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ENDOMETRIAL PERFUSION WITH GRANULOCYTE COLONY STIMULATING FACTOR IN INFERTILE WOMEN

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Abstract
The success rate of in vitro fertilization is still less than 40%. In implantation process, immunological mechanisms within the endometrium are very important and vital. Granulocyte-colony stimulating factor may play a crucial role in success of human reproduction by influencing implantation.
To evaluate the role of granulocyte-colony stimulating factor on pregnancy rate and endometrial receptivity by observing its effect on VEGF, TGF, Estradiol, progesterone and ultrasound Doppler Findings as endometrial thickness, pulsatile index, resistance index, V1/V2 and Zones.
A randomized clinical trial study conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University for a period of two years from 1st Sep. 2018 to 1st Sep. 2020. It included 40 women complaining from infertility with or without previous failure trial of Intracytoplasmic Sperm Injection and managed to undergo IVF / ICSI protocols reaching the day of embryo transfer with grade 1 (G1) embryos and received granulocyte-colony stimulating factor endometrial perfusion 6-12 hours before hCG trigger.
In this study, progesterone, VEGF, estradiol, endometrial thickness, RI, PI, V1/V2, zone and TGF were significantly improved at day of OPU compared to that at day of hCG. Pregnancy rate was 47.5%.
Granulocyte-colony stimulating factor has the potential to improve chemical and clinical parameters of the infertile females. This reflects increasing implantation rate, biochemical and clinical pregnancy rate.

Keywords: G-CSF, infertility, pregnancy rate, IVF, Iraq.

Introduction

Infertility has been considered as a public health priority [1]. It is more than a quality-of-life issue, with public health consequences including mental distress, financial strain, social stigmatization, and marital discord [2]. It affects 15% of reproductive age couples worldwide [3]. The rate of success of in vitro fertilization (IVF) is still < 40%, and it is time consuming technology and expensive [4]. Many factors could have impact on the IVF-embryo transfer success [5]. In spite of the fact that the endometrium and the embryo are two leading actors in the implantation, there are other powerful factors such as parental chromosome and anatomic structures, oocyte, and sperm parameters, immunologic factors, thrombophilic conditions and lifestyle, which means that for the treatment of infertility, a multidisciplinary approach is required [6]. In implantation process, immunological mechanisms within the endometrium are very important and vital [7]. Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic-specific cytokine produced by bone marrow cells, stromal cells, fibroblasts, and macrophages [6]. G-CSF participates in increasing phagocytosis and the oxidative process in mature neutrophils [8]. It has proposed to play an important role in human reproductive success by affecting implantation. The mechanism was assumed to invigorate neutrophilic granulocyte proliferation and differentiation and act on decidual cells macrophages [9]. Now, during the process of pregnancy forming, several physiological roles have been recommended for G-CSF i.e., improving embryo cleavage and formation of blastocyst [10], controlling endometrial expressions vital for a series of implantation processes including remodeling of endometrial vessels, local immune modulation and pathways of cellular adhesion [11], and focusing on development of follicle and ovulation [12]. It can be given either by infusing it in the uterus (womb) by a syringe around the time of transfer of embryo or subcutaneously after transfer of embryo. Interventional studies are needed to confirm the efficacy of G-CSF in improving endometrium and rates of pregnancy, as G-CSF treatment is still a new remedy. The aim of this study is to evaluate the role of G-CSF on pregnancy rate and endometrial receptivity by observing its effect on VEGF, TGF, Estradiol, progesterone and ultrasound Doppler Findings as endometrial thickness, pulsatile index, resistance index, V1/V2 and Zones.

Methods

Study design, setting, and time: A randomized clinical trial study that conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University for a period of two years from 1st Sep. 2018 to 1st Sep. 2020.

Study Population and sample size: The study included 40 women aged between 20 – 40 years who were selected from those attended the private clinic and High Institute for Infertility Diagnosis and Assisted Reproductive Technologies complaining from infertility with previous failure trial of Intracytoplasmic Sperm Injection (ICSI) and managed to undergo IVF / ICSI protocols reaching the day of embryo transfer with grade 1 (G1) embryos and underwent G-CSF (Neupogen) endometrial perfusion 6-12 hours before hCG trigger. Women with congenital malformation, intrauterine pathology (polyp, fibroid), hydrosalpinex, moderate to severe endometriosis, having a history of an autoimmune disease or thrombophilia were excluded from this study. To review patients' medical records for research purposes and to maintain patient anonymity and confidentiality of their medical records, they signed an informed consent.

Workup: A total of 40 patients undergoing IVF/ICSI cycle were evaluated following these steps:

- Full medical, surgical and obstetrical history with assessment of height & weight to obtain Body Mass Index (BMI).
- Clinical and gynecological examinations to exclude any abnormality.
- Hormonal analysis (FSH, LH, E2, Testosterone, Prolactin, TSH) for female partners at day 2 of the menstrual cycle
- Antagonist protocol was chosen as controlled ovarian hyperstimulation protocol for each patient in both groups.

