Original Article

Effect of Using Intra-Uterine Human Chorionic Gonadotropin Before and During Embryo Transfer in Cases of Intra-Cytoplasmic Sperm Injection

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ABSTRACT

Objectives: To evaluate the effect of intrauterine injection of Human Chorionic Gonadotropin (HCG) before and during embryo transfer on pregnancy rates in women with previously failed intra-cytoplasmic sperm injection (ICSI) trials.
Study Design: Prospective observational clinical study.
Subjects and Methods: A total of 39 women with at least one previously failed IVF/ICSI trial within the last year were included in the study. HCG was prepared by dissolving 5000 IU in 5 ml of normal saline, with 1 ml of this solution used to flush the endometrium at ovum retrieval and another 1 ml mixed with embryo culture media during transfer. Fresh embryos were transferred on day 3.
Results: Thirty-six patients were analyzed. The mean age was 29 ± 4.76 years, the mean BMI was 21.95 ± 1.20, and the mean duration of infertility was 6.34 ± 3.46 years. The mean number of follicles >16 mm was 6.32 ± 4.3, endometrial thickness was 9.21 ± 2.1 mm, the number of MII oocytes was 4.21 ± 3.6, fertilized oocytes was 3.41 ± 2.5, and transferred embryos was 2.11 ± 0.2. Chemical pregnancy was achieved in 16 out of 36 patients (44.4%), and clinical pregnancy in 15 out of 16 patients (41.6%). Fourteen pregnancies progressed, with one delivery and others continuing antenatal care.
Conclusion: Intrauterine injection of HCG before and during embryo transfer appears to increase pregnancy rates in women with previous failed ICSI attempts.

Key Words: Embryo transfer; human chorionic gonadotropin; ICSI; infertility; pregnancy rate.

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INTRODUCTION

Although assisted reproductive technologies (ART) have made great progress, the rate of successful embryo implantation is still below the desired level. This poses a serious barrier to the efficiency of in *vitro* fertilization with embryo transfer (IVF-ET) and intra-cytoplasmic sperm injection (ICSI) treatments. Implantation failure results in a significant number of pregnancies being lost, with a rate of over 50% in IVF-ET cycles, and contributes to a clinical pregnancy rate of just 30.4%[1,2].

In order to achieve successful implantation, it is important to possess embryos of superior quality and an endometrium that is receptive, thereby guaranteeing that the implantation takes place within the most favorable timeframe. Embryo implantation is a complex process that involves sophisticated molecular interactions between the embryo and the endometrium. Endometrial receptivity, embryo quality, and Human Chorionic Gonadotropin (HCG) are important factors that contribute to the successful implantation after IVF-ET[3].

HCG, functioning as a structurally similar version of LH, binds to the LHCGR (Luteinizing Hormone/ Chorionic Gonadotropin Receptors), which are present in both glandular and stromal cells of the endometrium. This interaction governs the process of endometrial receptivity and the successful implantation of an embryo. Implantation, a crucial biological connection between the embryo and the endometrium, is unsuccessful in 66% of IVF instances. The concentration of HCG strongly affects this process[4].

HCG is a crucial placental glycoprotein hormone that is necessary for the maintenance of pregnancy. It is created by the blastocyst around 6-8 days after fertilization. Primate embryos secrete HCG before implantation, as supported by evidence. HCG has the ability to attract inflammatory cells such as neutrophils, monocytes, and lymphocytes. It also increases the sensitivity of endothelial cells to interleukin 1, which leads to cell growth, movement, and the release of factors that promote the formation of new blood vessels[5].

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HCG has a vital role in stimulating the release of cytokines during the transition of the endometrium from the proliferative phase to the secretory phase. It also triggers the secretion of cytokines during the implantation window. This hormone promotes the formation of new blood vessels at the interface between the mother and fetus by increasing the expression of IL1R in cells of the endometrial stroma, which helps in the development of the embryo[6].

The presence of a direct relationship between the concentration of HCG in the media used for embryo culture and the rates at which embryos successfully implant indicates that HCG might serve as a valuable biomarker for selecting embryos in IVF. Sacchi *et al*. showed that extended exposure to low-dose HCG affects the ability of the endometrium to receive embryos by changing the phosphorylation of extracellular signal-regulated kinases 1 and 2, adherence to the extracellular matrix, and the integrity of tight junctions[7].

