thyroid autoimmunity coexists, LT4 can be considered when TSH >2.5 mIU/L.

For more than 50% of RSA cases in which all the couples' investigations are normal, couples need accurate information about their chances of giving birth, regardless of the underlying cause, and appropriate support should be provided to reduce the psychological burden associated with multiple miscarriages.[91] Studies have shown that with only supportive treatment, the cumulative probability of live births is 66.7% within 5 years.[92] Large-sample, multicenter, prospective RCTs are still particularly necessary to explore and formulate effective treatment plans. However, because of ethical restrictions, many prospective RCTs for RSA especially related to stem cell therapy, anatomical factors, and genetic factors cannot be carried out. Therefore, empirical and combined multiple treatments are still the main methods. Many new treatment directions are also still actively exploring, looking forward to the transformation to clinical applications as soon as possible. In addition, more researches are still needed to explore its pathogenesis more accurately and achieve therapeutic targets.

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