Randomised Controlled Trial

The effect of intrauterine hCG injection before embryo transfer on pregnancy rate in frozen embryo transfer cycles

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A B S T R A C T

Objective: HCG (human chorionic gonadotropin), which is secreted by cytotrophoblast cells, plays an important role in improving pregnancy outcomes among patients with infertility or related problems. In this study, we evaluate the effect of intrauterine hCG injection prior to frozen embryo transfer on pregnancy outcomes.

Methods: In this clinical trial study, among women with infertility problems referred to (XXX) and those with frozen embryos were included in the study. 155 patients in the intervention group received 500 units of hCG while 157 in control group received saline prior to embryo transfer. Along with demographic data, successful in vitro fertilization and clinical pregnancy, loss of pregnancy, successful transplantation, and biochemical parameters were compared among the two groups.

Results: The mean age of the patients included in the study was 32.97 ± 3.31 years. The level of anti-Mullerian hormone, follicle stimulating hormone and the grade of frozen embryos were not significantly different between the two groups (P > 0.05). The rate of laboratory pregnancy in the intervention group was significantly higher than in the control group (51% vs 35%), p = 0.006. The rate of successful implantation and clinical pregnancy in the intervention group was also significantly higher, p = 0.01 and p = 0.006, respectively. Overall loss of pregnancy in intervention group was 78.1% and 86.0% in control group which was not significantly different, p = 0.068.

Conclusion: The outcomes of our study showed that 500 IU of hCG prior to embryo transfer improves the rate of clinical and laboratory pregnancy. However, it does not reduce the rate of loss of pregnancy. Further studies are therefore required in this area.

1. Introduction

The rate of infertility is increasing worldwide and according to some studies, the prevalence of primary infertility in Iran seems to be higher than the global average which is about 20.2% [1]. Many methods and techniques have been developed to treat infertility. In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are assisted reproductive techniques that have been associated with the highest pregnancy success rates and in some studies their success rates have been reported to be up to 50% [2].

However, assisted reproductive technologies have not yet been able to lead to fertility in some patients. Several factors contribute to the differences in the rate of pregnancy reported with assisted reproductive technology, such as the methods used to control ovarian hyperstimulation (COH), egg retrieval, IVF, embryo culture, and embryo transfer (ET). The rate of embryo implantation depends on a wide range of factors related to endometrium, fetal quality and ET technique. The key to ET is to place the 'best quality' embryo in the proper position of the

Abbreviations: IVF, In vitro fertilization; ICSI, intracytoplasmic sperm injection; ET, embryo transfer; COH, control ovarian hyperstimulation.

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endometrial cavity exactly in the optimal implant window, when the best endometrial reception is provided. Many techniques have been developed in clinical and laboratory settings. However, the pregnancy rate per cycle remains around 30% [3,4]. Implantation failure is responsible for about 50–75% of pregnancy losses [5,6].

Frozen embryo transfer is one of the main components of assisted reproductive techniques that is widely used due to its low cost and lack of excessive ovarian stimulation. Due to the fact that, in addition to good quality embryos, a functional and receptive endometrium is needed for successful implantation, factors affecting endometrial acceptance play a key role in improving implantation [4,5]. hCG (human chorionic gonadotropin) is a glycoprotein hormone with three dimeric isoforms secreted by fetal cytotrophoblast age cells. It is responsible for the continuous production of progesterone from the corpus luteum to maintain pregnancy. Furthermore, hCG inhibits intrauterine insulin growth factor β-1 and stimulates leukocyte inhibition factor, vascular endothelial growth factor, and matrix metalloproteinase [7]. hCG is a primary mediator of the fetus to stimulate decidualization, angiogenesis, and maternal immune tolerance to accept the fetus and initiate trophoblastic invasion [8]. Considering the physiological role of this hormone in endometrial receptivity, it can be used as a beneficial intervention before embryo transfer in FET cycles. However, so far studies have shown conflicting results for the effect of this hormone on the endometrium.

Infertility treatment is associated with a great economic and psychological burden for families in return for an improvement in patient’s condition and an attempt of achieve pregnancy.

The aim of this study was to evaluate the effects of intrauterine hCG injection prior to embryo transfer on embryo transfer results.

