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Research Article

Effect of Intrauterine Infusion of Granulocyte Colony Stimulating Factor on IVF Outcomes in Infertile Women

Abstract

Objective: The primary goal of this study was to assess the G-CSF effects on IVF outcomes in women with normal endometrial thickness.

Patients and Methods: This was a randomized controlled study performed at Assisted Reproductive Techniques Center of Ain Shams University Maternity Hospital, over a 2-year period, between Jan 2014 and Jan 2016, and included 60 infertile women with normal endometrial thickness. Women were scheduled for IVF and randomized into two groups. Exclusion criteria were positive history of repeated implantation failure (RIF), endocrine disorders, severe endometriosis, congenital or acquired uterine anomaly and contraindication for G-CSF (renal disease, sickle cell disease, or malignancy). In G-CSF group (n=30), 300 µg trans-cervical intrauterine of G-CSF was administered at the oocyte retrieval day. Controls (n=30) were treated with standard protocol. Chemical, clinical and ongoing pregnancy rates, implantation rate, and miscarriage rate were compared between groups.

Results: Biochemical pregnancy had occurred in 27.3% of participants in group I and 21.3% of women in group II with a significant difference between the two groups. The clinical pregnancy rate was 22% and 16% in group I and II respectively with no significant difference between the two groups. The acceptability of women in group I was 68%, mild discomfort occurred in 44.7%, moderate discomfort was in 28.7%, severe discomfort was in 11.3% and 15.3% of women in group I had no discomfort at all. Difficult flushing had occurred in 14.7% of women.

Conclusion: It looks that the clinical pregnancy rates were significantly higher by intracavitary infusion of granulocyte colony stimulating factor

Introduction

Infertility is the failure of conception after one year or more of regular intercourse with no contraceptive method. Epidemiological studies had demonstrated that about 80% of couples had succeeded to conceive during that period. It is postulated that almost 15% of couples are infertile in developed countries [1]. There had not been reported a substantial increase in demand for the management of infertility in the last decade [2]. Embryo implantation largely depends on the quality of the embryo and the endometrial receptivity. It is estimated that implantation failure is responsible for approximately 50% to 75% of lost pregnancies [3].

Despite major advancements in assisted reproductive techniques, the implantation rates remain relatively not high. "Successful implantation requires good quality of embryos, receptive endometrium, and proper embryo transfer technique"

[1]. The receptive endometrium is defined as a healthy uterine milieu which support the transformation of endometrial cells into decidual cells, invasion of blastocysts, and fast growth of placenta [2]. This mechanism is helped by immune cells, growth factors, cytokines, and hormonal changes [3,4]. Immunological mechanisms in the endometrium are very crucial and important in implantation [5]. Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine appears in materno-fetal interface during embryo implantation and early pregnancy suggesting it may have a role in decidua and placental development [6]. It enhances granulocyte proliferation and differentiation [7].

Some studies had shown that systemic administration of G-CSF in patients with recurrent spontaneous pregnancy losses and repetitive implantation failures improves pregnancy outcomes [8-10]. Also, G-CSF transvaginal infusion successfully were utilized in women with low endometrial

thickness (<7 mm) and recurrent implantation failures recently [11-13]. Eftekhari et al., demonstrated that intrauterine G-CSF administration increased chemical and clinical pregnancy rates in infertile women with thin endometrium in frozen-thawed embryos transfer cycles but they showed in their study endometrial thickness in their patients did not improve [6]. Fewer trials have tested the G-CSF effect in women with normal endometrial thickness. Barad et al., reported that intrauterine G-CSF infusion in fresh embryo transfer cycles in women underwent IVF treatment did not improve endometrial thickness, implantation, and clinical pregnancy rates [7]. So, it is hypothesized that G-CSF inflammatory and immunological effects might improve the implantation rate and endometrial receptivity in infertile women. In this study, G-CSF effect on implantation and pregnancy rates in normal infertile women were examined.

Patients and Methods

This study was carried out in a private IVF centre in conjunction with Assisted Reproductive Techniques Center of Ain Shams University Maternity Hospital after the approval of the Research Ethics Committee, during the period between Jan 2014 to Jan 2016 and included 60 women, with unexplained infertility undergoing *in vitro* fertilization (IVF), were randomised into two groups. After receiving informed written consent from all participants and their spouse, according to enveloped pocket method women were allocated randomly in two groups (G-CSF and control group). Standard agonist or antagonist protocol was used for ovarian stimulation in groups [14]. When at least two follicles achieved 17 mm diameter, Human chorionic gonadotropin (hCG) (Choriomon 10000 IU, IBSA Institute, Switzerland) was administered for final oocyte maturation. Transvaginal oocyte retrieval was performed 36 hr after hCG injection. The oocytes were fertilized by intracytoplasmic sperm injection method.

