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The Effect of Intrauterine Infusion of Human Gonadotropin (HCG) on the Outcome of Embryo Transfer Cycles in Infertile Women: A Randomized Clinical Trial

Parvin Akbari Asbagh[1], Firouzeh Akbari Asbagh[2], *Mahshid Karimi[2], Samaneh Hosseini Quchani[3], Venus Hajialiakbar[4]

[1]Department of Pediatrics, School of Medicine, Imam Khomeini Hospital, Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Obstetrics and Gynecology, School of Medicine, Yas Hospital, Tehran University of Medical Sciences, Tehran, Iran.
[3]Ph.D of Reproductive Biology, IVF center of Yas hospital, Tehran University of Medical Sciences, Tehran, Iran.
[4]Yas Hospital, IVF unit, Department of OBS Gyn, Tehran University of Medical Sciences, Tehran, Iran.

Abstract

Background

The effects of using intrauterine human gonadotropin hormone (HCG) with different dose before embryo transfer cycles on pregnancy rate in infertile women had controversy results. Therefore, the present study was conducted to evaluate the effect of infusion 500 units of HCG 15 minutes before embryo transfer cycles on pregnancy outcome in infertile women.

Materials and Methods: The present randomized clinical trial study was conducted on 196 infertile women referred to Yas Infertility Center, Tehran, Iran, from June to November 2019. The patients randomly allocated to the groups by balanced block randomization (99 patients in each group). The intervention group received 500 IU of HCG 15 minutes before embryo transfer by intrauterine infusion and in the control group no intervention was performed before embryo transfer. The in-vitro fertilization (IVF) cycle outcomes between two groups was assessed 15 days after embryo transfer and compared with chi square test.

Results: The main causes of infertility in both groups were male factors and mixed factors. The chemical pregnancy rate in the HCG group was significantly higher than those of in the control group (30.92% vs. 18.18%, P=0.004). However, no significant difference was found in terms of clinical pregnancy, early miscarriage rate, ectopic pregnancy rate and twin pregnancy rate (P>0.05).

Conclusion

The infusion of 500 IU of HCG 15 minutes before embryo transfer improved the chemical pregnancy rate. However, due to conflicting result in other previous studies, further evidence with high sample size studies is needed to confirm the findings of this study.

Key Words: Embryo transfer, Infertility, in vitro fertilization, Human chorionic gonadotropin.

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Corresponding Author:

Mahshid Karimi (MD), Address: Yas Hospital, Tehran University of Medical Sciences, Tehran, Iran.

Email: mkarimi6471@yahoo.com

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1- INTRODUCTION

Successful implantation after in vitro fertilization (IVF) and embryo transfer depend on various factors related to embryo quality and endometrial acceptance. It is important that the fetus reaches the endometrial cavity and the endometrium is ready (1). Successful implantation requires good quality embryo, endometrial receptor and proper embryo transfer technique (2). It is estimated that 50 to 70% of pregnancies are lost due to implantation defects (3). Although significant improvements have been made in various techniques of IVF, but the probability of pregnancy by using this method is still about 30% (4).

Implantation is a very complex process that is regulated by many factors, the most important of which is human chorionic gonadotropin (HCG) (4). The HCG plays a very important role in promoting secretory endometrium after the proliferative phase. In addition, this hormone plays a very important role in the secretion of various endometrial cytokines during the implantation window. Xiao-Yan et al. found a positive correlation between beta-HCG concentration and implantation rate, indicating that HCG secreted by the fetus is used as a marker for fetal selection during the IVF cycle (5).

The results of a study showed that intrauterine infusion of HCG significantly increases the incidence of Insulin-Like Growth Factor-Binding Protein-1 in the uterus, thereby increasing the rate of leukemia inhibitory factor, vascular endothelial growth factor and the metalloproteinase matrix type 9 (6). Another study showed that intrauterine injection of 500 units of HCG before embryo transfer significantly improved both implantation and pregnancy rate in the in vitro fertilization / intracytoplasmic sperm injection (IVF / ICSI) cycle (7).

A meta-analysis study by Gao et al. in 2019 showed that injecting 500 units of HCG within 15 minutes before embryo transfer had the best results (8). Therefore, due to the lack of such a study in Iran and the results of this meta-analysis, we aimed to evaluate the results of pregnancy by infusion 500 units of human gonadotropin hormone 15 minutes before embryo transfer in infertile women referred to Yas Infertility Center in Tehran, Iran.