Embryos that secrete HCG early on have an advantage in implantation, whereas injecting HCG into the uterus can hinder the production of insulin-like growth factor binding protein-1 and macrophage colony-stimulating factor. This may directly affect the control of implantation, going beyond its traditional hormonal role. Therefore, HCG has the ability to control the signaling pathways of cytokines that are essential for the process of embryo implantation[8].

In the last 12 years, several clinical trials and meta-analyses have investigated the effects of intrauterine HCG injections on implantation rates. Nevertheless, the approaches and findings have been incongruous, resulting in ambiguous conclusions[9].

The objective of this study was to assess the impact of administering HCG through the uterus before and during the transfer of embryos on the success rates of pregnancy in women who have previously experienced unsuccessful attempts at ICSI.

SUBJECTS AND METHODS

This study was a prospective observational clinical study. It included 39 women with a history of at least one previously failed IVF/ICSI trial, with the current ICSI trial performed within one year of the last trial. The study was conducted from October 2022 to August 2023 at Assaf Fertility Center, Dokky, Cairo, Egypt.

All patients were under 40 years old, with at least 2 class A embryos transferred in the previous attempt. No HCG was used before or during embryo transfer in the previous attempt.

Primary Exclusion Criteria

Functional azoospermia, submucous uterine myomas or previous myomectomy, endometriosis, hydrosalpinx, and thin endometrium (<7 mm) at the time of HCG injection and/or before ovum retrieval.

Secondary Exclusion Criteria

Failed ovum retrieval, failed embryo development, and ovarian hyperstimulation syndrome.

Procedure

Human Chorionic Gonadotropin was prepared by dissolving 5000 IU of HCG in 5 ml of normal saline. One ml of this solution (equivalent to 1000 IU HCG) was used to flush the endometrium at the time of ovum retrieval using an embryo transfer catheter. At the time of embryo transfer, one ml of the prepared HCG solution was added to one ml of culture media, and the embryos were loaded in 20 µl of this solution. The embryos were then transferred to the mid-uterine cavity. Only fresh embryos were used in this study, and they were transferred on day 3, with a maximum of 3 embryos transferred to each patient.

Patients were positioned in the lithotomy posture during embryo transfer. The treatment was executed by the identical medical practitioners who carried out the prior endeavor. The transfer was conducted under the guidance of abdominal ultrasonography while the bladder was filled to capacity. The cervix was seen using a vaginal speculum and cleansed using embryo culture media. A semi-rigid catheter for embryo transfer, manufactured by Labotect GmbH in Gottingen, Germany, was utilized. The catheter was filled with 20 µl of embryo culture media containing HCG and the embryos. The tip of the catheter was positioned in the middle of the cavity. The embryos were injected with the HCG preparation in the embryo culture media, and the catheter was left within the cavity for a duration of four minutes.

A quantitative β-HCG sub-unit pregnancy test was conducted 14 days following embryo transplantation. If the test yielded a result of more than 50 miu, a transvaginal ultrasound was conducted two weeks later to detect the existence of a gestational sac. Following a further 2-week period, a vaginal ultrasound was performed again in order to detect the presence of fetal heart pulse.

Ethical Considerations

Approval from the ethical committee at the Ob/Gyn Department of Benha University and Assaf Fertility Center was obtained before the start of this study. After explaining the entire procedure, written consent was obtained from every participant in this study.

Statistical Analysis

The data management and statistical analysis will be conducted utilizing SPSS version 26 (IBM, Armonk,

New York, United States). The quantitative data were summarized using either means and standard deviations or medians and ranges. The categorical data were summarized using numerical values and percentages.

RESULTS

(Table 1) summarizes the basic and demographic characteristics of the patients, including age, body mass index, duration of infertility, basal LH, basal FSH, and AMH. The mean age of the patients was 29 years, the mean body mass index was 21.95, and the mean duration of infertility was 6.34 years. The mean basal LH and FSH measured on the second day of the menstrual cycle were 4.88 and 5.2 miu, respectively. The mean AMH value was 1.46 ng/ml.

