

Effect of Intrauterine Recombinant Human Granulocyte Colony-stimulating Factor (rhG-CSF) Infusion on Pregnancy Outcomes in Patients With Repeated Implantation Failure During Freeze-Thaw Embryo Transfer Cycles: A Retrospective Cohort Study

Meng Lyu

The First school of Clinical Medicine of Lanzhou University

Xiaoling Ma

The Reproductive Medicine Center, the First Hospital of Lanzhou University

Junping Hu

The Reproductive Medicine Center, the First Hospital of Lanzhou University

Hongjuan Zhan

The Reproductive Medicine Center, the First Hospital of Lanzhou University

Lin Liu (✉ ldyy_jiul@lzu.edu.cn)

The Reproductive Medicine Center, the First Hospital of Lanzhou University

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Abstract

Background: Repeated implantation failure (RIF) is one of the difficulties that hinder the further improvement of clinical pregnancy rate by assisted reproductive technology. RIF has become an urgent clinical problem and hot research topic in the field of assisted reproduction, which is also a challenge for clinicians.

Objective: The aim of this study was to evaluate the effects of intrauterine recombinant human granulocyte colony-stimulating factor (rhG-CSF) to improve implantation, clinical pregnancy, early abortion, multiple pregnancy and live birth rate (LBR) rate in women with RIF.

Methods: A retrospective clinical analysis involving 142 women with RIF was conducted in the reproductive Medicine Center, the First Hospital of Lanzhou University between 1 January 2015 and 30 June 2018. They were divided into two groups: rhG-CSF group and control group, according to whether or not intrauterine rhG-CSF. In rhG-CSF group (n=47) granulocyte colony-stimulating factor (300 micrograms in 1 mL) was infused into the uterus within five minutes by embryo transfer cathete during the proliferative period of menstrual cycle before freeze-thaw embryo transfer (FET), while the control group was not given intrauterine perfusion. The implantation, clinical pregnancy, early abortion and multiple pregnancy were compared between the two groups.

Results: The mean age for whole study group was 35.3 ± 4.2 years old. There were no significant differences between demographic characteristics in two groups ($p > 0.05$). The successful implantation (28.44% vs 12.44%, $p = 0.012$), and clinical pregnancy (48.95% vs 27.35%, $p = 0.011$) rates were significantly higher in the rhG-CSF group than in the control group. Binary logistic regression indicated that rhG-CSF treatment remained significantly associated with successful clinical pregnancy (OR=2.979, 95% CI=1.262-7.003).

Conclusion: Intrauterine infusion of rhG-CSF can increase embryo implantation rate, clinical pregnancy rate in patients with RIF. In addition, the age and rhG-CSF are the independent risk factors affecting pregnancy outcomes.

Introduction

With the maturity of in vitro fertilization (IVF) technology and the increasing success rate, the clinical pregnancy rate of each transplantation cycle has exceeded 50%[1]. The normal ovulatory patients can even reach 69%[2]. Although assisted reproductive technology (ART) has improved pregnancy outcomes for infertile couples, many couples still remain unsuccessful after several IVF attempts causing deep effect on quality of life and a heavy financial burden[3]. Repeated implantation failure (RIF) is defined as the failure to achieve a pregnancy after transferring high-grade embryos through at least three in vitro fertilization (IVF) cycles to the endometrium[4]. These patients are challenges for the infertility specialist, and the defects of endometrial receptivity is the mainly causes of RIF[5].

Granulocyte-colony stimulating factor (G-CSF) is a glycoprotein that mainly acts on neutrophils to promote their proliferation, differentiation and activation glycoprotein[6], which is routinely used to mobilize hematopoietic stem cells and promote the proliferation of bone marrow mesenchymal stem cells to treat related diseases[7]. In recent years, it has been found that the biological function of G-CSF is not limited to the blood system[8]. G-CSF seems play an important role in reproductive medicine, which is being administered mainly in women with thin unresponsive endometrium undergoing IVF[9], repeated IVF failures[10], unexplained recurrent pregnancy losses[11], improve the process of embryo implantation[12] and endometrial receptivity[13]. Many studies have confirmed that the pregnancy outcomes have been improved in patients undergoing IVF-ET[14, 15]. Therefore, G-CSF has attracted the interest and attention of reproductive medicine enthusiasts and was used to change endometrial thickness. Several studies with different results have assessed the effect of G-CSF on endometrial thickness and IVF success [16, 17]. In the studies of N. Gleicher et al.[18] and Dayong Lee et al.[19], the endometrial thickness, implantation and pregnancy rates were significantly higher in IVF patients treated with G-CSF, whereas in the studies of Eftekhari M et al.[20] and Shivani Jain et al.[21], treatment with G-CSF did not affect endometrial thickness and pregnancy outcomes in IVF patients. This difference may be related to the inconsistent with selection criteria of patients. At present, the effect of G-CSF has been evaluated mostly focus on thin endometrium. The study of G-CSF on pregnancy outcomes in patients with RIF is relatively little. It is currently uncertain whether G-CSF is effective in improving results following the patients with RIF. The conclusion remains controversial and needs further to be explored.

