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ORIGINAL ARTICLE

Retrospective Study

Effect of intrauterine perfusion of granular leukocyte-colony stimulating factor on the outcome of frozen embryo transfer

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Abstract

BACKGROUND

Treatment of thin endometrium with granular leukocyte-colony stimulating factor (G-CSF) remains controversial.

AIM

To investigate the effect of G-CSF on the outcome of frozen embryo transfer in patients with thin endometrium.

METHODS

A retrospective propensity score matching (PSM) study was performed to assess patients administered frozen embryo transfer at the Reproductive Medicine Center of the Affiliated Drum Tower Hospital of Nanjing University Medical School, in 2012-2018. The patients were divided into G-CSF intrauterine perfusion (G-CSF) and non-G-CSF groups, and clinical pregnancy, implantation, ectopic pregnancy, and early abortion rates between the two groups were compared.

RESULTS

Before PSM, 372 cycles were enrolled, including 242 and 130 cycles in the G-CSF and non-G-CSF groups, respectively. Age (34.23 ± 5.76 *vs* 32.99 ± 5.59 years; P = 0.047) and the blastula/cleavage stage embryo ratio (0.68 *vs* 0.37; P = 0.011) were significantly elevated in the G-CSF group compared with the non-G-CSF group; however, clinical pregnancy (46.28% *vs* 51.54%; P = 0.371) and embryo implantation (35.21% *vs* 35.65%; P = 0.910) rates were similar in both groups. After PSM by age and blastula/cleavage stage embryo ratio, 244 cycles were included (122 cases each in the G-CSF and non-G-CSF groups). The clinical pregnancy (50.82 % *vs* 48.36%; P = 0.701) and embryo implantation (37.38% *vs* 34.11%; P = 0.480) remained similar in both groups.

CONCLUSION

Intrauterine infusion of G-CSF does not improve the clinical outcome of frozen embryo transfer in patients with thin endometrium.

Key Words: Thin endometrium; Granular leukocyte-colony stimulating factor; Intrauterine perfusion; Frozen embryo transfer

Core Tip: Granular leukocyte-colony stimulating factor (G-CSF) administration for the treatment of thin endometrium remains controversial. A retrospective study of patients with thin endometrium who underwent frozen embryo transfer (FET) at the Reproductive Medicine Center of the Affiliated Drum Tower Hospital of Nanjing University Medical School, from January 1, 2012 to December 31, 2018, was performed. This study suggested that G-CSF intrauterine infusion does not increase clinical pregnancy and embryo implantation rates after FET in patients with thin endometrium. Early abortion may be somewhat decreased by G-CSF administration.

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INTRODUCTION

Successful embryo implantation depends on two key factors, including a well-developed embryo and a well-receptive endometrium[1]. Endometrial receptivity refers to the ability of the endometrium to receive embryos, and encompasses endometrial thickness, morphology, and blood flow distribution[2]. Endometrial thickness is one of the most important factors predicting the outcome of pregnancy in assisted reproduction[3,4]. Studies have shown that women with a thin endometrium have more difficulty obtaining good pregnancy outcome compared with those with normal endometrium[5]. Indeed, an endometrium thinner than 7 mm results in decreased odds ratio of achieving pregnancy both in women with pathologic and idiopathic factors[6]. Clinically, approximately 2.4% of patients undergoing *in vitro* fertilization (IVF) show refractory thin endometrium with no response to treatment, and represents a challenging issue[4].

Various treatment options for thin endometrium have been proposed, including high estradiol doses, human chorionic gonadotropin, tamoxifen, pentoxifylline, vitamin E, L-arginine, aspirin, nitroglycerin patches, vaginal sildenafil, acupuncture and neuromuscular electric stimulation, intrauterine administration of granulocyte colony stimulating factor (G-CSF), and stem cell therapy[6-12]. However, G-CSF administration for the treatment of thin endometrium remains controversial. It was first applied by Gleicher and colleagues in patients with thin endometrium in 2011, with increased endometrial thickness and successful pregnancy[13]. However, other studies suggested that G-CSF does not improve pregnancy outcome in patients with thin endometrium[6,14,15].

The above reports clearly indicated that further investigation is required before the wide application of G-CSF in patients with thin endometrium. Therefore, the current study aimed to investigate the effect of G-CSF on the outcome of frozen embryo transfer in patients with thin endometrium, using a propensity score matching (PSM) design.