- At the day of trigger: Blood sampling for (VEGF, TGF-B1, E2, Progesterone), power Doppler US for subendometrial blood flow and US evaluation for endometrial thickness and pattern were done.
- Intrauterine Insemination (IUI) Catheter was used for G-CSF (Neupogen) perfusion 6-12hours before hCG trigger (300µg/1ml of G-CSF (Neupogen) was infused into the uterus within five minutes by IUI catheter 6-12 hours before hCG administration).
- Protocol of G-CSF Perfusion:
  1. The IUI catheter was connected to the pre-filled syringe after small amount of air aspirated and let the air at the distal end of the syringe above the piston.
  2. The IUI catheter was introduced into the endometrial cavity gently through the cervical canal.
  3. We injected the content of the syringe slowly into the uterine cavity, while the catheter was gently moved backward and forth. The air was injected to deliver the small amount of solution remained inside the catheter into the uterine cavity.
- Trans-vaginal US guided oocyte retrieval done after triggering of ovulation with hCG about35-36 hours.
- At the day of Oocytes pick up: Blood sampling for (VEGF, TGF-B1, E2, Progesterone) for both groups.
- Embryo transfer was done 2-3 days after Oocyte pickup according to number and grading of embryos.

IVF /ICSI Procedures: Step by step descriptions of the IVF procedures:
Step 1: Controlled Ovarian Hyperstimulation (COH).
Step 2: Oocyte Retrieval (OCR).
Step 3: Oocyte Grading and Quality.
Step 4: Fertilization and Embryo Culture.
Step 5: Embryo Quality.
Step 6: Embryo Transfer (Two days after oocyte retrieval and by using embryo transfer catheter, 2-3 embryos were transferred).
Step 7: Luteal Phase Support and Follow up.
Pregnancy results were evaluated based on positive serum βhCG test, two weeks after transfer of embryo and observation of gestational sac on transvaginal U/S examination (clinical pregnancy), 21 days after positive serum βhCG.
Endometrial receptivity was evaluated at day of trigger and at day of oocyte pickup by using VEGF, TGF, Estradiol, progesterone and ultrasound Doppler Findings (Endometrial thickness, Pulsatile index, Resistance Index, V1/V2 and Zones).

Statistical analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 26. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Paired t-test (two tailed) was used to compare the continuous variables at day of OPU compared to that at day of hCG. A level of P – value less than 0.05 was considered significant.

Results

In this study, mean age of study patients was 29.8 ± 5.4 years. We noticed that 62.5% of them had normal BMI level; 70% of cases complained from primary infertility; the most common cause of infertility was male factor (35%); 40% of them were complained from infertility for a period < 5 years; and 67.5% of them didn't underwent ICSI before as shown in table (1).
Table 2 shows the comparison by hormones and U/S findings at day of OPU compared to that at day of hCG. Regarding hormonal parameters, it was obvious that means of progesterone, VEGF, and TGF were significantly increased (P < 0.05) and mean of estradiol was significantly decreased (P= 0.001) at day of OPU compared to that at day of hCG. Concerning Doppler U/S, means of endometrial thickness and zone were significantly increased (P< 0.05) and means of RI, PI, and V1/V2 were significantly decreased (P < 0.05) at day of OPU compared to that at day of hCG.

Regarding ICSI cycle outcome, 19 of study patients (47.5%) were got pregnant as shown in figure (1). Regarding those who got pregnant, means of progesterone, VEGF, and TGF were significantly increased (P < 0.05) and mean of estradiol was significantly decreased (P= 0.001) at day of OPU compared to that at day of hCG. Concerning Doppler

U/S, means of endometrial thickness and zone were significantly increased (P < 0.05) and mean of V1/V2 was significantly decreased (P = 0.001) while RI was significantly decreased. No significant changes in means of PI at day of OPU compared to that at day of hCG as shown in table (3).

Regarding those who failed to be pregnant, mean of progesterone, was significantly increased (P=0.001) and mean of estradiol was significantly decreased (P= 0.001) at day of OPU compared to that at day of hCG; while no significant changes in means of VEGF and TGF.

Concerning Doppler U/S, means of endometrial thickness and zone were significantly increased (P< 0.05) and means of PI and V1/V2 were significantly decreased (P = 0.001) while no significant change in mean of RI at day of OPU compared to that at day of hCG as shown in table (4).

