ORIGINAL RESEARCH

Impact of Recombinant Human Granulocyte Colony-Stimulating Factor Combined with Aspirin on Clinical Pregnancy Outcomes and Endometrial Receptivity in Patients with Recurrent Abortion: A Retrospective Study

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ABSTRACT

Objective • This study investigates the impact of recombinant human granulocyte colony-stimulating factor (rhG-CSF) and aspirin on endometrial receptivity and clinical pregnancy outcomes in individuals with a history of recurrent abortions.

Methods • In this retrospective study, 131 individuals with recurrent abortions treated at our facility from July 2019 to December 2020 were split into two groups: mixed therapy and control. The mixed therapy group received aspirin and rhG-CSF, while the control group had no specific treatment. Primary endpoint: live birth rate; secondary: pregnancy rate at 20 weeks. We also evaluated abortion rates, newborn weight, pre-eclampsia, premature delivery, fetal/newborn congenital malformations, and maternal drug adverse reactions. Additionally, we analyzed endometrial blood flow three weeks post-treatment.

Results • The analysis encompassed 131 individuals, with 65 in the control group and 66 in the mixed therapy group. Notably, the mixed therapy group (n = 54) exhibited a markedly higher live birth rate than the control group ($P < .05$). In terms of medication-related side effects, the control group showed no adverse reactions, while the mixed therapy group reported mild effects (skin itching in three cases, leukocytosis in seven, and bone pain in one case) that did not significantly impact outcomes. Pre-treatment, the mixed therapy group had a notably lower resistive index, pulsatility index, and systolic-to-diastolic ratio compared to the control group, with statistical significance ($P < .05$). The control group's indices remained unchanged ($P > .05$).

Conclusions • In women with a history of recurrent abortions, the administration of recombinant human granulocyte colony-stimulating factor and aspirin can effectively and safely improve live birth rates. This improvement may be associated with enhanced endometrial receptivity. (*Altern Ther Health Med.* 2024;30(1):205-209).

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INTRODUCTION

Recurrent abortion, often referred to as Recurrent Spontaneous Abortion (RSA), is the occurrence of three or more spontaneous abortions before reaching 20 to 28 weeks of gestation.[1] Women of childbearing age experience this condition, with an incidence ranging from approximately 0.05% to 3%.[2,3] Nearly half of these cases lack a known etiology, and medical professionals classify them as unexplained recurrent miscarriages.[4] The factors identified in past studies include genetic influences,[5] abnormal reproductive system anatomy in pregnant women,[6] reproductive system infections,[7] atypical endocrine function in pregnant women,[8] a tendency towards thrombosis,[9] and abnormalities in immune function.[10,11]

Studies have considered these factors and investigated various treatment methods.[12] However, substantial evidence supporting the effectiveness of these treatments remains inconclusive. Studies suggest that recombinant human granulocyte colony-stimulating factor (rhG-CSF) may enhance the activation of cells, promote decidualization in endometrial stromal cells, facilitate embryo implantation, and increase pregnancy success rates when it reaches a specific concentration.[13]

Granulocyte colony-stimulating factor (G-CSF) has demonstrated its effectiveness as a treatment for unexplained recurrent abortion in a single-center randomized controlled trial. This study reported a significantly higher rate of successful, healthy births after G-CSF therapy, with 29 out of 35 women (82.8%) experiencing successful pregnancies. In contrast, the control group achieved a success rate of 48.5%. Moreover, individuals who received G-CSF not only achieved pregnancy but also exhibited significantly higher hCG levels than those in the control group.[14] The results of another historical cohort study further support the potential of G-CSF to elevate the live birth rate in women with a history of recurrent abortions.[15]

However, in a multi-center, randomized, double-blind, placebo-controlled study, the researcher employed the clinical pregnancy rate at 20 weeks of gestation as the primary endpoint. Their findings revealed that, compared to the placebo, G-CSF proved ineffective in enhancing the pregnancy rate at 20 weeks and the ultimate live birth rate.[16] The underlying reasons for the outcomes of these studies remain incompletely understood, and the findings exhibit inconsistency. Therefore, to comprehensively assess the therapeutic potential of G-CSF in cases of recurrent abortion, further research is imperative.

In a previous randomized controlled study, the therapeutic impact of aspirin on recurrent abortion was investigated. The findings indicated that, compared to a placebo, a daily dose of 100 mg aspirin can safely and efficiently enhance the live birth rate.[17] However, a subsequent meta-analysis did not support these results.[18] Aspirin has demonstrated therapeutic potential in enhancing endometrial receptivity among women experiencing unexplained recurrent abortions and implantation failures in previous studies. These findings indicate a significant improvement in endometrial thickness due to aspirin administration.[19,20] However, further investigation regarding its influence on pregnancy outcomes is lacking.