2- MATERIALS AND METHODS

2-1. Study design and population

The present study is a randomized clinical trial that was performed on 196 infertile women referred to Yas Infertility Center, Tehran, Iran, from June to November 2019. The available sampling method was used to select the samples. The sample was randomly allocated to one of the two groups using balanced block randomization method with a ratio of 1: 1. The intervention group received 500 IU of HCG before embryo transfer within 15 minutes before embryo transfer by intrauterine infusion and in the control group no intervention was performed before embryo transfer.

2-2. Methods

Infertile women scheduled for embryo with either fresh or frozen-thawed cycles were enrolled. For endometrial preparation**,** oral estradiol valerate was initiated on day 2–4 of the menstrual cycle, at a dose of 4 mg per day for 4 days, in continue by 6 mg per day during the next following 4 days. On day 12 of estradiol supplementation, transvaginal ultrasound was done to measure the endometrial thickness. Endometrial thickness $\geq$ 8 mm was deemed as an ideal thickness. Then progesterone 100 mg was administered and the embryo transfer was performed with the coordination of the embryologist (9). In the intervention group, the patient was placed in a lithotomy position and in

this position the cervix was visible using a speculum. The cervical mucus was removed using a piece of sterile gauze, then the mucus was partially removed using gentle suction with a 1 ml syringe. Embryo transfer was performed using a Hook catheter. After the catheter is passed through the inner cervix, 40 microliters of tissue culture medium containing 500 units of HCG was injected intrauterine. Approximately 15 minutes after intrauterine infusion of HCG, the embryos were loaded into a catheter and transferred into the uterine cavity. About 5 minutes after the embryo transfer, the vaginal speculum remains in its place and then gently removed by pressing on the vaginal angle of the cervix.

In the control group, embryo transfer was performed without intrauterine injection of HCG. Pregnancy test was performed 2 weeks after embryo transfer and 3 weeks after positive pregnancy test, ultrasound examination was performed to observe the number and location of the pregnancy sac and to confirm the fetal heart rate. Two weeks after the day of transfusion, with a BHCG blood test and 4-6 weeks after the transfusion, in BHCG positive cases, transvaginal ultrasound was performed. The primary outcome in the present study was pregnancy rate and implantation rate, and the secondary outcome was abortion rate. Chemical pregnancy was characterized by an increase in serum HCG levels without detecting a pregnancy sac. Clinical pregnancy was considered in the presence of a pregnancy sac and the presence of a pulse rate in the fetus. Also, the abortion was considered as termination of pregnancy before the 20th week of pregnancy.

2-3. Measuring tool

Data collection was based on interviews with patients and laboratory (such as AMH), and ultrasound results registered in the researcher-made checklist for each patient referred to the center. The result of the treatment was recorded by the researcher with the Infertility Fellowship education this form.

2.4-Ethical consideration

The ethics committee of Tehran University of Medical Sciences was approved the study (ethics code: IR.TUMS.MEDICINE.REC.1398.872).
All the participants gave written informed consent before entering the study. The RCT registration number: IRCT20200213046475N1.

2-5. Inclusion and exclusion criteria

All women with primary infertility who had at least one failed embryo transfer and at least one grade A or B frozen embryo were included in the study. Female over 40 years or with body mass index (BMI) over 35 kg/m^2, women with severe male infertility such as azoospermia in her husband, uterine abnormalities such as septum, unicorn uterus, uterine myoma or history of myomectomy, grade 3 and 4 endometriosis, hydrosalpinx, and the storage time of freezing more than 2 years, were excluded from the study.

2-6. Data Analyses

Descriptive statistics were reported as number (%) for categorical variables and mean (SD) for continuous variables across participant's background in both groups. In order to compare the qualitative variables between the two groups, Chi-square test was used. Quantitative variables in the two groups were compared using independent t-test. All analyses were performed using Stata 14. The significant level was considered less than 5%.

3- RESULTS

A total of 246 patients were evaluated to participate in the study. 9 patients did not consent to participate and 37 patients did not meet the criteria and 2 patient were excluded due to thin endometrium. Therefore finally, 198 patients allocated

into two groups randomly and 99 infertile pregnant women devoted to each group. Two patients were loosed to follow-up in intervention group and therefore 97 subjects from this group were entered to the final analysis. The follow diagram of the allocation of patients to the studied groups is shown in **Figure. 1**.

