Influences of granulocyte growth factor in uterine perfusion on pregnancy outcome of patients with failure of embryo implantation for unknown reason

Jun He, Juan Liu, Hua Zhou and Chao jun Chen*
Guizhou Provincial People's Hospital, Shandong Province, China

Abstract: To investigate the influence of granulocyte growth factor in uterine perfusion on the pregnancy outcome of patients with failure of embryo implantation for unknown reason. Then, 68 patients with failure of embryo implantation for unknown reason were enrolled in our hospital from November 2013 to February 2015, which were divided into observation group and control group by random (34 patients in each group). Patients in observation group received basic treatment for granulocyte growth factor in uterine perfusion on the next day, while patients in control group received basic treatment with placebo. Then, endometrial preparation, adverse reaction and pregnancy outcome of patients were compared between the two groups. Comparing the endometrial preparation and average endometrial thickness of patients in control group (9.87±2.12) with those in observation group [(9.87±2.12), there is no significant difference (P>0.05). After treatment, patients in both groups performed diabetes, hypertension and other pregnancy complications without difference of statistical significance (P>0.05). The embryo implantation rate and clinical pregnancy rate of patients in observation group were significantly higher than those in control group [(82.35%) and (44.12%) vs (52.94%) and (17.65%)]. Moreover, the live birth rate of patients in observation group performed significantly higher than that in control group [(41.18%) vs (14.71%)] with significant difference (P<0.05). By taking treatment of granulocyte growth factor, patients with failure of embryo implantation can effectively improve clinical pregnancy rate and embryo implantation rate without severe complication. Therefore, treatment of granlocyte growth factor can improve the pregnancy outcome of patients.

Keywords: Granulocyte growth factor, uterine perfusion, failure of embryo implantation, pregnancy outcome.

INTRODUCTION

In current medical field, the main method to treat infertility is *in vitro* fertilization – embryo transfer (IVF-ET). However, the biggest problem in this process is implantation failure (Peng *et al.,* 2015). Clinically, there are a lot of conditions to implant embryo successfully, while the most important conditions are superior embryo, good endometrial receptivity and good synchronous development of embryo and endometrium. Relevant researches on reproductive medicine shows that the major reasons to affect embryo implantation are embryo quality and endometrial receptivity (Bondarenko *et al.,* 2015; Marlow *et al.,* 2015). Therefore, superior embryo can improve the pregnancy rate of patients, good endometrial receptivity can increase embryo adhesion and implantation opportunity. Meanwhile, endometrial receptivity is closely related to blood flow, thickness and microenvironment of endometrium. During the implantation of embryo, both of endocrine and immune systems participate in the adjustment of embryo implantation (Wang *et al.,* 2015; Morishita *et al.,* 2015). At present, recognized pathogenesis of implantation failure includes: Abnormal uterus, infection disease, father chromosome abnormality, or other immune diseases. However, 40% patients have failure of embryo implantation for unknown reason. There are a lot of clinical methods to treat patients with failure of embryo implantation for unknown reason, but the most methods are not widely accepted in clinic for controversial curative effects. Some researches showed that granulocyte growth factor has positive effects on trophoderm during embryo implantation (Shpiz *et al.,* 2015). This work discussed the role of granulocyte growth factor in embryo implantation for patients with implantation failure for unknown reason to study its influence on curative effect and pregnancy outcome. The report is as follows:

**Corresponding author:* e-mail: hejun136@gmail.com

Clinical data

68 patients with failure of embryo implantation for known reason received by Guizhou Provincial People's Hospital were enrolled from November 2013 to November 2015. Ethical approval was given by the Medical Ethics Committee of Guizhou Provincial People's Hospital. All of these patients were in age of 22~37 years old with average age of (26.09±4.45). They had 1~3 times of abortion with average time of (1.04±0.55) and 5~11 gestational weeks when abortion with average weeks of (9.56±0.25). Inclusion criteria (Shahrokh *et al.,* 2015): (1) age<39 years old; (2) abortion or ineffectiveness for more than 4 times with traditional treatment; (3) no abnormality for husband or wife (seminal fluid of husband, uterus of wife, and chromosome, serum hormone, insulin and sugar

tolerance of both were normal); (4) normal autoantibody; (5) negative test results for relevant infectious diseases. Exclusion criteria: (1) abortion caused by internal secretion, immune, heredity, anatomy, infection, etc. (2) having impaired fertility and taking infertility treatment. The whole study was well-informed to patients and their family members who signed written consent and approved and implemented by the ethic committee of our hospital. All the patients were divided into observation group and control group by random number table with 34 patients each group. Patients had no significant difference between the two groups in aspects of age, body mass index, pregnancy times, superior embryo number and other clinical data (P>0.05). Therefore, the comparability is strong.