2. Methods

In this clinical trial study, women with infertility problems and frozen embryo referred to (XXX) were included in the study. Inclusion criteria were women aged 20–39 years; good quality embryos (A and B) and those with the first frozen embryo transfer. Exclusion criteria were women aged greater than 40 years; male infertility leading to TESE (Testicular sperm extraction), PESA (percutaneous epididymal sperm aspiration) or azoosperma; history of myomectomy, existence of hydrosalpinx, existence of fibroma with compressive effect on endometrium and endometriosis. Patients who did not consent to participate in the study were also excluded. 312 eligible patients completed the study in two groups of control (157) and intervention (155). The patients were randomly assigned into two groups using a simple randomization method in which the patients were blindly asked to select 1 or 3 (1 = intervention and 3 = control).

Patients were studied in two groups: The intervention group received 2 mg estradiol every 12 h after baseline ultrasound and confirmed uterine and ovarian suppression and absence of ovarian pathologies. The dose was adjusted based on endometrial response. From endometrial preparation and embryo transfer, seen by serial ultrasound; patients received progesterone suppositories for 4 days. 4 min before embryo transfer, 500 hCG units (volume 0.1 cc) were injected into the uterine cavity with Soft-Pass™ Embryo Transfer Catheter (COOK MEDICAL LLC, USA), and the embryo was transferred by grade A-B cleavage step.

In the control group, after preparing the endometrium with the conventional HRT protocol, when the thickness of the endometrium reached the appropriate level, seen by serial ultrasound; patients received progesterone suppositories for 4 days and 4 min before embryo transfer, a volume of 0.1 cc of media solution was injected into the uterine cavity with Soft-Pass Embryo Transfer Catheter (COOK MEDICAL LLC, USA), and the embryo was transferred by grade A-B cleavage step.

In both the groups, treatment with estradiol and progesterone was continued and 2 weeks later, pregnancy test (hCG test) was performed. Pregnant patients were followed up with ultrasound 2 weeks after the presence of pregnancy sac and fetal heartbeat. The rate of laboratory and clinical pregnancy and loss of pregnancy and DPR pregnancy were recorded in each group.

Quantitative variables were described by mean and standard deviation. To analyze the data, first the normality of the data distribution was checked using the Shapiro-Wilk test. If the data distribution was normal, the analysis of variance (ANOVA) was used. To determine the effect of the intervention, the difference between the values before and after treatment was calculated and in the case of normal distribution of the difference of the data, a paired t-test was used. In the other case, Wilcoxon test was used. Data were analyzed using SPSS v22 (IBM, Chicago, IL, USA) and a p-value less than 0.05 was considered statistically significant.

The methods are stated in accordance with STROCSS guidelines 2021 [9].

The unique identification number is: researchregistry7533.

Written consent was obtained, and the study was approved by the institutional review board under the code of ethics (XXX).

3. Results

Mean age of 312 women included in the study was 32.97 ± 3.31 years, which was 32.9 ± 3.30 and 33.04 ± 3.32 years for the intervention and control groups, respectively. 98 women (31.4%) were in the age range 35–39 years, which was 43 (27.7%) and 55 (35%) for the intervention and control groups, respectively. Other variables of the subjects, such as height, weight, body mass index, and duration of infertility, were examined and presented in (Table 1).

Basic laboratory information of the patients, such as the level of anti-Mullerian hormone (AMH) and follicle stimulating hormone (FSH) and the grade of frozen embryos, was examined, which did not show a significant difference between the intervention and control groups. (P > 0.05) (Table 2).

In this study, successful pregnancy in the two study groups (intervention and control) were examined and compared. Two weeks after embryo transplantation, according to the study protocols, pregnancy tests were performed on subjects and if positive, patients were followed up for implantation and clinical pregnancy and loss of pregnancy during the study. Based on the findings of the present study, the rate of laboratory pregnancy in the intervention group was higher than in the control group (51% vs. 35%), which was statistically significant. (P = 0.006), however, in two groups, among patients aged 35 years or older, laboratory pregnancy was not significant, p = 0.27.

Table 1

| Demographic variables in patients participating in the study. | Patients with available data | Total population (n = 312) | Intervention Group (n = 155) | Control Group (n = 157) |
|---------------------------------------------------------------|-----------------------------|---------------------------|-----------------------------|-------------------------|
| Demographic characteristics                                   |                             |                           |                             |                         |
| Age (years), mean (SD)                                        | 312                         | 32.97 (3.31)              | 32.90 (3.30)                | 32.90 (3.32)            |
| Age Category, Years, n (%)                                    | 312                         | 214 (68.6)                | 112 (72.3)                  | 102 (65)                |
| ≥35                                                           | 312                         | 98 (31.4)                 | 43 (27.7)                   | 55 (35)                 |
| Height (Cm), mean (SD)                                        | 312                         | 164.42 (6.10)             | 164.61 (6.36)               | 164.24 (6.82)           |
| Weight (Kg), mean (SD)                                        | 312                         | 73.91 (11.81)             | 74.27 (12.06)               | 73.55 (11.58)           |
| BMI (kg/m²), mean (SD)                                        | 312                         | 27.61 (4.04)              | 27.58 (4.18)                | 27.64 (3.92)            |
| Duration of infertility, years, mean (SD)                    | 312                         | 6.28 (3.14)               | 6.30 (2.84)                 | 6.26 (3.14)             |