Group I (n=30) at the day of oocyte retrieval, after oocytes collection, 300 mg G-CSF (300 µg/mL, Neupogen; Hoffmann-La Roche Ltd, Basel Switzerland) was administered by slow transcervical intrauterine infusion with IUI catheter (AINSEGREGY, RIMOS, Italy) as described in a previous clinical trial [6]. In controls, the cycles were continued without G-CSF infusion. In all patients, 2-3 embryos were transferred by using embryo transfer catheter (Cook USA), two days after oocyte retrieval.

Group II (n=30) received the same protocol of induction but no intrauterine infusion of G-CSF was done. Pregnancy outcomes were assessed based on positive serum βhCG test (chemical pregnancy), 14 days after embryo transfer and observation of gestational sac on transvaginal ultrasound examination (clinical pregnancy), three weeks after positive serum βhCG. Implantation rate was assessed by the number of gestational sacs divided by the number of transferred embryos in each group. The ongoing pregnancy rate was defined as the presence of fetal heart activity by ultrasonography after 12 wks of pregnancy. The miscarriage rate was assessed by the number of miscarriages before 20 wks gestation per number of women with positive βhCG test.

Inclusion criteria

1. Primary or secondary infertility ≥ one year
2. Participant age: 18 - 37
3. Diagnosis of unexplained infertility ≤ 36 months
4. Anti-Müllerian hormone ≥ 0.4 ng/mL and/or folliclestimulating hormone ≤13 IU/L in early follicular phase
 - a. Regular cycle of 25–35 days, positive ovulation tests, and/or midluteal progesterone ≥25 nmol/L in an unstimulated cycle
 - b. Normal semen analysis according to WHO 2010 criteria
 - c. No uterine cavity abnormalities
 - d. Normal Fallopian tubes
 - e. Negative genitourinary test for chlamydia and gonorrhoea ≤ one year

Exclusion criteria

1. Body mass index (BMI) ≥35 kg/m²
2. Ongoing conception

All included women were subjected to revising history and examination sheets with particular emphasis on personal history: age, residence, education level and socioeconomic status, Complaint regarding infertility, obstetric history including parity and gravidity and ultrasound for any uterine or tubal abnormality, the number of ovarian follicles and the diameter of the dominant follicle. The endometrium was measured at the greatest anteroposterior dimension under a longitudinal section.

A simple computer-generated randomization was done by an independent statistician in a ratio of 1:1 and transferred into sealed opaque envelopes.

Statistical methods

Data were analyzed using IBM® SPSS® Statistics version 22 (IBM® Corp., Armonk, NY, USA) and XLSTAT™ version 2014.5.03 (Addinsoft™, NY, USA). Normally distributed numerical variables were presented as mean (SD) and intergroup differences were compared using the unpaired *t* test. Skewed numerical variables and discrete variables were presented as median (interquartile range) and between-group comparisons were done using the Mann-Whitney test. Categorical variables were presented as number (%) and intergroup differences were compared using the chi-squared test with Yates' continuity correction or Fisher's exact test, when appropriate. Ordinal data were compared using the chi-squared test for trend. A two-sided *p*-value <0.05 was considered statistically significant.

Results

Baseline characteristics, 60 couples suffering from infertility were enrolled in this study, after being randomly assigned to two groups, 30 in each [Tables 1-4].

Discussion

In the present clinical trial, G-CSF effects on implantation and pregnancy rates in infertile women candidate for IVF treatment were tested. It was found that pregnancy outcomes did not increase significantly after intra uterine G-CSF infusion in women with normal endometrial thickness. G-CSF is a factor that improving the synchronization between uterine milieu and embryo development during endometrial remodeling [15,16]. Previous studies had showed that G-CSF can improve recurrent pregnancy losses by increasing the inflammation process and subsequently endometrial receptivity [8-11].