We conducted this study on infertile patients with repeated IVF failure, the efficacy of intrauterine rhG-CSF perfusion on pregnancy outcomes was evaluated.

Materials And Methods

Study population and design

This study is designed as a retrospective clinic trial with 142 patients diagnosed with RIF and was carried out in The Reproductive Medicine Center, the First Hospital of Lanzhou University. They were divided into two groups including rhG-CSF group (n = 47 cases) and control group (n = 95 cases) according to whether intrauterine rhG-CSF perfusion during FET cycle.

In our study, RIF was defined as failure of implantation in at least three consecutive IVF attempts, in which three embryos of high-quality are transferred in each cycle [22]. The patients were considered eligible if they had the following inclusion criteria: a. implantation failure who undergoing IVF cycle at least three times; b. at least 3 high-quality embryos were transferred in all transfer cycles; c. vitrification freezing. Exclusion criteria: a. congenital uterine malformations, Asherman's syndrome, uterine fibroids and polyps, endometriosis and moderate to severe intrauterine adhesions; b. patients with endometrial organic lesions; c. chromosomal abnormalities and male infertility factors; d. rhG-CSF allergies and diabetes, hypertension, cardiovascular diseases, active infections, malignant tumors, sickle cell anemia and other major diseases.

Subjects

Embryo transfer was done for all patients who underwent FET cycle. Patients in the rhG-CSF group received intrauterine rhG-CSF perfusion before FET. The procedure involved the administration of 300ug rhG-CSF(Ruibai,Shandong Qilu Pharmaceutical) through slow infusion into the endometrial cavity using an embryo transfer catheter, after urinary suffocation, lithotomy was taken and placed on the examination bed and the cervix was exposed with a peep, and the vulva and vagina were disinfected with iodophor. The control group did not receive any additional treatment before the embryo transfer. Follicle development was monitored by ultrasonography. Follicle size and endometrial thickness were up to the standard and then decided to be transplanted this week. All patients received routine luteal support after transplantation.

Criteria

Implantation rates were determined by the number of gestational sac on the total number of transferred embryos. Clinical pregnancy was defined as the presence of gestational sac with fetal heart beat by ultrasound 4 weeks following the FET. The early abortion rate was assessed by the number of miscarriages before 12 weeks gestation on the total number of clinical pregnant patients. The multiple pregnancy rate was defined as the gestation of the number of patients with multiple pregnancy to the number of patients with clinical pregnancy.

Statistical Analysis

All of statistical analysis was done by SPSS 22.0. With 95% confidence level in each group was considered. The normal distribution of data was checked. Mean \pm SD were calculated for descriptive analysis. Independent t-test and χ^2 test were used. The clinical pregnancy rate was analyzed by binary logistic regression analysis. Statistical tests were carried out bilaterally. The statistical significances considered as $p < 0.05$.

Results

Descriptive statistics

In total, 416 infertile women with RIF were selected to participate in the study, 274 of whom were excluded based on inclusion and exclusion criteria. Therefore, 142 infertile women with RIF remained in the study. Of the 142 women, 47(33.1%) persons were in rhG-CSF group and 95(66.9%) individuals were in control group. Data acquisition and selection of the cases are shown in Fig. 1.