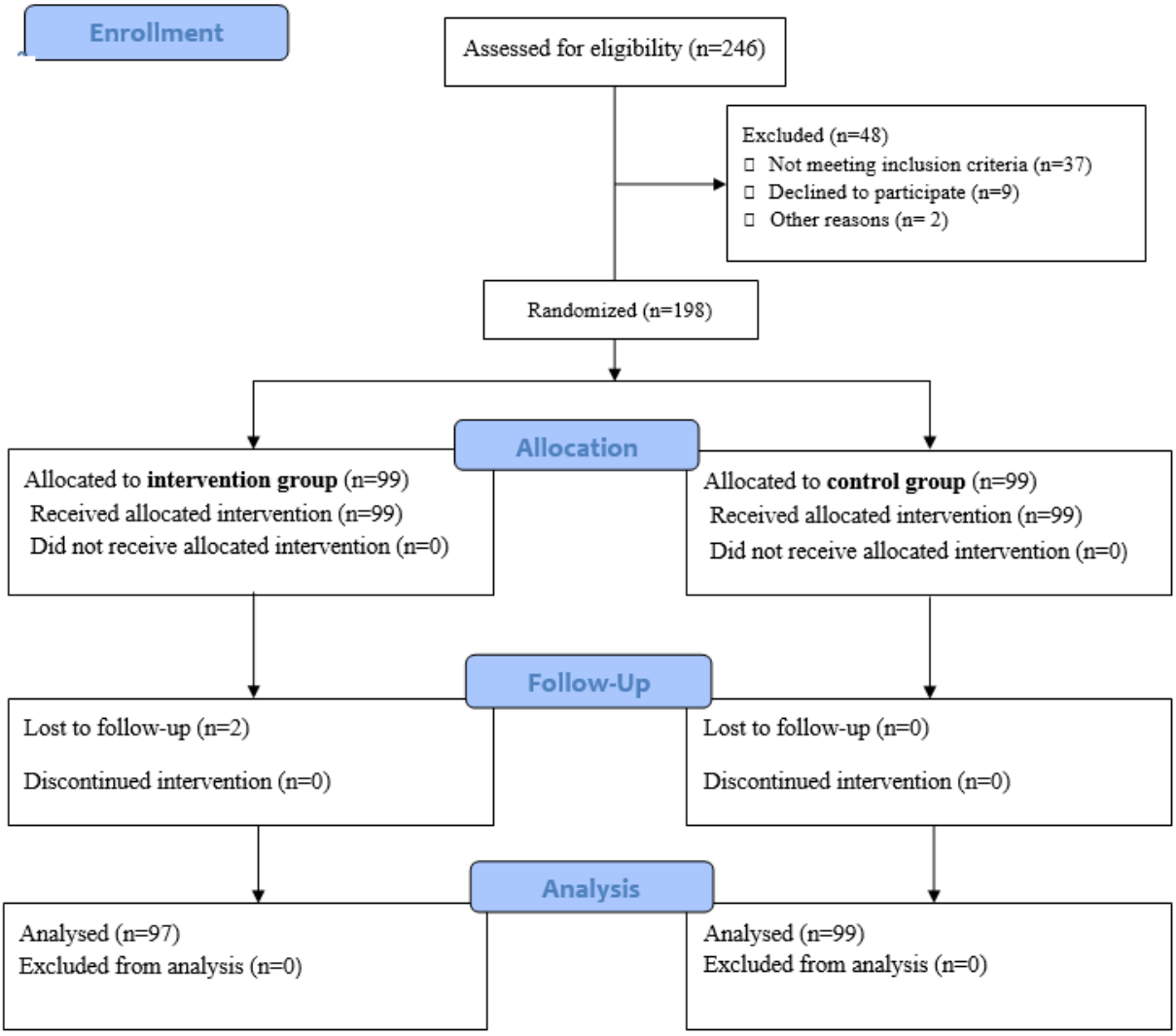

Fig.1: Flowchart on the allocation of patients to the studied groups.

Baseline and cycle characteristics of infertile women in two groups is presented in **Table.1**. As shown, there was no significant difference between two groups in baseline characteristics including: BMI, age and AMH and number of embryo transferred (P>0.05). Meanwhile, the mean duration of infertility in HCG group was significantly higher (13.09±10.11 years vs. 9.86±5.409.86±5.40 years, p=0.006).

Table-1: Baseline and cycle characteristics of infertile women in two groups, n=196.

Variables	HCG group, n=97 Mean (SD)	Control group, n=99 Mean (SD)	*P*-value
Age (Year)	33.49±4.90	32.46±5.34	0.16
BMI (Kg/m^2)	28.54±3.57	28.92±3.78	0.47
AMH	2.98±2.81	3.08±2.35	0.78
Duration of infertility (year)	13.09±10.11	9.86±5.40	0.006
Number of embryo transferred	1.92±0.70	2.05±0.73	0.20

SD: Standard deviation, BMI: Body Mass Index, AMH: Anti-mullerian hormone.