In the context of women with a history of recurrent abortions, we hypothesize that combining the various modes of action of G-CSF and aspirin can enhance the likelihood of successful pregnancies. Our research primarily focuses on assessing the impact of rhG-CSF and aspirin on endometrial receptivity and clinical pregnancy outcomes in individuals who have experienced recurrent abortions.

MATERIALS AND METHODS

Study Design

This prospective cohort study investigated the combined effects of rhG-CSF and aspirin on endometrial receptivity and pregnancy outcomes in women with a history of recurrent abortions. The case selection period for this research extended from July 2019 to December 2020, and the study was conducted at the Reproductive Center of Yanda Hospital in Hebei Province. Informed consent was obtained from each participant, and ethical approval for this research was granted by the Ethics Committee of Hebei Yanda Hospital (Approval No: 2020-003). The study strictly adhered to the principles outlined in the Declaration of Helsinki.

Inclusion and Exclusion Criteria

The following inclusion and exclusion criteria were applied to recruit women of childbearing age for this study. Inclusion criteria: (1) age between 18 to 40 years; (2) history of more than three consecutive unexplained abortions; (3) normal menstrual cycle and ovulation confirmed through b-ultrasound monitoring, with an endometrial thickness not exceeding 7.0 mm on the day of conception; (4) husband's semen analysis indicating no abnormalities; (5) absence of uterine cavity procedures in the past three months.

Exclusion criteria were as follows: (1) uterine malformations, uterine cavity adhesions, uterine fibroids, or other conditions known to affect endometrial growth; (2) known allergies to medications used in this study; (3) unexplained vaginal bleeding; (4) pre-existing infertility diagnoses; (5) incomplete clinical data; (6) presence of other medical conditions recognized to cause miscarriages, such as rheumatic immune diseases.

Treatment Regimen and Protocols

In the mixed therapy group, individuals received a combination of rhG-CSF and aspirin. Subcutaneous injections of rhG-CSF were initiated from the sixth day following ovulation until either the onset of menstruation or the conclusion of the ninth week of pregnancy. The dosage administered was 1 milligram (100 000 IU))/kg/day.

Aspirin was initiated at a daily dose of 75 milligrams and continued until the 36th week of pregnancy. Treatment with aspirin and rhG-CSF was administered concurrently or terminated in the event of abortion, ectopic pregnancy, or premature delivery. Patients in the control group refused to receive targeted treatment for various reasons, and they only underwent regular examinations in the outpatient department.

Study Endpoints and Diagnostic Measurements

The primary endpoint of this study was the live delivery rate, with secondary endpoints inludes the 20-week pregnancy rate, abortion rates, newborn weights, incidents of pre-eclampsia, premature deliveries, occurrences of fetal and newborn congenital malformations, and maternal adverse drug reactions. Additionally, we conducted a comparative assessment of endometrial blood flow in patients after three weeks of treatment. On the 22nd day post-treatment, Doppler ultrasound was employed to measure the endometrial blood flow resistance index, pulsation index, and contraction ratio.[20]

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 26.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous data were summarized using the mean and standard deviation ($\bar{x} \pm s$) for inter-group comparisons, which were conducted using the two-tailed Student's t test. Categorical variables were compared across groups using Fisher's exact test and the chi-square (χ^2) test, and the results were presented as counts or proportions. Statistical significance was defined as a two-sided $P < .05$.

RESULTS

Baseline Characteristics of Study Participant

A total of 131 women were enrolled in this study, adhering to the specified inclusion and exclusion criteria. Among them, 66 women were allocated to the combined therapy group, while 65 women were placed in the control group. There were no statistically significant differences between the two groups in terms of baseline data ($P > .05$), indicating their comparability, refer to Table 1. In the final study, participants exhibited varying prior live birth experiences, ranging from 0 to 2, with 73 (55.7%) having no history of childbirth. The number of prior abortions ranged from 3 to 6, with 71 cases (54.2%) having experienced more than 4 abortions.

Pregnancy Outcomes

At the end of the study, the combined therapy group, consisting of 54 individuals, exhibited a significantly higher live birth rate in comparison to the control group ($P < .05$). Additionally, the combined treatment group experienced fewer miscarriages than the control group ($P < .05$). However, there were no statistically significant differences between the two groups regarding the timing of miscarriages ($P > .05$) or the newborns' birth weights ($P > .05$).

No discernible adverse reactions were observed in the control group regarding the therapy-induced side effects. In contrast, the combined treatment group reported mild adverse effects, including skin itching in three cases, leukocytosis in seven cases, and bone pain in one case, refer to Table 2. Importantly, these adverse reactions were of mild intensity and did not adversely impact the course of treatment.