Background characteristics of rhG-CSF and control group were compared. Mean age in control group was 34.39 ± 5.15 versus 34.38 ± 4.68 for rhG-CSF group. No significant difference was found in age($p =$

0.994). Other demographic characteristics of participants are shown in Table 1. There were no significant differences in the demographics of the patients between the two groups ($p > 0.05$)

Table 1
Demographic characteristics of participants in two groups of the patient with RIF

Characteristics	rhG-CSF Group (n = 47)	Control Group (n = 95)	χ^2/t	p-value
Age(y)*	34.38 ± 4.68	34.39 ± 5.15	-0.007	0.994#
BMI(kg/m ²)*	22.24 ± 2.63	22.12 ± 2.76	0.243	0.808#
hCG day endometrial thickness (mm)*	0.84 ± 0.13	0.79 ± 0.15	1.841	0.068#
Transferred embryos (n)*	2.32 ± 0.60	2.28 ± 0.56	0.336	0.737#
History of miscarriage**	11(23.4%)	20(21.1%)	0.102	0.750\$
History of ectopic pregnancy**	6 (12.8%)	5 (5.3%)	2.477	0.116\$
Duration of infertility*	4.31 ± 2.51	4.60 ± 2.92	-0.565	0.573#
Type of infertility**			2.298	0.145\$
Primary	25(17.6%)	67(47.2%)		
Secondary	22(44.4%)	32(22.5%)		
Fertilization**			2.701	0.129\$
IVF	27(19%)	67(47.2%)		
ICSI	20(14.1%)	28(19.7%)		
FET cycle**			2.349	0.309\$
Artificial cycle	46(32.4%)	90(62.4%)		
Natural cycle	1(0.7%)	5(3.5%)		
Transplantable embryos were all high quality embryos [grade I and II embryos or blastocysts above 3BC or 3CB(Gardner grading method)]				
* Data are presented as mean ± SD. ** Data are presented as n (%).				
# Student t-test \$ Chi-square test				
G-CSF: granulocyte colony-stimulating factor. hCG = human chorionic gonadotropin				

The effect of rhG-CSF on IVF/ICSI outcomes in patients with RIF

The implantation rate (28.44% vs 12.44%, $p = 0.012$) and clinical pregnancy rate (48.95% vs 27.35%, $p = 0.011$) in the rhG-CSF group were significantly higher than that in the control group. There were no significant differences in the early abortion rate, multiple pregnancy rate and live birth rate of the patients with RIF (Table 2).

Table 2
Outcome measures of patients in rhG-CSF and control groups

Outcomes	rhG-CSF Group	Control Group	OR 95%CI	<i>p</i> -value
Implantation rate**	28.44%(31/109)	12.44%(27/217)	2.79(1.567 4.992)	0.012 [#]
Clinical pregnancy**	48.95%(23/47)	27.35%(26/95)	2.54(1.228 5.269)	0.011 ^{\$}
Early abortion rate**	21.7%(5/23)	34.6%(9/26)	1.91(0.531 6.884)	0.319 ^{\$}
Multiple pregnancy rate**	30.4%(7/23)	15.4%(4/26)	0.416(0.104 1.664)	0.208 ^{\$}
Live birth rate**	69.6%(16/23)	57.8%(15/26)	0.597(0.183 1.943)	0.554 ^{\$}
** Data are presented as n (%).				
# Student t-test \$ Chi-square test G-CSF: granulocyte colony-stimulating factor				

Dependency Of The Obtained Results

Binary logistic regression model was used to determine the dependency of the effect of rhG-CSF treatment on IVF/ICSI outcomes. When adjusted for the participants' age, BMI, hCG day endometrial thickness, duration of infertility, number of embryos transferred, history of miscarriage and ectopic pregnancy, type of infertility, fertilization and FET cycle, we observe that intrauterine rhG-CSF infusion (OR = 2.979, 95%CI = 1.262 ~ 7.003) was positively correlated with clinical pregnancy rate, and the difference was statistically significant. Age (OR = 0.889, 95%CI = 0.808 ~ 0.977) was another one of the main risk factors affecting clinical pregnancy rate, and negatively correlated with pregnancy outcome as shown in Table 3. Visualization of binary regression analysis using GraphPad Prism 8 (Figure 2). Then, ROC curve analysis, reaching an AUC of 0.9559, and $p = 0.0001$, respectively, revealed that frequency of intrauterine perfusion was a predictive factor for the outcome of clinical pregnancy rate (Figure 3).

Table 3
Analysis of influencing factors of clinical pregnancy outcome (Dual Logistic Regression Analysis, Multiple Factors)

Variable	B	Wald	p	OR 95%CI
rhG-CSF	1.092	6.207	0.013	2.979(1.262 7.003)
Age	-0.118	6.020	0.014	0.889(0.808 0.977)
Constant	3.584	1.889	0.169	-
Adjustment of age, BMI, hCG day endometrial thickness, History of miscarriage, History of ectopic pregnancy, duration of infertility, Transferred embryos, type of infertility, rhG-CSF, fertilization and FET cycle.				