Discussion

G-CSF is a factor that elevating the synchronization between uterine environment and embryo development during endometrial remodeling [13]. Previous studies have showed that G-CSF can manage repeated implantation failure and recurrent abortion by improving the inflammatory process and receptivity of the endometrium [14]. In this study, 19 of study patients (47.5%) were got pregnant. It has suggested that G-CSF administration may improve the clinical results after ART treatment, but it is still vague which particular infertility conditions or through which route of administration does the G-CSF management play a useful role [15]. In the current study, progesterone, estradiol, VEGF, endometrial thickness, zone, RI, PI, and V1/V2 and TGF were significantly improved at day of OPU compared to that at day of HCG. This is agreed with a result found in Gleicher N et al study in 2013 [16]; while in Lv Y et al study in 2020, they observed that mean of estradiol at the day of hCG was reduced in those with absolute pregnancy than those without pregnancy, but there was no significant relation between them. On the day of OPU, despite non-significant relation between both groups, mean of estradiol at pregnant group was higher than group of non-pregnant women. Additionally, they noticed a non-significant increase in endometrium thickness on hCG day in the pregnant group [17]. They concluded that high level of estradiol had a negative effect on pregnancy outcome [18]. It was reported by previous studies that GCSF isn't mitogenic for endometrial cells, whether glandular or epithelial, but in an interactive fashion, it regulates its own expression with transforming growth factor (TGF)-b1 and the expression of TGF-b1 in the endometrium [19]. Moreover, G-CSF management may recover the normal growth of trophoblast and development, as well as a direct impact on the expression of VEGF [20]. In pregnant women of the current study, progesterone, VEGF, estradiol, endometrial thickness, V1/V2, zone and TGF were significantly improved at day of OPU compared to that at day of HCG. In non-pregnant women of the present study, progesterone, endometrial thickness, PI, V1/V2, zone, and estradiol were significantly improved; while no significant changes in means of VEGF and TGF at day of OPU compared to that at day of HCG. Different results found in a study conducted by Gao MZ et al in 2013, as found at day of OPU, the pregnancy group had higher average concentrations of VEGF in the serum than that in non-pregnancy group, while no significant differences in VEGF or TGF-β1 concentrations between them in FF and serum [21]. Administration of G-CSF provides endometrium expansion, especially in thin endometrium, and increases implantation and rates of pregnancy. Also, it was found out by some studies that G-CSF administration doesn't change endometrium and pregnancy rates, although it is seen that endometrial thickness is increased [6]. The discrepancies observed among above studies, are multifactorial, it related to different age (particularly in older women, which appears irrational with the diminished follicular development in the elderly), cause of infertility, ovarian reserve and starting dose of gonadotrophin etc., so concentrations of E2 were not always dependent on gonadotrophin in patients with distinctive ovarian reactions [22]. In fact, the growth factors contained or produced inside the ovary may act alone or in concert to regulate the follicles' growth. They are eventually controlled by endocrine, paracrine and autocrine regulation, and may influence oocyte quality and fertility potential if aberrantly expressed. For judging the ovarian function and in managing infertility, further illustration of specific physiological role of factors concerned in the pre-ovulatory follicle may be helpful [21]. In conclusion, G-CSF has important role in

improving chemical and clinical parameters of the infertile females. This reflects increasing implantation rate, biochemical and clinical pregnancy rate.

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Table 1: Distribution of study patients by clinical information

Variable	No. (n=40)	Percentage (%)
Age (Year)		
< 25	5	12.5
25 – 34	25	62.5
≥ 35	10	25.0
BMI Level		
Normal	25	62.5
Overweight	15	37.5
Type of infertility		
Primary	28	70.0
Secondary	12	30.0
Cause of infertility		
Male factor	14	35.0
Female factor	9	22.5
Combined	10	25.0
Unexplained	7	17.5
Duration of infertility (Year)		
< 5	16	40.0
5 – 9	13	32.5
≥ 10	11	27.5
Number of previous ICSI		
No	27	67.5
1	7	17.5
≥ 2	6	15.0

Table 2: Comparison by hormones and US findings at day of Ovum Pick-Up compared to that at day of hCG

Parameter	Time		P - Value
	At day of hCG Mean ± SD	At day of OPU Mean ± SD	
Hormonal parameter			
Estradiol (pg/ml)	2042.18 ± 370.7	1687.45 ± 329.2	**0.001**
Progesterone (ng/ml)	1.1 ± 0.3	6.48 ± 1.2	**0.001**
VEGF (pg/ml)	391.36 ± 120.4	534.74 ± 133.0	**0.001**
TGF (pg/ml)	961.12 ± 386.1	1204.91 ± 423.4	**0.001**
Doppler U/S finding			
Endometrial thickness (mm)	7.57 ± 1.1	9.84 ± 1.9	**0.001**
Resistance Index	1.07 ± 1.4	0.46 ± 0.11	**0.012**
Pulsatile Index	1.43 ± 0.5	1.13 ± 0.7	**0.026**
V1/V2	3.5 ± 1.7	2.15 ± 0.7	**0.001**
Zone	2.07 ± 0.3	3.6 ± 0.6	**0.001**