Abbreviations: SD, standard deviation; n, number.
Successful implantation in the intervention group was 38.1% and the control group was 24.2%, which was statistically significant, p = 0.006. However, the rate of clinical pregnancy among the two groups in patients aged 35 years or more was not different, p = 0.17. Similarly, rate of clinical pregnancy in intervention and control group was 28.4% and 15.3%, which was significantly different, p = 0.006. However, the rate of clinical pregnancy among the two groups in patients aged 35 years or more was not different, p = 0.29. Overall loss of pregnancy rate in intervention group was 78.1% and 86.0% in intervention group. The difference was not statistically significant, p = 0.068, (Table 3).

4. Discussion

During the past four decades, considerable effort has been done on improving the success rate of IVF through the development of new treatments [10]. These studies and research range from the techniques used to improve IVF laboratory performance, namely metabolomics to evaluate and select the best and most suitable embryo, to the approaches of the clinical protocol [11]. The concept of HCG administration in the uterine cavity before embryo transfer is based on evidence from the past two decades. Based on the findings of previous validated studies, it has been proven that HCG is the first molecule to participate in the interaction between the fetus and maternal decidua. HCG is expressed even at the stage of 8-cell embryos [12,13]. Following a specific pattern of increase in implantation time and trophoblast invasion, cytотrophoblasts differentiate from synctiotrophoblasts. At the same time, standard HCG is converted to the hyperglycosylated HCG isofrom (H-HCG), which is involved in the proliferation and invasion of extra-villous trophoblasts [14,15]. HCG is associated with the suppression of insulin-like growth factor binding protein-1 and macrophage colony stimulating factor while promotes the secretion of leukemia inhibitory factor, vascular endothelial growth factor, and matrix metalloproteinase-9. It also inhibits oxidative stress and apoptosis in stromal cells of endometrium [16]. The beneficial effect of intratüretinal HCG to improve fertility outcomes have been reported in several studies [17]. This study was designed and performed to evaluate the effect of infusion of 500 hCG units (volume 0.1 cc) in IVF conditions on pregnancy outcomes. Our findings showed that infusion of 500 hCG (volume 0.1 cc) after achieving endometrial thickness had significant benefits in improving pregnancy outcomes after IVF. The rate of in vitro pregnancy in the intervention group showed a significant improvement compared to the control group (51 vs. 35%). This positive effect of HCG infusion was also reported in other similar studies [17–22]. The rate of implantation and clinical pregnancy was also reported in the intervention group more than in the control group, which was similar to the findings of other studies in this field [17–22]. However, age differences of patients, differences in transfer cycle, different sample size, and proficiency in performing practical techniques can justify a slight difference in the reported results. On the contrary, studies using blastocyst transfer did not support the efficacy of hCG injection prior to endometrial thickness [21,23].

On the other hand, our results showed that the effect of hCG infusion in the age group under 35 years in laboratory pregnancy, implantation, and clinical pregnancy was much better and more significant than the age group 35 years and older. Similarly, the results of the study by Laokirkkiat et al. [18] supported the effectiveness of hCG infusion in the age group under 40 compared to the age group over 40 years, this slight difference in the two studies was due to the age difference in the study population. Also, in line with the results of other existing studies, the results of our study in terms of loss of pregnancy did not show a significant difference, although in the intervention group, the rate of loss of pregnancy was much lower than the control group, however for more valid and generalizable results, further studies with greater sample sizes seem necessary.