Discussion

Embryo implantation is a complex and delicate process, which requires high-quality embryos, well-tolerated endometrium and appropriate immune privilege, in order to achieve successful pregnancy. In our study, the implantation and clinical pregnancy in rhG-CSF group were significantly improved compared with control group, and no differences was observed between the two group regarding early abortion and multiple pregnancy rate. The study shows that rhG-CSF is beneficial to the pregnancy outcome of patients with RIF. which provides reliable clinical evidence for the use of rhG-CSF in patients with RIF and are basically consistent with the related research results abroad.

Comparison with previous studies

In recent years, pregnancy outcomes have been reported to improve in these trials in which G-CSF was used in patients with RIF. Fatemeh Davari-tanha and colleagues[23]published a randomized double blind placebo control trial including 80 patients who allocated to three group(G-CSF, saline and placebo group) indicated that intrauterine G-CSF may increase chemical pregnancy and implantation rate in patients with recurrent implantation failure. This was in accordance with our results in implantation rate. And meanwhile, the study of Maryam Eftekhar[24]in women with repeated IVF failure involving 89 patients who allocated to two group(G-CSF and control group) that indicated higher implantation and clinical pregnancy in RIF who had received rhG-CSF compared with the control group. The conclusion is completely consistent with ours. However, in recent study by Ziya Kalem and colleagues[25]used intrauterine G-CSF administration before ART in the RIF group had an effect on clinical pregnancy and live birth rates. Although the results of this study were no statistically significant, the G-CSF group had better implantation and pregnancy rate than the control group. In addition, different routes of G-CSF administration have also been studied in IVF. In a study on IVF cycles with received subcutaneous G-CSF before implantation by Ashraf Aleyasin and colleagues[26], they concluded administration of single-dose systemic subcutaneous G-CSF increases the implantation and pregnancy rates in infertile women with repeated IVF failure. In another study by Soheila Arefi and colleagues[27], demonstrates the possibility that pregnancy outcome is better in women with repeated unexplained IVF who are subcutaneously

treated with G-CSF but no significant between two groups. And in a randomized clinical trial study in 2019, A. Eapen and colleagues[11] investigated 150 women with unexplained recurrent pregnancy loss, they reported that no significant increase in clinical pregnancy with the subcutaneous injection rhG-CSF. They have an equal conclusions compared the previous studies in implantation and pregnancy rates.

Different conclusions were reached in these studies. The differences in administration route, participants' age, endometrial thickness, and sample sizes could have contributed to the observed differences in the results. In the study of Maryam Eftekhar[24], Aleyasin A[26]and our study, the sample size was larger, rhG-CSF was administered systemically through Intrauterine perfusion pathway, the study populations were younger, and had normal proliferating function. The difference is that saline group was set as the control in Maryam Eftekhar's study, and the effect of mechanical stimulation on endometrium was avoided. Moreover, our Binary logistic regression analysis concluded that intrauterine perfusion rhG-CSF was an independent risk factor for pregnancy outcomes in RIF patients. The route of administration of G-CSF is also controversial. A meta-analyses published by Ling Zhang et al.[28]concluded that both intrauterine G-CSF and systemic administration treatment had good clinical outcomes after embryo transfer in the patients with RIF. Another, a consistent conclusion has been reached by Ying Jiang et al.[27], in which subcutaneous injection was better. Nevertheless, a retrospective study in women undergoing IVF/ICSI treatments by Hulusi Bulent Zeyneloglu[10] showed that dual administration of G-CSF was significantly more effective than the only method. Combined with our study, the positive effect of G-CSF on pregnancy outcomes in patients with RIF women should be affirmed, but the route of administration needs further study. An article on the correlation between G-CSF dose and pregnancy outcome has no consensus yet.. Using a single dose of 300 ug before the implantation was positive for the pregnancy outcome[29], and 150ug as an intrauterine infusion on day of oocyte recovery did not show a benefit in improving pregnancy outcomes[30]. In our findings, we demonstrate that the predictive effect of intrauterine perfusion on clinical pregnancy rate and it provides a statistical basis for clinical treatment. Live birth rate was no significant differences between the two groups. Could be that whether treatment with rhG-CSSF is no longer a necessary condition for for their continued pregnancy, but it can affect the embryo implantation through its different physiological function before the embryo successfully implantation.