Table 3: Comparison by hormones and US findings in pregnant women at day of Ovum Pick-Up compared to that at day of hCG

Parameter	Pregnant women (n= 19)		P - Value
	At day of hCG Mean ± SD	At day of OPU Mean ± SD	
Hormonal parameter			
Estradiol (pg/ml)	2237.59 ± 300.5	1849.1 ± 269.3	**0.001**
Progesterone (ng/ml)	0.93 ± 0.24	7.16 ± 0.9	**0.001**
VEGF (pg/ml)	432.9 ± 129.9	599.4 ± 149.8	**0.001**
TGF (pg/ml)	1138.36 ± 388.6	1418.27 ± 347.4	**0.001**
Doppler U/S finding			
Endometrial thickness (mm)	7.25 ± 1.0	9.48 ± 2.4	**0.001**
Resistance Index	1.22 ± 1.8	0.37 ± 0.05	**0.046**
Pulsatile Index	1.57 ± 0.6	1.19 ± 1.0	**0.173**
V1/V2	3.74 ± 2.2	2.13 ± 0.8	**0.001**
Zone	2.11 ± 0.3	3.94 ± 0.2	**0.001**

Table 4: Comparison by hormones and US findings in not pregnant women at day of Ovum Pick-Up compared to that at day of hCG

Parameter	Not pregnant women (n=21)		P - Value
	At day of hCG Mean ± SD	At day of OPU Mean ± SD	
Hormonal parameter			
Estradiol (pg/ml)	1882.31 ± 350.3	1555.2 ± 319.1	**0.001**
Progesterone (ng/ml)	1.24 ± 0.25	5.92 ± 1.2	**0.001**
VEGF (pg/ml)	357.37 ± 102.8	387.56 ± 125.5	**0.188**
TGF (pg/ml)	816.11 ± 325.0	923.44 ± 565.1	**0.121**
Doppler U/S finding			
Endometrial thickness (mm)	7.83 ± 1.1	10.14 ± 1.4	**0.001**
Resistance Index	0.99 ± 1.2	0.49 ± 0.1	**0.076**
Pulsatile Index	1.31 ± 0.5	1.08 ± 0.4	**0.022**
V1/V2	3.3 ± 1.3	2.17 ± 0.6	**0.001**
Zone	2.04 ± 0.2	3.31 ± 0.6	**0.001**

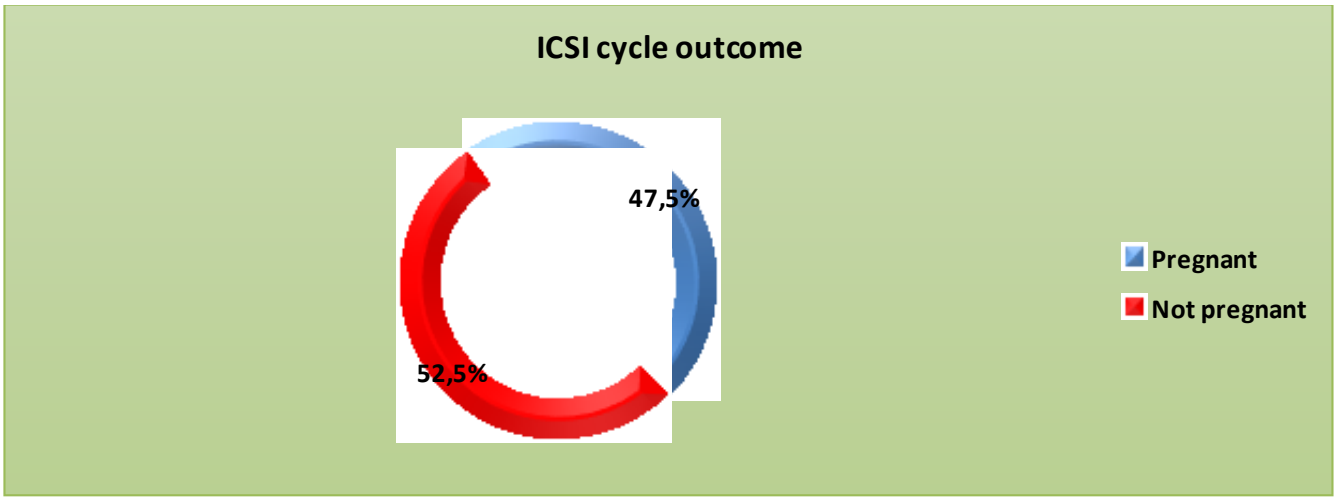

Figure 1: Distribution of study patients by ICSI cycle outcome