Akbari Asbagh, Akbari Asbagh [24] reported that low dose hCG (500 IU) 15 min before embryo transfer improves chemical pregnancy rate, however, does not affect ectopic pregnancy rate, miscarriage, clinical pregnancy. These findings are also reported in a study by Santibañez, Garcia [25] Intratüretinal hCG administration is a simple and affordable method that can be used to improve pregnancy outcome. This method does not take more time than clinical staff and does not require complex training. In our study, complications such as ectopic pregnancy or multiple pregnancy were not reported. Due to the impossibility of long-term follow-up owing to outbreak of COVID-19 and focus of medical centers on reducing the volume of elective referrals, clinical pregnancy was used as a result criterion instead of live birth. This is a limiting factor for this study. More live birth tests are needed as a

Table 2
Baseline laboratory variables in patients participating in the study.

| Patients with available data | Total population (n = 312) | Intervention Group (n = 155) | Control Group (n = 157) | P value |
|-----------------------------|---------------------------|-------------------------------|-------------------------|--------|
| AMH (ng/ml) mean (SD)       | 312                        | 3.65 (2.37)                  | 3.51 (2.41)             | 3.80   |
| FSH (mIU/ml) mean (SD)      | 312                        | 6.02 (2.12)                  | 6.12 (2.15)             | 5.93   |
| Embryo Grade, n (%)        | 312                        | 225 (72.1)                   | 116 (74.8)              | 109    |
| A                           | 87 (27.9)                  | 39 (25.2)                    | 69 (46.4)               | 48 (30.6) |

Abbreviations: SD, standard deviation; n, number; AMH, Anti Mullerian Hormone; FSH, Follicle Stimulating Hormone; ng, nano grams; ml, milli liter; mIU, milli International Unit.

Table 3
Relationship between study intervention and pregnancy outcome.

| Study Findings | Total population (n = 312) | Intervention Group (n = 155) | Control Group (n = 157) | Relative risk (95% CI) | P value |
|----------------|---------------------------|-------------------------------|-------------------------|------------------------|--------|
| Laboratory Pregnancy (yes), n (%) | 104 (48.6) | 63 (56.2) | 41 (39.4) | 1.23 (1.02-1.45) | 0.021 |
| years <35 | 30 (30.6) | 16 (17.1) | 14 (25.5) | 0.95 (0.75-1.2) | 0.270 |
| Total | 134 (42.9) | 79 (59.0) | 55 (45.0) | 1.21 (1.06-1.39) | 0.006 |
| Implantation (yes), n (%) | 45 (40.2) | 27 (26.5) | 18 (29.2) | 1.22 (0.98-1.57) | 0.043 |
| years <35 | 25 (25.5) | 14 (24.2) | 11 (20.0) | 0.95 (0.75-1.2) | 0.170 |
| Total | 79 (31.1) | 59 (38.1) | 38 (24.2) | 1.28 (1.06-1.49) | 0.010 |
| Clinical Pregnancy (yes), n (%) | 39 (34.8) | 21 (20.6) | 18 (11.6) | 1.38 (1.10-1.72) | 0.021 |
| years <35 | 8 (8.2) | 5 (5.5) | 3 (5.5) | 0.93 (0.67-1.31) | 0.293 |
| Total | 68 (21.8) | 44 (28.4) | 24 (15.3) | 1.35 (1.09-1.67) | 0.006 |
| loss of pregnancy (yes), n (%) | 83 (74.1) | 52 (49.4) | 31 (20.0) | 0.63 (0.47-0.84) | 0.251 |
| years <35 | 90 (91.8) | 52 (49.4) | 38 (88.4) | 1.37 (1.17-1.59) | 0.293 |
| years | 256 (2.1) | 121 (78.1) | 135 (86.0) | 1.64 (1.34-2.00) | 0.008 |

a Chi-square test.
b Statistical significance.
primary outcome to identify the women who benefit most from this intervention.

5. Conclusion

Intrauterine injection of a small volume of hCG during embryo transfer significantly enhances the implantation rate. It also improves the rate of laboratory and clinical pregnancies. However, the results in the age group of 35 years and older were not significant in the age group of less than 35 years. Therefore, this method may be useful for selected patients undergoing clinical infertility treatment.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Source of funding

No funding was secured for this study.

Author contribution

Dr. Moghadaseh Jahanshahi and Dr. Sedigheh Hosseinimousa: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. Dr. Ashraf Aleyasin and Dr. Marzieh Aghahosseini: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. Dr. Aida Najafian and Dr. Maryam Shabani Nashtaei: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Registration of Research Studies

Name of the registry: IRCT20110602006689N4.

1. Unique Identifying number or registration ID: IR.TUMS.MEDICINEREC.1399.1258. Hyperlink to the registration (must be publicly accessible): https://ethics.research.ac.ir/ProposalCertificateEn.php?id=186195

Guarantor

Moghadaseh Jahanshahi.

Consent

Not applicable

Consent to participate

From the under 16 years old was given by a parent or legal guardian.

Availability of data and material

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Declaration of competing interest

The authors deny any conflict of interest in any terms or by any means during the study.

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