MATERIALS AND METHODS

Subjects

A retrospective study of patients with thin endometrium who underwent frozen

treatment by written consent.

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embryo transfer (FET) at the Reproductive Medicine Center of the Affiliated Drum Tower Hospital of Nanjing University Medical School, from January 1, 2012 to December 31, 2018 was performed.

Inclusion criteria were: (1) At least one transplantation cycle with an endometrium thickness < 8 mm; (2) At least one high-quality embryo available for freezing; and (3) Endometrium prepared with estrogen replacement treatment. Exclusion criteria were: uterine malformations, intrauterine adhesions, adenomyosis, endometrial polyps, and endometrial tuberculosis.

This study was approved by the ethics committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School. The patients who received intrauterine infusion of G-CSF provided signed informed consent before treatment. For this retrospective study, the requirement for informed consent was waived.

Endometrial preparation protocol

Both groups of patients underwent estrogen and progesterone replacement therapy. From the third day of menstrual bleeding, oral Femoston (Solvay Pharmaceuticals, Netherlands) at 6-8 mg/d was administered, and endometrial thickness was observed at 18-20 d of medication by vaginal ultrasound (Voloson; GE, United States) with a RIC5-9W-RS intracavity probe. Patients with endometrium thickness below 8 mm were asked about their willingness to try the G-CSF perfusion treatment. Intrauterine infusion of G-CSF was therefore performed according to the patient's wishes (G-CSF group); the remaining patients formed the non-G-CSF group. Then, Femoston and progestin (6 mg/d, respectively) and progesterone injection (Zhejiang Xianyu Pharmaceutical Co., Ltd., China) at 60 mg/d (20 mg/branch) were administrated for endometrial transformation. On the fifth day of progestogen administration, intrauterine transplantation of cleavage stage embryos was performed; alternatively, intrauterine transplantation of blastulas was performed on the seventh day of progestogen administration.

Intrauterine perfusion of G-CSF

In the G-CSF group, 1 mL of recombinant human G-CSF (150 μg/branch, 0.5 mL, 0.9 × 10^9 IU; Jie Xin, Jiangsu Wuzhong Pharmaceutical Group and Suzhong Zhongkai Biopharmaceutical Company, China) were transvaginally administered into the uterine cavity through artificial insemination tubes (J-IUIC-351304; COOK, United States) on the day before progestin treatment.

The patient was placed in the lithotomy position on the transplantation bed. After scrubbing the vulva and placing a sterile drape and the speculum, the vagina and the cervical canal were gently scrubbed. An artificial insemination tube was placed into the uterine cavity, and withdrawn after drug administration. Transplantation surgeries were performed by the same team. All participating physicians had more than 2 years of work experience, with job titles of junior and above.

Embryo transplantation

On the day of transplantation, after observing bladder filling by abdominal ultrasound, the patient was placed in the lithotomy position on the transplantation bed. After scrubbing the vulva and placing a sterile drape and the speculum, the vagina and cervical canal were gently scrubbed. A transfer catheter was gently placed under the guidance of abdominal ultrasound, followed by embryo injection. The transfer catheter was then withdrawn, ensuring that the embryo was released. All transplantations were performed by the same team, and the participating physicians and embryologists had > 3 years of work experience, with titles of intermediate senior or senior. The clinical pregnancy rate of artificial insemination in this center was stable as assessed by week, month and year.

Criteria for high quality embryo

Embryos were graded according to the Istanbul Consensus (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011)[16]. High-quality embryos at the cleavage stage included Class I (translucent cells with equal size, with no particles in the cytoplasm and ≤ 5% fragments) and class II (cells with slightly uneven size, with particles in the cytoplasm and 6%-20% fragments) embryos after thawing. High quality blastulas were those with an expansion grade ≥ IV and the trophoblast and inner cell mass not containing C (IVAA, IVAB, IVBB, VAA, VAB and VBB).