The role of GCSF in implantation and pregnancy

Our results fully illustrates that G-CSF might play an important role in the implantation and pregnancy process. As we all know, G-CSF can improve endometrial receptivity via increasing endometrial thickness[31], in order to make the embryo implantation and clinical pregnancy rate go up[32]. At present, there is no unified view of the specific mechanism of rhG-CSF in assisted reproduction. Salmassi et al. proposed that G-CSF level in serum and follicular fluid was a predictor of IVF outcome[33]and its participation in the mechanism of ovulation as described above is quite clear[34]. The authors concluded that G-CSF could have an important function in

achieving positive pregnancy. In another, G-CSF is an integral part of the utero-placental cytokine network needed to establish and maintain pregnancy, at the maternal-foetal interface. Rahmat et al. indicated that

the expression of G-CSF receptor was increased in endometrium, at the highest dose of rhG-CSF stimulation[35]. What's more, G-CSF and its receptor have been widely expressed in germ cells, such as placenta, endometrial epithelial cells, stromal cells and decidualized tissues, they play their biological roles by binding to G-CSF receptors[36].

With the increasing understanding of G-CSF in reproduction, the majority of researches on the mechanism of G-CSF began to emerge. The study indicated G-CSF seems able to influence endometrial expressions in implantation process involving endometrial vascular remodelling, local immune modulation and cellular adhesion pathways[35]. And G-CSF was reported to have positive effects on oocyte maturation and embryonic development[37]. Decidualization of endometrial stromal cells is one of the key factors for successful implantation. T.Tanaka and coworkers demonstrates that G-CSF enhances the decidualization process in ESCs[38]. Also G-CSF contributes to the improvement of the implantation by adding G-CSF into the cultural medium[39]. The present results suggest that G-CSF may play a significant role in follicular development[40]aid to embryo implantation.

For many decades, mounting evidence from studies in mice and humans supports the idea that G-CSF have positive effect in embryo implantation and pregnancy development. However, the specific target of G-CSF on endometrial cells is not clear. Clinicians must consider the patient's condition comprehensively and choose the appropriate time and dose to make the patients with RIF obtain better pregnancy outcomes.

Limitations

The limitation of our study is that it is a retrospective clinical analysis, and the control group is lack of strict matching(No saline infusion as control); And the number of patients aged between 20 and 29 in the study was very small, and no further hierarchical analysis was performed. Among the subjects we included ,2 cases were perfused 4 times and all obtained clinical pregnancy,.and there were 12 cases with 3 times of perfusion ,9 of which obtained clinical pregnancy. Although this study established that the number of perfusion times has a high degree of specificity in predicting the outcome of clinical pregnancy, the sample size of the intervention group was small, We need to be further confirmed by large sample, multi-center randomized controlled trials.

Declarations

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Author contributions

Meng Lyu Wrote the original draft and manuscript, Xiaoling Ma and JunpingHu collect and analyze dataJunpingHu and Hongjuan Zhan prepared figures 1-3 and table1-3; Lin Liu was responsible for the conception and revision of the manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that they have nothing to disclose.

Ethical approval

The project was approved and supervised by the Ethics Committee of the First Hospital of Lanzhou University. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. With waiver of informed consent due to the retrospective, observational design of the study, and the Ethics Committee of the First Hospital of Lanzhou University abandoned the informed consent procedure for the study