In 2011, Gleicher et al., demonstrated a new option for thin endometrium treatment. They tested the G-CSF effect in four women who underwent IVF that endometrial thickness had not improved with routine regimen. They demonstrated successful endometrial thickness to at least 7 mm after G-CSF uterine infusion and all women were conceived [12]. Also, Tehraninejad et al in a study on fresh embryos transfer cycles in women with history of IVF cycle cancellation because of thin endometrium

Table 1: The clinic-demographic differences between group I (G-CSF + ICSI) and Group II (ICSI alone).

	Group I (30)	Group II (30)	P- value
Age	31.4 ± 3.2	32.1 ± 3.5	> 0.05
Menarche age	11.3 ± 3.2	11.5 ± 3.8	> 0.05
Body mass index (kg/m ²)	28.2 ± 3.8	27.6 ± 3.6	> 0.05
Previous gravidity	1 ± 0.8	1 ± 0.6	> 0.05
Type of infertility			
1ry	12	11	> 0.05
2ry	18	19	
Duration of infertility	7.8 ± 3.1	7.6 ± 2.8	> 0.05
Education			
≤High school	18	16	> 0.05
>High school	12	14	
Occupation			
House wife	19	16	> 0.05
Employed/business Woman	11	14	
Number of developing follicles at insemination	2.7±1.6	2.8±1.3	> 0.05
Mean diameter of dominant follicles at insemination	20.2 ± 1.5	19.2± 2.6	> 0.05
Mean endometrial thickness	8.2 ± 1.9	8.3 ± 1.7	> 0.05

* Analysis using independent student's t-test. NS = non-significant.

Table 2: Shows a comparison between the two studied groups as regards the biochemical and clinical pregnancy rates.

Pregnancy rates	Group I (%)	Group II (%)	P
Biochemical pregnancy	27.3	21.3	< 0.05(sig)
Clinical pregnancy	22	16	< 0.05(sig)

Table 3: Patient compliance and acceptability during and after G-CSF flush.

Patient compliance	(%)
Mild discomfort	44.7
Moderate discomfort	28.7
Severe discomfort	11.3
No discomfort	15.3
Difficult flushing	14.7
Acceptability	68

Table 4: side effects of G-CSF flush.

	Group I (%)	Group II (%)	P- value
Nausea	(21.3)	(6.7)	< 0.05
Vomiting	(10)	(2)	< 0.05
Abdominal cramping	(37.3)	(6)	< 0.05
Vaginal bleeding	(12.7)	(2.7)	< 0.05
No. of patients reporting > 1 of the above side effects (n)	(22.7)	(4.7)	< 0.05

reported that the pregnancy chances and endometrial thickness was improved after G-CSF infusion [13].

While Eftekhar et al in their non-randomized clinical study reported that G-CSF increased implantation and clinical pregnancy rates in infertile patients with thin endometrium in frozen-thawed embryos transfer cycles without increasing endometrial thickness [6].

In this study endometrial thickness in participants was in normal range (8-15 mm). We did not get significant differences between two groups as regards chemical, clinical, ongoing pregnancy, implantation, and pregnancy loss rates. There are few studies on the effect of G-CSF in women with a normal endometrial thickness.

In agreement to our results, Barad et al., demonstrated that intrauterine G-CSF infusion in fresh embryo transfer cycles in IVF women with normal endometrial thickness did not affect endometrial thickness, implantation, and clinical pregnancy rate [7]. So, it seems when there is evidence of spoiled endometrial receptivity, like low thickness, RIF, or early miscarriage, G-CSF had beneficial effects on pregnancy and implantation rates. Transvaginal ultrasound evaluation of endometrium could be used to determine preparation of the endometrium before embryo transfer. It was unclear that these assessments were helpful in determining whether the endometrium is optimally prepared [17].

A systematic review and meta-analysis of 14 studies demonstrated that there may be a relationship between endometrial thickness and conception, but implantation is more complex than be assessed by single scan [18]. Now, during the management an infertile couple, the Endometrial Receptivity Array (ERA test) leads to the assessment, at molecular level, of the endometrial factors [19]. So, it is assumed that for better G-CSF assessment effects on endometrial receptivity and implantation, the molecular G-CSF effects e.g. proteomics, integrins, transcriptomics and ERA test be utilized in further studies [19,20].

In summary, we demonstrated that, in normal IVF women who had normal endometrium, the intrauterine instillation of G-CSF did not increase pregnancy outcomes. The available evidence did not support the routine use of G-CSF in infertile women with normal endometrial thickness. More randomized controlled studies are required for comparison of G-CSF effects on women with thin and normal endometrial thickness.

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