Assessment of pregnancy outcome

The serum human chorionic gonadotropin (HCG) index was detected at 14 days after transplantation. HCG-positive patients were those with HCG amounts above the reference range for non-pregnant women (0-5 IU/mL in our center). Ultrasound examination was performed at 28 d after transplantation, and patients with a gestational sac were diagnosed with clinical pregnancy. Successful embryo implantation was reflected by a gestational sac (including intrauterine and ectopic pregnancy) observed by ultrasound. Each pregnancy sac was counted as an embryo. Gestational sacs located in the fallopian tube, pelvis, uterine horn, cervix, cesarean scar, and abdominal cavity indicated ectopic pregnancy. Termination of a pregnancy at less than 12 gestation weeks due to non-human factors was considered early spontaneous abortion.

Observation indicators

Demographic features, including age, BMI, infertility years, primary or secondary infertility, were collected from medical records. Observation outcomes included clinical pregnancy (clinical pregnancy cycles/transplantation cycles × 100%), embryo implantation (number of embryos implanted/total number of embryo transplanted × 100%), ectopic pregnancy (number of cycles with ectopic pregnancy/total number of clinical pregnancy cycles × 100%), and early abortion (number of early spontaneous abortion cycles/total number of clinical pregnancy cycles × 100%) rates.

Statistical analysis

Data analysis was performed with the SPSS 24.0 software (IBM Corp., Armonk, NY, United States). Continuous variables were presented as mean ± SD, and categorical data were shown as frequency and percentage. The propensity score matching method was used to match baseline data with 1: 1. The t-test was carried out for comparing continuous variables. The chi-square test was used for comparing categorical data; variables not meeting the criteria for the chi-square test were compared by the Fisher's exact test. $P < 0.05$ was considered statistically significant.

RESULTS

Baseline patient characteristics

A total of 372 cycles (in 348 patients) were assessed in this study, including 242 and 130 cases in the G-CSF and non-G-CSF groups, respectively. Age (34.23 ± 5.76 *vs* 32.99 ± 5.59 years; $P = 0.047$) and the blastula/cleavage stage embryo ratio (0.68 *vs* 0.37; $P = 0.011$) were significantly elevated in the G-CSF group compared with the non-G-CSF group. The remaining baseline characteristics were similar in both groups (Table 1).

Pregnancy outcomes in the whole patient population

Comparing the 242 and 130 cases in the G-CSF and non-G-CSF groups, there were no statistically significant differences in clinical pregnancy (46.28% *vs* 51.54%; $P = 0.371$), embryo implantation (35.21% *vs* 35.65%; $P = 0.910$), ectopic pregnancy (2.68% *vs* 1.49%; $P > 0.999$) and early abortion (14.29% *vs* 22.39%; $P = 0.166$) rates (all $P > 0.05$; Table 1). There were no adverse reactions in either groups.

Pregnancy outcomes after PSM

As shown above, there were statistically significant differences in age and blastula/cleavage stage embryo ratio between the two groups in the whole patient population. Therefore, the propensity score matching method was used to match both groups of these parameters. As a result, 122 cases were included in each of the G-CSF and non-G-CSF groups. As shown in Table 2, there were no statistically significant differences in clinical pregnancy (50.82% *vs* 48.3%; $P = 0.701$), embryo implantation (37.38% *vs* 34.11%; $P = 0.480$), ectopic pregnancy (3.23% *vs* 1.69%; $P = 0.965$), and early abortion (12.90% *vs* 23.73%; $P = 0.123$) rates between the two groups.

Pregnancy outcomes in cycles with blastula transplantation or cleavage stage embryo transplantation after PSM

Considering the possible effect of embryo transfer type on pregnancy outcome, the results were compared between blastula transplantation and cleavage stage embryo transplantation. Totally 35 blastula transplantation cases were included in each of the

Table 1 Baseline patient features and pregnancy outcomes in the whole study population