The main causes of infertility in both groups were male factors and mixed factors. Such a way that the cause of infertility in 35.05% of cases in HCG group and 22.22% of cases in control group was male factors. Moreover, mixed factors with the 26.8% of cases in HCG group and 29.29% in control group was the second cause of infertility in two groups. There was not a significant different between two groups in regards of embryo quality (*P*=0.28) (**Table. 2**).

Table-2: Comparison the cause of infertility and quality of embryo between two groups, n=196.

Variables		HCG group, n=97 Number (%)	Control group, n=99 Number (%)	*P*-value
Cause of infertility	PCOS	14 (14.43)	18 (18.18)	0.19
	Tubal factor	13 (13.40)	11 (11.11)	
	Unexplained	10 (10.31)	19 (19.19)	
	Male factor	34 (35.05)	22 (22.22)	
	Mixed (ovulatory and/or tubal + male factor)	26 (26.80)	29 (29.29)	
Embryo Quality	A	34 (35.05)	40 (40.4)	0.28
	B	41 (42.27)	31 (31.31)	
	C	22 (22.68)	28 (28.28)	

PCO: Polycystic Ovary Syndrome.

The cycle outcomes in two groups is compared in **Table. 3**. The analysis showed that chemical pregnancy rate in the HCG group was significantly higher than those of in the control group (30.92% vs. 18.18%, *P*=0.004). However, no significant difference was found in terms of clinical pregnancy, early miscarriage rate, ectopic pregnancy rate and twin pregnancy rate (*P*>0.05).

Table-3: Comparison of IVF/ICSI cycle outcomes between two groups, n=196.

Variables	HCG group, n=97 Number (%)	Control group, n=99 Number (%)	*P*-value
Chemical pregnancy rate	30 (30.92)	18 (18.18)	0.04
Clinical pregnancy rate	26 (26.80)	16 (16.16)	0.07
Early miscarriage rate	4 (1.12)	2 (2.20)	0.34
Ectopic pregnancy rate	3 (3.10)	2 (2.20)	0.63
Twin pregnancy rate	3 (3.10)	1 (1.10)	0.3

IVF/ICSI: in-vitro fertilization/intracytoplasmic sperm injection.

4- DISCUSSION

This study was conducted to investigate the effect of intrauterine infusion of HCG before embryo transfer on pregnancy outcome in infertile women. The results of the study showed that infusion of 500 IU of HCG 15 minutes before embryo transfer are associated with increase of chemical pregnancy rate but has no beneficial effect early miscarriage rate, ectopic pregnancy rate and twin pregnancy rate. Results of the meta-analysis conducted by Xie et al. (9) by pooling findings of 6 trials consisted of 1,432 women showed that chemical pregnancy rate was significantly improved in HCG groups compared to the control group (41.8% vs. 31.2%, P<0.001), that this finding is in line with the results of our study. Meanwhile, results of the meta-analysis does not support the use of intrauterine HCG administration before embryo transfer (11). Beneficial effects of using intrauterine HCG with different dose before embryo transfer on chemical pregnancy has been shown in several previous conducted studies (12-16). Whereas, in some other studies not found improvement on implantation rate (17, 18). Against with our findings, Hafezi et al. in their study was not found a significant difference in terms of clinical pregnancy between HCG and control groups. They conclude that the luteal phase defects does not occur in the vitrified-warmed cleavage-stage embryo transfer in programmed cycles which be overcome by intrauterine infusion of HCG (19). However, different in the study population as well as using different HCG doses or HCG preparations can be justified these differences in findings. The positive effect of HCG due to its molecular properties in interactions between the maternal and fetal interface have been shown previously. HCG has an important role on trophoblast differentiation (20), extravillous cytotrophoblast (EVT) cell proliferation, EVT invasion (21), endometrial decidualization and vascularization (22). Also, HCG keeps the decidualized endometrial stromal cells (ESC) from apoptosis induced by oxidative stress (23). Additionally, hCG regulates the immune system (24), HCG is also is associated in inducing inhibiting T lymphocyte (25), regulating macrophage migration and uterine natural killer cell proliferation (26). By summarizing the results of the above studies, it is inferred that HCG is important for trophoblast differentiation, EVT invasion, endometrial receptivity, and maternal-fetal immune tolerance. However, this study had some limitations. First was the low sample size of the study which may not be able to observe significant differences between the two groups. Moreover, due to the short follow-up period we could not able to the effect of intrauterine infusion of HCG before embryo transfer on late miscarriage rate and live birth rate.

5- CONCLUSION

We found that infusion of 500 IU of HCG 15 minutes before embryo transfer improved the chemical pregnancy rate. However, due to conflicting result in other previous studies, further evidence with high sample size studies is needed to confirm the findings of this study.

6- ACKNOWLEDGEMENTS

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7- CONFLICT OF INTEREST: None.

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