Table 1: Basic and demographic characteristics

Characteristics	Mean ± SD	Range
Age in years	29± 4.76	21- 38
BMI	21.95 ± 1.20	20- 24
Duration of infertility in years	6.34 ± 3.46	2- 14
LH (miu)	4.88 ± 1.36	3- 8
FSH (miu)	5.20 ± 0.99	4- 8
AMH (ng/ml)	1.46 ± 0.5	1-3

Data were presented as mean ±standard deviation (SD) and range, BMI: Body Mass Index, LH: Luteinizing Hormone, miu: milli-international units, FSH: Follicle Stimulating Hormone, AMH: Anti-Müllerian Hormone, ng/ml: nanograms per milliliter.

Regarding types of infertility, 14 had a female factor, nine had a malefactor, seven had mixed, and 6 had unexplained infertility. (Figure 1).

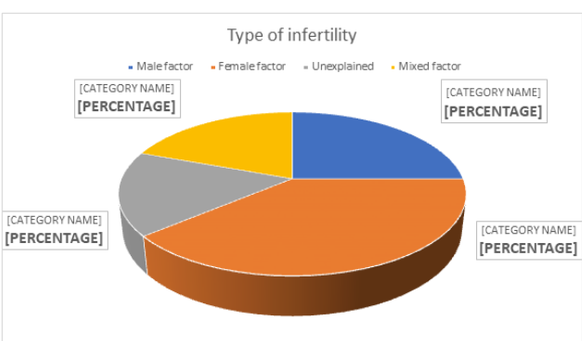

Fig. 1: Types of infertility

The primary outcome of the study is summarized in (Table 2).

Table 2: Primary outcome.

Characteristics	Mean ± SD	Range
Number of follicles > 16 mm	6.32 ± 4.3	2-12
Endometrial thickness (mm) On day of HCG injection.	9.21 ± 2.1	8- 12
Number of MII oocytes.	4.21±3.6	2-10
Number of Fertilized oocytes.	3.41 ± 2.5	2- 8
Number of Transferred Embryos	2.11 ± 0.2	2-3

Data were presented as mean ±standard deviation (SD) and range, mm: millimeters, HCG: Human Chorionic Gonadotropin, MII: Metaphase II.

All patients had fresh embryos transferred on day three and had at least one failed ICSI trial performed within one year at our center. The number of failed ICSI trials is shown in (Table 3).

Table 3: Number of failed ICSI trials.

ICSI Trial	Number of patients
2	24
3	8
4	4

ICSI: Intracytoplasmic sperm injection.

The secondary outcome showed that chemical pregnancy (β-HCG > 50 miu) occurred in 16 out of 36 patients (44.4%). (Figure 2).

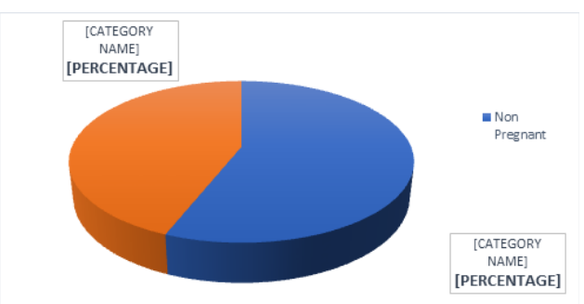

Fig. 2: Chemical pregnancy

Clinical pregnancy occurred in 15 out of the 16 patients with chemical pregnancy, with a gestational sac seen by vaginal ultrasonography performed 2 and 4 weeks after embryo transfer. The clinical pregnancy rate was 41.6%. (Figure 3).

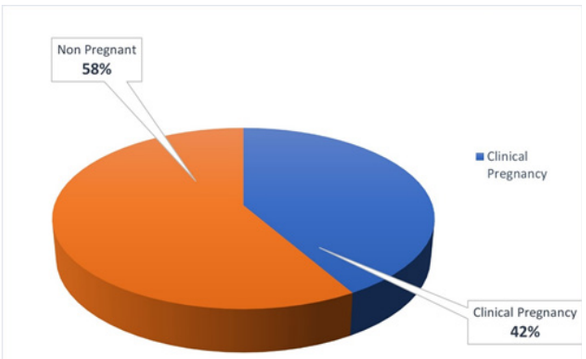

Fig. 3: Clinical pregnancy

Ongoing pregnancies

One patient was found to have a blighted ovum with an empty gestational sac, while the remaining 14 had regular gestational sacs with fetal echo and cardiac pulsations. One patient delivered a healthy baby on July 27, 2023; eight patients continued their antenatal care with us, having different gestational ages until August 2023. Five patients failed to comply with follow-up. (Figure 4).