Characteristics	G-CSF group (*n* = 242)	Non-G-CSF group (*n* = 130)	*P* value
Age (yr), mean ± SD	34.23 ± 5.76	32.99 ± 5.59	0.047
BMI (kg/cm^2), mean ± SD	21.35 ± 3.97	20.89 ± 2.63	0.182
Average infertility years (yr), mean ± SD	5.13 ± 1.75	4.92 ± 1.81	0.281
Primary infertility cycle, *n* (%)	79 (32.64)	53 (40.77)	0.118
Secondary infertility cycle, *n* (%)	163 (67.36)	77 (59.23)	
Average endometrial thickness (mm), mean ± SD	7.16 ± 0.40	7.18 ± 0.43	0.655
Cycle with one transplanted embryo, *n* (%)	71 (29.34)	30 (23.08)	0.195
Cycle with two transplanted embryos, *n* (%)	171 (70.66)	100 (76.92)	
Cycle with blastula transplantation, *n* (%)	97 (40.08)	35 (26.92)	0.011
Cycle with cleavage stage embryo transplantation, *n* (%)	145 (59.92)	95 (73.08)	
Clinical pregnancy rate, %	46.28 (112/242)	51.54 (67/130)	0.371
Embryo implantation rate, %	35.21 (144/409)	35.65 (82/230)	0.910
Ectopic pregnancy rate, %	2.68 (3/112)	1.49 (1/67)	> 0.999[1]
Early abortion rate, %	14.29 (16/112)	22.39 (15/67)	0.166

[1]Fisher's exact test. G-CSF: Granular leukocyte-colony stimulating factor; BMI: Body mass index.

Table 2 Baseline patient features and pregnancy outcomes after propensity score matching based on age and blastula/cleavage stage embryo ratio

Characteristics	G-CSF group (*n* = 122)	Non-G-CSF group (*n* = 122)	*P* value
Age (yr), mean ± SD	33.54 ± 5.97	33.45 ± 5.45	0.902
BMI (kg/cm^2), mean ± SD	21.17 ± 2.33	20.79 ± 2.71	0.241
Average infertility years (yr), mean ± SD	4.77 ± 1.98	4.95 ± 1.93	0.473
Primary infertility cycle, *n* (%)	45 (32.64)	50 (40.77)	0.512
Secondary infertility cycle, *n* (%)	77 (67.36)	72 (59.23)	
Average endometrial thickness (mm), mean ± SD	7.13 ± 0.43	7.17 ± 0.43	0.468
Cycle with one transplanted embryo, *n* (%)	30 (24.59)	30 (24.59)	> 0.999
Cycle with two transplanted embryos, *n* (%)	92 (75.41)	92 (75.41)	
Cycle with blastula transplantation, *n* (%)	35 (28.69)	35 (28.69)	> 0.999
Cycle with cleavage stage embryo transplantation, *n* (%)	87 (71.31)	87 (71.31)	
Clinical pregnancy rate, %	50.82 (62/122)	48.3 (59/122)	0.701
Embryo implantation rate, %	37.38 (80/214)	34.11 (73/214)	0.480
Ectopic pregnancy rate, %	3.23 (2/62)	1.69 (1/59)	0.965
Early abortion rate, %	12.90 (8/62)	23.73 (14/59)	0.123

G-CSF: Granular leukocyte-colony stimulating factor; BMI: body mass index.

G-CSF and non-G-CSF groups. As shown in Table 3, there were no statistically significant differences in clinical pregnancy (68.57% *vs* 57.14%; $P = 0.322$), embryo implantation (57.41% *vs* 44.64%; $P = 0.181$), ectopic pregnancy (4.17% *vs* 0%; $P > 0.999$) and early abortion (20.83% *vs* 22.50%; $P = 0.974$) rates between the two groups. A total of 87 cases with cleavage stage embryo transplantation were included in each of the G-CSF and non-G-CSF groups. As shown in Table 4, there were no statistically significant differences in clinical pregnancy (43.68% *vs* 44.83%; $P = 0.879$), embryo implantation

Table 3 Baseline patient features and pregnancy outcomes in cycles with blastula transplantation after propensity score matching based on age and blastula/cleavage stage embryo ratio

Characteristics (blastula)	G-CSF group (*n* = 35)	Non-G-CSF group (*n* = 35)	*P* value
Age (yr), mean ± SD	31.26 ± 4.80	31.71 ± 4.46	0.686
BMI (kg/cm^2), mean ± SD	21.93 ± 2.41	21.01 ± 2.65	0.133
Average infertility years (yr), mean ± SD	4.68 ± 1.91	4.80 ± 1.98	0.797
Primary infertility cycle, *n* (%)	13 (37.14)	16 (45.71)	0.467
Secondary infertility cycle, *n* (%)	22(72.86)	19(54.29)	
Average endometrial thickness (mm), mean ± SD	7.10 ± 0.53	7.07 ± 0.54	0.815
Cycle with one transplanted embryo, *n* (%)	16 (45.71)	14 (40.00)	0.233
Cycle with two transplanted embryos, *n* (%)	19 (54.29)	21 (60.00)	
Clinical pregnancy rate, %	68.57 (24/35)	57.14 (20/35)	0.322
Embryo implantation rate, %	57.41 (31/54)	44.64 (25/56)	0.181
Ectopic pregnancy rate, %	4.17 (1/24)	0 (0/20)	> 0.999[1]
Early abortion rate, %	20.83 (5/24)	22.50 (5/20)	0.974[2]