Evaluation of Endometrial Receptivity

Before therapy, the two groups had no statistically significant difference in the endometrial receptivity scores. However, following the completion of therapy, the combined treatment group exhibited a substantial reduction in the resistive index, pulsatility index, and systolic-to-diastolic ratio compared to the values observed in the control group. This difference was highly significant ($P < .05$). Conversely, the control group demonstrated no statistically significant changes in their resistive index, pulsatility index, or systolic-to-diastolic ratio compared to their pre-treatment values ($P > .05$).

Table 1. Baseline Characteristics of Participants

Parameters	rhG-CSF+ASP	Control	t/χ^2 value	*P* value
n	66	65		
Age (years)	32.6±4.2	33.2±4.3	0.808	.421
BMI (kg/m2)	25.9±3.8	25.6±3.7	0.458	.648
Alcohol Use (n, %)	17 (25.8)	12 (18.5)	1.011	.315
Previous Live Births (n, %)	39 (59.1)	41 (63.1)	0.219	.640
Previous Miscarriages	3.8±0.4	3.7±0.5	1.265	.208
Gestational Week of Miscarriage (w)	7.7±1.9	8.1±2.2	1.114	.267

Note: BMI: Body Mass Index; n: number; %: percentage; w: weeks. *P* value indicates statistical significance ($P < .5$). rhG-CSF+ASP represents the group of participants who received combined therapy with recombinant human granulocyte colony-stimulating factor (rhG-CSF) and aspirin (ASP).

Table 2. Outcome of Participants

Parameters	rhG-CSF+ASP	Control	t/χ^2 value	*P* value
n	66	65		
Live Births (n, %)	54 (81.8)	40 (61.5)	4.645	.010[a]
Miscarriage (n, %)	12 (18.2)	25 (38.5)	4.645	.010[a]
Gestational Week of Miscarriage (w)	6.3±1.2	6.0±1.0	0.801	.429
Newborn Weight (g)	3107±238	3074±229	0.675	.501

Note: n: number; %: percentage; w: weeks; g: grams. *P* values indicate statistical significance ([a]$P < .05$); rhG-CSF: recombinant human granulocyte colony-stimulating factor and ASP: aspirin.

Table 3. Assessment of Endometrial Receptivity

Parameters	rhG-CSF+ASP	Control	t/χ^2 value	*P* value
n	66	65		
Before Treatment				
Pulsatility Index	1.14±0.27	1.12±0.23	0.456	.649
Resistive Index	0.61±0.10	0.59±0.09	1.203	.231
Systolic-To-Diastolic Ratio	2.43±0.41	2.47±0.43	0.545	.587
After Treatment				
Pulsatility Index	0.91±0.23	1.10±0.22	4.830	<.001
Resistive Index	0.50±0.08	0.58±0.09	5.379	<.001
Systolic-To-Diastolic Ratio	2.12±0.29	2.46±0.40	5.576	<.001

Note: n: number; Before Treatment: measurements taken prior to therapy; After Treatment: measurements taken after therapy. *P* values indicate statistical significance ($P < 0.5$); rhG-CSF: recombinant human granulocyte colony-stimulating factor and ASP: aspirin.

DISCUSSION

Recurrent abortion, characterized by three or more successive spontaneous miscarriages, typically presents with symptoms of vaginal bleeding, abdominal pain, and uterine cramping.[21] Its pathophysiology involves complex interactions between genetic, immunological, hormonal, and anatomical factors, which make the exact etiology challenging to determine. The emotional and psychological impact on patients is profound, often leading to increased stress, anxiety, and potential complications in future pregnancies.[24,25] The multifaceted etiology of recurrent abortion presents significant challenges in its clinical treatment. The exact underlying causes behind recurrent abortion are not yet fully understood.

Several studies have suggested that embryo successful implantation is intricately linked to luteal function and endometrial receptivity.[22,23] In recent years, there has been a marked increase in studies examining endometrial receptivity and its relationship with recurrent abortion, capturing significant attention from experts and scholars in the field. Past studies have explored the potential of rhG-CSF and aspirin in aiding individuals with a history of recurrent abortion to achieve pregnancy. However, the findings from these studies exhibit considerable variability.[14,15,19,20,24]

In this study, we administered a combined treatment of rhG-CSF and aspirin to women with recurrent abortion. The results indicated that the combined therapy, compared to the control group, significantly enhanced pregnancy outcomes in women with a history of recurrent abortions. This improvement may be attributed to an increase in endometrial receptivity. Several studies have also highlighted the significance of uterine blood perfusion during the mid-luteal phase as a critical factor influencing embryo implantation and development.[25] When maternal blood exhibits a hypercoagulable state, it can result in inadequate uterine

blood perfusion, potentially leading to the deposition of fibrin in the placenta and uterus. This, in turn, can give rise to microthrombosis, impacting placental blood flow, and ultimately contributing to miscarriage.[26,27]