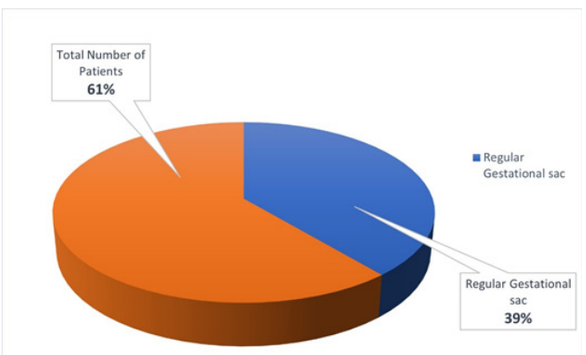

Fig. 4: Ongoing pregnancy

DISCUSSION

Multiple investigations have demonstrated the benefit of the embryo secreting HCG early, prior to implantation. Previous studies have shown the crucial function of HCG in regulating the inflammatory reaction and facilitating the growth of new blood vessels during the process of embryo implantation. The adverse effects of IVF treatment on endometrial receptivity can be alleviated by administering HCG before embryo transfer[3,10]. Research by Xiano *et al*. identified a positive correlation between high HCG levels in embryo culture media and implantation success rates[11].

In the past 12 years, many studies have investigated the impact of endometrial flushing with HCG prior to embryo transfer on pregnancy outcomes. Most of these studies were randomized controlled trials involving study and control groups[12,13]. In our study, each patient acted as her own control. We included 39 patients who had experienced one or more previously failed trials. The reviewed studies utilized HCG doses ranging from 100 to 1000 IU, with our study employing a dose of 1000 IU.

Regarding the timing of HCG administration, some studies performed endometrial flushing immediately before embryo transfer, while others did so 3 to 15 minutes beforehand. A few researchers administered HCG three days before the transfer. In one study, the endometrium was not flushed before the transfer; instead, the embryo was placed in a medium containing HCG[14]. In our study, we performed endometrial flushing with HCG at the time of ovum retrieval to maintain consistent endometrial fluid content during embryo transfer. During the transfer, we also included the embryos in a medium with HCG, following the method suggested by Santibañez *et al*. This approach was not widely used in other studies[14].

We employed an embryo transfer catheter for the flushing process, as other studies have shown that it is more effective than the IUI catheter due to its length and smaller diameter, allowing for more efficient HCG injection. Most studies used fresh cleaving-stage embryos, whereas Jahanshahi *et al*. and Huang *et al*. used frozen embryos[15,16]. Wirleitner *et al*. and Zarei A focused their studies on blastocysts[17,18].

For this research, newly divided embryos were moved. Multiple studies have demonstrated that the use of HCG can improve pregnancy rates. In their study, Mansour *et al*.[13] found that injecting 500 IU HCG just before cleavage stage embryo transfer (ET) resulted in a substantial increase in pregnancy rates. The study group had a pregnancy rate of 41%, compared to 29.5% in the control group. Comparable results were noted for cleavage-stage embryo transfers (ETs), however, in the case of blastocyst transfers, the beneficial impact of intrauterine HCG treatment was only evident in low-quality blastocysts.

In research utilizing a greater dosage of HCG, Zarei *et al*.[18] observed enhanced pregnancy rates after administering an intrauterine injection of 250 µg of recombinant HCG (equal to 6,500 IU) before day three transfer. The researchers discovered that administering intrauterine rHCG injection prior to embryo transfer greatly improved the rates of implantation, clinical pregnancy, and continued pregnancy.

Aaleyasin *et al*.[19] discovered that the study group had a pregnancy rate of 54.6%, whereas the control group had a pregnancy rate of 35.8%. Prior to the transfer of fresh cleaving embryos, the endometrium was flushed with 500 IU of HCG. In addition, the administration of HCG injections was associated with a greater incidence of multiple pregnancies when compared to the control group.