[1]Fisher's exact test.
[2]Corrective Chi-square test. G-CSF: Granular leukocyte-colony stimulating factor; BMI: body mass index.

Table 4 Baseline patient features and pregnancy outcomes in cycles with cleavage stage embryo transplantation after propensity score matching based on age and blastula/cleavage stage embryo ratio

Characteristics (cleavage stage embryo)	G-CSF group (*n* = 87)	Non-G-CSF group (*n* = 87)	*P* value
Age (yr), mean ± SD	35.29 ± 6.18	34.91 ± 5.67	0.673
BMI (kg/cm^2), mean ± SD	20.86 ± 2.25	20.70 ± 2.73	0.674
Average infertility years (yr), mean ± SD	4.81 ± 2.13	5.01 ± 1.90	0.514
Primary infertility cycle, *n* (%)	32 (36.78)	34 (39.08)	0.755
Secondary infertility cycle, *n* (%)	55 (63.22)	53 (60.92)	
Average endometrial thickness (mm), mean ± SD	7.17 ± 0.39	7.26 ± 0.38	0.125
Cycle with one transplanted embryo, *n* (%)	14 (16.09)	16 (18.39)	0.161
Cycle with two transplanted embryos, *n* (%)	73 (83.91)	71 (81.61)	
Clinical pregnancy rate, %	43.68 (38/87)	44.83 (39/87)	0.879
Embryo implantation rate, %	30.63 (49/160)	30.38 (48/158)	0.962
Ectopic pregnancy rate, %	2.68 (1/38)	1.49 (1/39)	> 0.999[1]
Early abortion rate, %	14.29 (3/38)	22.39 (9/39)	0.066

[1]Fisher's exact test. G-CSF: Granular leukocyte-colony stimulating factor; BMI: body mass index.

(30.63% *vs* 30.38%; $P = 0.962$), ectopic pregnancy (2.68% *vs* 01.49%; $P > 0.999$) and early abortion (14.29% *vs* 22.39%; $P = 0.066$) rates between the two groups.

DISCUSSION

Reports have shown that CRL is larger in the first trimester in frozen-thawed embryo transfer compared with fresh embryo transfer[17]. Decreased risks of small for gestational age, low birth weight and preterm delivery have also been found in frozen-thawed embryo transfer[18] . In addition, uterine artery PI is lower in frozen-thawed embryo transfer compared with fresh embryo transplantation both in the first

trimester and across the whole gestation[19]. After conception, when uterine artery resistance index does not decrease correspondingly, central arterial blood pressure increases, resulting in pressure vasospasm, in turn causing increased blood pressure, forming a vicious cycle[20]. However, a large number of retrospective studies have confirmed that frozen-thawed embryo transfer has a greater risk of pregnancy hypertension compared with fresh embryo transfer[21]. In addition, embryonic cryo-thawing does not seem to prevent preterm birth in IVF pregnancies[22]. Many scholars believe that frozen-thawed embryo transfer can reduce the occurrence of pregnancy-related complications. Frozen-thawed embryo transfer is often used as a supplement after failed fresh embryo transfer cycle, and the mother's state at conception is closer to that of natural conception. Moreover, frozen-thawed embryo transfer is less affected by other confounders than fresh embryo transfer, so it was examined in this study.

The present study demonstrated that intrauterine infusion of G-CSF does not improve the clinical outcome of frozen embryo transfer in patients with thin endometrium.