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Figures

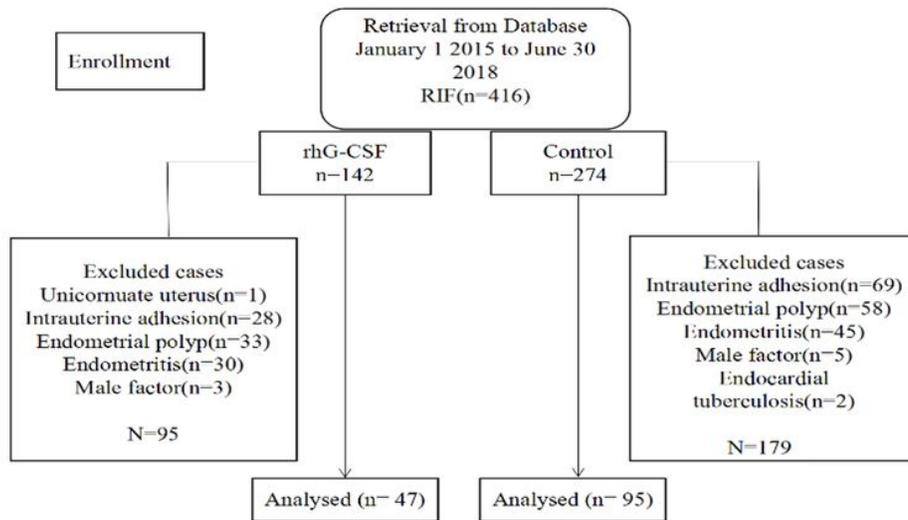


Figure 1

Consort flowchart

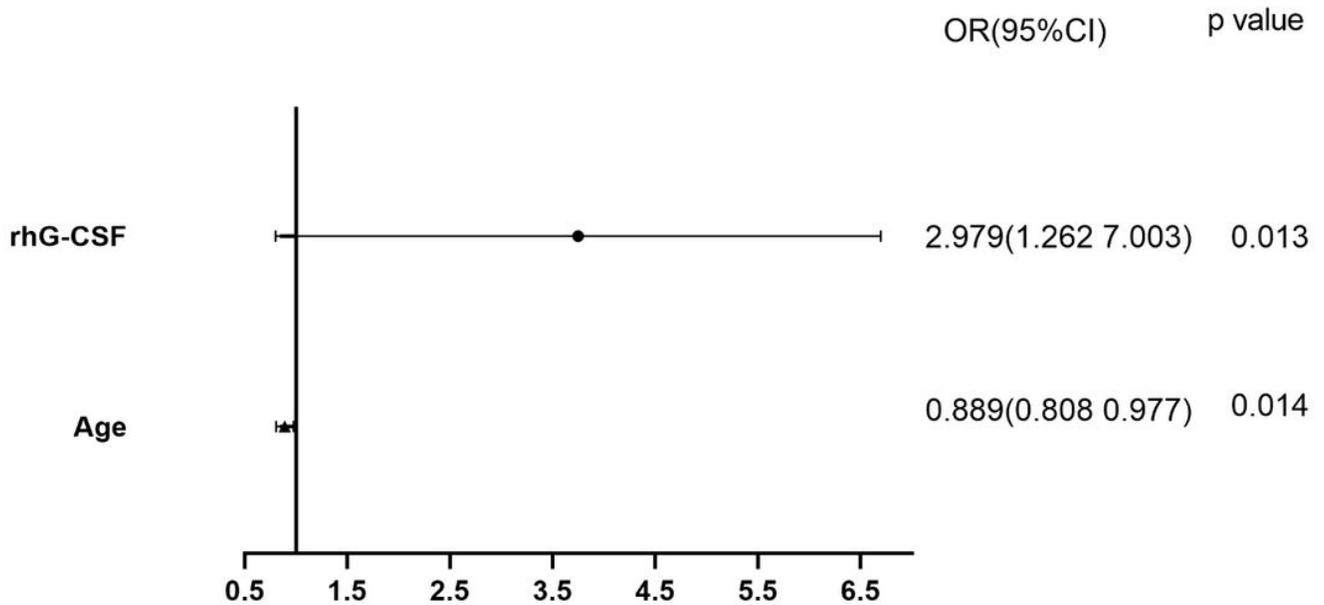


Figure 2

Visualization of binary logistic regression analysis

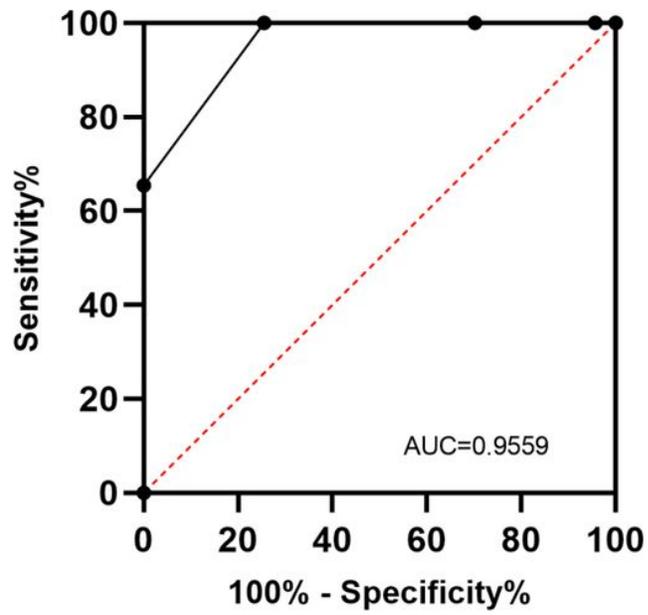


Figure 3

ROC analysis of perfusion times in predicting clinical pregnancy rate