Aspirin exerts its anti-thrombotic effects by modulating arachidonic acid-thromboxane A2 interactions. This mechanism theoretically aids in restoring the pro-thrombotic condition in patients with recurrent abortion, consequently enhancing uterine blood perfusion.[24] Additionally, recombinant human granulocyte colony-stimulating factor, produced by lymphocytes and endothelial cells, facilitates the differentiation and proliferation of hematopoietic cells in the bone marrow. It plays an important role in various processes, including follicular growth, ovulation, and pregnancy, effectively enhancing endometrial receptivity.[28]

The findings of our study provided compelling evidence that the simultaneous administration of these two medications resulted in a significant reduction in both the endometrial blood flow resistance index and the pulsatility index when compared to the control group. This reduction suggests an improvement in the overall vascular dynamics of the endometrium.

Studies have highlighted the multifaceted beneficial effects of rhG-CSF. Notably, it exhibits potent anti-inflammatory properties, promoting an environment conducive to improved endometrial health. It also promotes vascular growth, a key factor in enhancing the circulatory network within the endometrium. Additionally, rhG-CSF has been shown to stimulate the proliferation and regeneration of endometrial cells, contributing to structural integrity. Ultimately, these combined effects result in an increase in endometrial blood perfusion, further setting its role in optimizing the uterine environment for successful pregnancy outcomes.[29]

In our research, the combined therapy group achieved a live birth rate of 81.8%, a significant improvement compared to the control group's rate of 61.5%. Compared to the findings of Scarpellini et al.,[14] it is worth noting that the live birth rate in the combined treatment group of our study was slightly lower. This variance could potentially be attributed to disparities in factors such as the participants' ethnicity, BMI, and age between the two studies. Additionally, differences in sample sizes might also contribute to these variations.

However, our findings are inconsistent with the results of a study conducted by Eapen et al.,[16] In our research, the combined therapy group exhibited a substantially higher live birth rate than the 59.2% rate reported in Eapen's study. This difference can be attributed to a critical difference in treatment approaches between the two studies. While our research incorporated the combined use of rhG-CSF and aspirin, Eapen's study exclusively employed rhG-CSF. This contrast highlights the potential impact of aspirin in our research.

Our findings suggest the potential of combined rhG-CSF and aspirin therapy to enhance endometrial receptivity and improve pregnancy outcomes in women with recurrent abortion. Aspirin's inclusion in our treatment regimen may have contributed to improving the state of microthrombosis, ultimately leading to enhanced uterine blood perfusion. This improvement in blood perfusion could, in turn, have played a pivotal role in elevating the live birth rate observed in our combined therapy group.

Strengths and Limitations of the Study

The strengths of our study lie in its robust participant group size and the comprehensive evaluation of endometrial receptivity. This extensive sample enhances the reliability and generalizability of our findings. Furthermore, our research contributes to the development of an effective and safe strategy for treating women with recurrent miscarriages, addressing a critical clinical need.

However, this research does exhibit some important limitations. Firstly, it was conducted at a single location, potentially introducing bias among the included patients. Secondly, the study did not assess the reproductive immune function of the participants. Thirdly, there was no post-delivery follow-up of the patients, and newborn evaluation was omitted. To address these limitations, we recommend that future research endeavors employ diverse randomized controlled designs, concurrently assess a broader array of immune, endocrine, and other relevant indicators, monitor patients' responses to the combined therapy plan over an extended follow-up period, and ensure the safety and well-being of both mothers and newborns. Such comprehensive investigations will further advance our understanding of recurrent miscarriage treatments.

CONCLUSION

In conclusion, our study has unveiled promising insights into managing recurrent miscarriages. The combined therapy involving recombinant human granulocyte colony-stimulating factor and aspirin showed significant advantages, particularly in elevating endometrial receptivity and improving pregnancy outcomes. Despite certain limitations, such as single-location research and unexplored aspects of reproductive immune function, our findings pave the way for a more effective and safe strategy in the treatment of women dealing with recurrent miscarriages. We suggest that future research endeavors adopt diverse randomized controlled designs, encompass a broader spectrum of immune and endocrine indicators, incorporate comprehensive patient follow-up, and assess maternal and newborn well-being over extended periods. It will facilitate us to collectively advance our understanding of recurrent miscarriage treatments and ultimately provide better care for affected individuals.

DATA AVAILABILITY

The data used to support this study is available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Shuyan Sun and Jing Chen are Co-First Authors: These authors contributed equally to this work.

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