The FET technology reduces ovarian hyperstimulation syndrome (OHSS) occurrence[23] and the number of repetitive egg retrievals, improving embryo utilization and increasing the rate of cumulative pregnancy, as an important part of assisted reproductive technology (ART). Endometrial thickness represents one of the most important parameters for evaluating endometrial receptivity, and is closely related to the pregnancy outcome of assisted reproduction technology[2]. Currently, no uniform definition of thin endometrium is available. Generally, it is considered that an endometrium with a thickness below 7-8 mm cannot effectively support embryo implantation and maintain subsequent pregnancy. Therefore, the pregnancy rate of patients with thin endometrium is significantly reduced[3,24,25]. We consider an endometrial thickness < 8 mm to indicate a thin endometrium. In FET cycles, clinical pregnancy and live birth rates are significantly lower in patients with endometrial thickness ≤ 8 mm compared with the > 8 mm group[26]. Therefore, thin endometrium severely affects the success rate of IVF and imposes tremendous mental and economic burdens on patients.

Gleicher firstly infused G-CSF into the uterine cavity in 4 patients with thin endometrium, who all showed increased endometrial thickness and achieved clinical pregnancy[27]. This technique was repeated for 21 similar cases; although endometrial thickness was increased, only 19.1% of patients achieved clinical pregnancy[13]. However, Gleicher *et al*[27] used a self-control design, and the sample size was small. Since then, G-CSF has been assessed in multiple studies, with discrepant findings. For example, Sarvi *et al*[28] administered G-SCF in the superovulation cycle, and endometrial thickness and implantation rate were significantly improved, while Barad *et al*[29] reported opposite results. In FET, Gao *et al*[30] indicated that intrauterine perfusion of G-SCF achieves significant improvements in endometrial thickness and implantation rate, while Check *et al*[31] has a different conclusion. It should be noted that study protocols, perfusion times, and G-CSF doses were not uniform among the above studies.

To investigate whether intrauterine G-CSF infusion improves the clinical outcome of FET in patients with thin endometrium, this study retrospectively analyzed 372 FET cycles (in 348 patients). The results showed that clinical pregnancy, implantation, ectopic pregnancy and early abortion rates were similar in the G-CSF and non-G-CSF groups, corroborating previous reports[14,30]. Early abortion rate in the G-CSF group was 14.29% *vs* 22.39% for the non-G-CSF group, suggesting a trend of reduction after G-CSF treatment. In baseline data, age and blastula/cleavage stage embryo ratio showed statistically significant differences between the two groups, with higher values in the G-CSF groups. Age is an important factor affecting the clinical outcome of IVF. As age increases, clinical pregnancy and live birth rates show a downward trend[32]. Meanwhile, it is generally admitted that the implantation rate of blastulas is higher than that of cleavage stage embryos. In agreement, previous data in our center showed that the clinical pregnancy rate of cleavage stage embryos is significantly lower than that of blastulas[33]. Considering that age and blastula/cleavage stage embryo ratio differences may bias the clinical results, corrections were made by the propensity score matching method. After matching for age and blastula/cleavage embryo ratio in this study, pregnancy outcomes were not significantly different between the two groups. Similar results were obtained after subgroup analysis (in cycles with blastula transplantation or cleavage stage embryo transplantation). However, a 10.83% reduction in the early abortion rate was found in the G-CSF group (12.90%) compared with the non-G-CSF group (23.73%), suggesting that early abortion may be somewhat decreased by G-CSF administration. This could be explained by the fact that G-CSF can improve endometrial angiogenesis and effectively promote the regeneration of

endometrial cells[34,35]. In addition, G-CSF is considered to be closely related to the menstrual cycle and pregnancy[36]. Our center has demonstrated that intrauterine perfusion of G-CSF significantly increases vascular endothelial growth factor (VEGF) amounts in human endometrial stromal cells by 2.25 times, suggesting that G-CSF may upregulate endometrial VEGF and increase blood flow (data submitted elsewhere). VEGF is a key factor in the regulation of endometrium vascular growth. It was shown that VEGF expression and microvessel density are significantly reduced in glandular epithelial cells of thin endometrium[35]. In addition, VEGF amounts in serum, villi and decidual tissues from patients with spontaneous abortion are lower than those of normal pregnant women[37,38]. The elevated trend of abortion rate in patients with thin endometrium may be related to reduced VEGF amounts. Therefore, we speculate that the slight decrease observed in abortion rate after intrauterine perfusion of G-CSF may be related to the above mechanism.