Santibañez *et al*.[14] did not use HCG to flush the endometrium before embryo transfer but included the embryos in a medium containing HCG. They observed a significantly higher pregnancy rate in the study group, with an increase of 15% compared to the control group. In their Cochrane Database systematic review, Craciunas *et al*.[20] concluded that using intra-cavity HCG (IC-HCG) at a dose of 500 IU or more for cleavage-stage ETs has promising outcomes. Mostajeran *et al*.[21] reported a pregnancy rate of 36.4% in the study group compared to 19% in the control group. They used 700 IU HCG to flush the endometrium 10 minutes before transferring fresh cleaving embryos.

In their meta-analysis, Simopoulou *et al*.[22] found a significant increase in pregnancy rates following the intrauterine injection of 1000 IU HCG 5-12 minutes before embryo transfer.

Huang *et al*.[16] used frozen cleaving embryos in women with previously failed ICSI trials and injected 1000 IU HCG into the study group 3 days before embryo transfer. They reported a pregnancy rate of 59.6% in the study group versus 32% in the control group. Similarly, Jahanshahi *et al*.[15] used frozen cleaving embryos. They concluded that the intrauterine injection of 500 IU HCG just before embryo transfer significantly increased the implantation rate, with 51% in the study group compared to 35% in the control group.

In a study conducted by Torky *et al*.[23], the researchers examined the impact of injecting granulocyte colony-stimulating factor (100 mcg/1cc), 5000 IU HCG, or saline during ovum retrieval in cases where there was a repeated failure of implantation. The first group had the greatest pregnancy rate at 56%, followed by the second group at 46% and the saline group at 27%. Conforti *et al*.[9] found in their comprehensive review and meta-analysis that HCG may be an effective strategy for women undergoing cleavage stage embryo transfer. In their study, Abdallah *et al*.[24] found that administering 500 IU of HCG during blastocyst transfer did not have a significant effect on the rates of successful implantation or delivery. They observed that the blastocysts in their experiment were already anticipated to release HCG, indicating that the extra supplementation was insufficient to produce a positive outcome.

Wirleitner *et al*.[17] performed research on blastocysts, where they administered a dose of 500 IU of HCG two days before and immediately before embryo transfer. The study found no significant increase in clinical outcomes in blastocyst transfer cycles, irrespective of the embryo quality, as evidenced by pregnancy rates of 50% in the study group and 53.3% in the control group. In a research conducted by Hosseinisadat *et al*.[25], it was discovered that administering 100 IU of HCG after oocyte extraction did not enhance the rates of clinical or chemical pregnancy in ART cycles. The study group had a pregnancy rate of 13.7%, whereas the control group had a rate of 13.04%. Naghshineh *et al*.[12] conducted research where patients were given 500 IU HCG intrauterine 15 minutes before transplanting fresh or vitrified embryos. They found that the pregnancy rate in the experimental group was 22%, whereas it was 14% in the control group. However, this difference was not statistically significant.

In our study, we used each patient as her own control, flushing the endometrium with 1000 IU HCG on the day of ovum retrieval and including the cleaving embryo in a medium containing HCG. The clinical pregnancy rate was nearly 42%.

Beyond the beneficial effect of HCG, the high pregnancy rate may also be due to the cumulative pregnancy rate. A positive psychological impact might have also contributed, as patients may feel that modifying the embryo transfer technique could improve outcomes.

In this study, we avoided flushing the endometrium with HCG immediately before embryo transfer to maintain the endometrial milieu. This approach contrasts with many studies that performed the flushing just before embryo transfer.

All participants in this study had experienced a previous failed attempt within one year at the same fertility center, serving as their own controls. The results demonstrated a clinical pregnancy rate of 41.6% (15 out of 36 patients). This study suggests that endometrial flushing with HCG at the time of ovum retrieval, without altering the endometrial environment and including the embryos in a medium containing HCG, may increase pregnancy rates.

CONCLUSIONS

Flushing the endometrium with HCG at the time of ovum retrieval, without altering the endometrial milieu, and including the embryos in a medium containing HCG may increase the pregnancy rate, possibly due to the positive psychological impact on patients and the cumulative pregnancy rate, but larger, multicentric studies are needed to confirm these findings.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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