Methods

Therapeutic method

Patients in observation group took basic treatment by uterine perfusion for granulocyte growth factor on the next day. Basic treatment included immunotherapy of progesterone, immune globulin and lymphocyte of husband. From the day of ovulation to menses or the 7^{th} week of pregnancy, all the patients took 10mg/time/d progesterone by intramuscular injection (manufacturer: Guangzhou Baiyun Shan Ming Xing Pharmaceutical Co., Ltd.; batch number: 20130922; specification: 1ml: 30mg) and 3ml/time/month immune globulin by intramuscular injection (manufacturer: Shanghai Institute of Biological Products Co., Ltd., batch number: 20121218; specification: 10% 3ml). Immunotherapy by lymphocyte of husband: 30ml peripheral blood of subject was extracted for standby and then separated with heparin anticoagulant under aseptic condition to extract lymphocyte. After washing lymphocytes for 3 times with normal saline, 3ml cell suspension was injected at position 6-8 on intradermal arm of woman by intracutaneous injection once a week with 3 times for one course. During the treatment, contraceptives should be used. Based on this, taking uterine perfusion for granulocyte growth factor (manufacturer: Jintan Biotechnology Co., Ltd. of North China Pharmaceuticals; batch number: 20111021; specification: 0.3ml: 75ug): 0.1% iodine was used to wash vagina and vulva for all the patients. Then, endometrium was observed by abdomen B ultrasound. Granulocyte growth factor was pushed to uterine cavity slowly with artificial insemination pipe connecting with injection syringe having 1mlgranulocyte growth factor in it. Insemination pipe should be taken out 15s later and patients were asked to lie in bed for 15~20min.

For control group, basic treatment and treatment with placebo were taken. Basic treatment was as the same as that used by observation group, and 9% sodium chloride (manufacturer: Shandong Hualu Pharmaceutical Co., Ltd.; batch number: 20121021; specification: 100ml) 3~4.5g (40-60mmol), q.o.d was intravenously dripped.

Observation indices

Curative effects on patients were analyzed between both groups by comparing endometrial preparation, pregnancy complication and pregnancy outcome.

Endometrial preparation. In natural cycle: the menstruation of patients was regular with normal ovulation, or endometrial thickness≥8mm; in artificial cycle: the menstruation of patients was irregular with abnormal ovulation, or endometrial thickness<8mm.

Gestation period of patients in both groups was strictly monitored in the whole progress. Two weeks after treatment, human chorionic gonadotropin in serum was examined to determine if patients had pregnancy. Four weeks after treatment, ultrasound was taken to check if there was gestational sac or fetal heart beat and determine if patient had clinical pregnancy. Embryo implantation rate = (implanted embryo/total number) ×100%; clinical pregnancy rate = (clinical pregnancy cycle/implanted cycle) ×100%.

STATISTICAL ANALYSIS

Measuring data that were expressed as ($\bar{\chi}$ ±s) should be tested by t test. Enumeration data, such as pregnancy complications and pregnancy outcome of patients after treatment, were compared by χ^2 test. When P<0.05, the difference has statistical significance.

RESULTS

Comparison of endometrial preparation between the two groups

Compared with the endometrial preparation and average endometrial thickness of patients in control group, those in observation group had no significant difference (P>0.05) after treatment (table 2).

Comparison of pregnancy complications and pregnancy outcome between the two groups

After treatment, all the patients in both groups had diabetes, hypertension and other pregnancy complications. However, there was no obvious statistical significance in difference between the two groups (P>0.05). By comparing the pregnancy outcome of two groups, it was found that the embryo implantation rate and clinical pregnancy rate of patients in observation group were higher than those in control group. Moreover, 1 patient in both groups had development arrest of twins. However, the live birth rate of infants of patients in observation group was also significantly higher than that in control group with significant difference (P<0.05) (table 3).

DISCUSSION

The progress of embryo implantation is complex and affected by various factors. Common reasons to cause the

Table 1: Comparison of Clinical Data between the two groups

Group	Cases	Age (years)	Body mass index(kg/m^2)	Smoking (cases)	Pregnancy frequency	Number of spontaneous abortions (time)	Spontaneous abortion gestational age (week)	Number of high quality embryo
Observation	34	26.43±3.32	27.81±1.93	2(5.88)	3.52±0.35	1.56±0.64	9.69±0.64	7.61±0.82
Control	34	26.57±3.58	27.89±1.97	4(11.76)	3.41±0.43	1.58±0.68	9.55±0.61	7.56±0.79
t/χ^2		0.1672	0.1691	0.7312	1.1569	0.1249	0.9233	0.2560
P		0.8677	0.8662	0.3925	0.2515	0.9010	0.3592	0.7987