G-CSF, a cytokine that can stimulate the differentiation of bone marrow hematopoietic cells, was initially mainly used in the treatment of blood system diseases associated with leukopenia; recently, it was found to promote angiogenesis and immune regulation and inhibit inflammatory reactions with overt neurotrophic effect, and has been widely applied in nervous, cardiovascular and endocrine system diseases[39].The expression of G-CSF receptor was found in ovarian granulosa cells, the endometrium and the placenta. G-CSF plays an important role in pregnancy. It not only affects embryo implantation and ovarian function, but also promotes endometrial thickening, and can even be used as a remedy for embryo implantation failure[40]. One of the most important causes of implantation failure is the thin endometrium[41, 42]. G-CSF can increase the recruitment of regulatory T cells and DC cells in the endometrium, affecting the remodeling of endometrial blood vessels, the regulation of the immune environment in the uterus, and the expression of key genes in the cell adhesion pathway during the process of transplantation[43]. In recent years, many studies have suggested that G-CSF, as a glycoprotein, improves endometrial thickness and facilitates embryo implantation[44,45]. A meta-analysis also showed that G-CSF administration has a beneficial role in clinical outcome after embryo transfer by both routes of local infusion and systematic administration, especially in cases with RIF[46].

G-CSF promotes embryo cleavage and blastocyst formation[47], controlling endometrial vascular remodeling, local immune modulation and cellular adhesion pathways, thereby playing an important role in embryo development and implantation. However, intrauterine surgery has a certain risk of infection, especially in case of infection by pathogenic microorganisms such as mold and bacteria in the vagina, and has a close relationship with the doctor's operation. Although there is still a lack of studies assessing the drug toxicity and teratogenicity of intrauterine G-CSF infusion, the possibility of serious adverse reactions cannot be completely ruled out. Therefore, whether this technique should be selected as a means to improve the clinical outcome should consider its advantages and disadvantages in the light of each patient's situation.

In the fresh embryo transplantation cycle, the endometrium is generally thicker due to stimulation by ovulation therapy, and thin endometrium is relatively rare. In addition, in the fresh cycle, patients with thin endometrium are often treated with cycle abandonment and frozen-thawed embryo transplantation. The data are insufficient. It is not clear whether the use of G-CSF is more beneficial to the thin endometrium of the fresh embryo transplantation cycle.

The current study had limitations. First, it was a retrospective trial, with inherent shortcomings. In addition, it was a single-center study, and selection bias could not be ruled out. Finally, the sample was relatively small. Therefore, large well-designed multi-center studies are warranted to comprehensively determine the effects of G-CSF on pregnancy outcome in patients with thin endometrium.

CONCLUSION

This study suggested that G-CSF intrauterine infusion does not increase clinical pregnancy and embryo implantation rates after FET in patients with thin endometrium.

ARTICLE HIGHLIGHTS

Research background

Endometrial thickness is one of the most important factors predicting the outcome of pregnancy. Women with a thin endometrium have difficulty obtaining good pregnancy outcome. Treatment of thin endometrium with granular leukocyte-colony stimulating factor (G-CSF) remains controversial.

Research motivation

G-CSF administration for the treatment of thin endometrium remains controversial.

Research objectives

This study aimed to investigate the effect of G-CSF on the outcome of frozen embryo transfer in patients with thin endometrium.

Research methods

A retrospective propensity score matching (PSM) study was performed to assess patients administered frozen embryo transfer at the Reproductive Medicine Center of the Affiliated Drum Tower Hospital of Nanjing University Medical School, in 2012-2018. The patients were divided into G-CSF intrauterine perfusion (G-CSF) and non-G-CSF groups, and clinical pregnancy, implantation, ectopic pregnancy, and early abortion rates between the two groups were compared.

Research results

After PSM by age and blastula/cleavage stage embryo ratio, 244 cycles were included (122 cases each in the G-CSF and non-G-CSF groups). The clinical pregnancy (50.82 % *vs* 48.36%; $P = 0.701$) and embryo implantation (37.38% *vs* 34.11%; $P = 0.480$) remained similar in both groups.

Research conclusions

This study suggested that G-CSF intrauterine infusion does not improve the clinical outcome of frozen embryo transfer in patients with thin endometrium.

Research perspectives

It provides some ideas for the G-CSF treatment of patients with thin endometrium.However, large well-designed multi-center studies are warranted to comprehensively determine the effects of G-CSF on pregnancy outcome in patients with thin endometrium.

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