Table 2: Comparison of Endometrial Preparation between the two groups

Group	Cases	Artificial cycle	Natural cycle	Average thickness of endometrium (mm)
Observation	34	14(41.18)	20(58.82)	9.87±2.12
Control	34	12(35.29)	22(64.71)	9.85±2.04
t/χ^2		0.2491	0.2491	0.0396
P		0.6177	0.6177	0.9685

Table 3: Comparison of Pregnancy Complications and Pregnancy Outcome between the two groups [n (%)]

Group	Cases	Diabetes	Hypertension	Preeclampsia	Embryo rate of planting	Clinical pregnancy rate	Number of live infant birth
Observation	34	2(5.82)	2(5.82)	1(2.940	28(82.35)	15(44.12)	14(41.18)
Control	34	1(2.94)	0(0.00)	2(5.82)	18(52.94)	6(7.65)	5(14.71)
t/χ^2		0.3487	2.0606	0.3487	6.7194	5.5805	5.9162
P		0.5548	0.1511	0.5548	0.0095	0.0182	0.0150

failure of embryo implantation include uterus abnormality, infectious diseases, chromosome abnormality of father or other autoimmune diseases, while about 40% patients have implantation failure for unknown reason. At present, there are many methods to clinically treat patients with implantation failure, mainly including implanting superior embryo, improving endometrial receptivity, enhancing implanted embryo, intensifying the development synchronization of implanted embryo and endometrium and improving implantation technology (Shevchuk *et al.,* 2015; Tada *et al*., 2015). Among these methods, improving endometrial receptivity is the most important because endometrial receptivity is directly affected the embryo implantation (Lattova *et al.*, 2015). Meanwhile, endocrine system and immune system all participate in embryo implantation. Therefore, endometrial receptivity can be regulated by adjusting immune system under the precondition of good embryo quality to improve embryo implantation rate and clinical pregnancy rate of patients with failure of embryo implantation for unknown reason (Lashley *et al.,* 2014; Haller-Kikkatalo *et al.,* 2014).

With the development of economic society, pregnancy tends to be younger in average age, and artificial abortion and operations also increase year by year. All of these factors cause severe injury to endometrium. In general, the thickness of endometrial tissue will directly affect the pregnancy condition and outcome of patients. The repair and growth of endometrium after injury are closely related to the stem cells on stratum layer. When stem cell is used for lack of endometrium, granulocyte growth factor can repair injured parts and endometrial tissue with mesenchymal stem cells to some extent. In this way, the embryo implantation rate and clinical pregnancy rate of patients can be improved (Simón *et al.*, 2014; Enghelabifar *et al.,* 2014). Featured with analgesia, convenient operation and high compliance, intrauterine infusion is a new-type and non-invasive treatment method which has been applied to the failure of embryo implantation for unknown reason (Plaks *et al.,* 2014; Gores-Lindholm *et al.,* 2013). Relevant research results show that the embryo implantation rate and clinical pregnancy rate of patients with implantation failure for unknown reason are all significantly higher than those of patients taking placebo treatment before embryo implantation and after uterine perfusion for granulocyte growth factor (Nelson and Fong, 2013; Pinedo *et al.,* 2015). Based on this research, this work further concluded that the embryo implantation rate and clinical pregnancy rate of patients taking uterine perfusion for granulocyte growth factor were 82.35% and 44.12%, respectively, significantly higher than those of patients taking placebo treatment, 52.94% and 17.65%. This result further verified that uterine perfusion for granulocyte growth factor is beneficial to improve the success rate of embryo implantation in treating the failure of embryo implantation for unknown reason and increase the

possibility of clinical pregnancy. After taking uterine perfusion for granulocyte growth factor, various bioactive molecules on endometrium can be activated to change molecular functions and structures. Therefore, the growth of embryo and endometrium can be synchronized, endometrial receptivity adjusted and embryo implantation rate improved.

This work also found that the birth rate was 41.18% with 14 live infants born after taking uterine perfusion for granulocyte. This rate was significantly higher than placebo treatment group with 5 infants (14.71%). Moreover, infants of both groups had no malformation. However, it requires further observation and analysis to see if the treatment affects infant development of physique and intelligence.

In conclusion, uterine perfusion for granulocyte combined with basic treatment can improve the embryo implantation rate and clinical pregnancy rate without severe pregnancy complication in treating patients with failure of embryo implantation for unknown reason. This method can improve the pregnancy outcome of patients to some extent. However, the sample size involved in this work was small, and this work was conducted in a single center, therefore, large sample and multi-centered researches are required to obtain further results.

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