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Intrauterine Instillation of Human Chorionic Gonadotropin with Intrauterine Insemination Catheter Around the Golden Time of Embryo Transfer Does Not Improve *In Vitro* Fertilization /Intracytoplasmic Sperm Injection Outcomes in Infertile Women: A Randomized Controlled Trial

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Abstract

Background: We set out to explore the effect of intrauterine human chorionic gonadotropin (hCG) instillation by intrauterine insemination (IUI) catheter before embryo transfer (ET) on assisted reproductive technologies (ART) outcomes of infertile women.

Methods: One hundred women with infertility who were scheduled for *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles were included in the study. They were randomly devoted to two groups: experimental (n= 50) and control (n= 50). In the experimental group, 500 IU hCG passed into the internal cervical orifice via IUI catheter within 15 minutes before the transfer of fresh or vitrified cleavage-stage embryos. The control group underwent the same ET procedure without prior injection of hCG.

Results: None of the outcomes showed a statistically significant difference between the two groups. In the intervention and control groups, respectively, biochemical pregnancies rates were 26% and 18%, implantation rates were 13.5% and 8.6%, clinical pregnancies rates were 22% and 14%, ongoing pregnancies rates were 18% and 14%, and live birth rates were 14% and 12%.

Conclusions: Intrauterine injection of hCG via IUI catheter is not recommended in a clinical routine setting at this stage. Future efforts are warranted to further refine the applicability of this modality.

Keywords: Assisted reproductive technologies, Embryo transfer, Human chorionic gonadotropin, Intrauterine insemination catheter, Randomized clinical trial.

Introduction

Approximately 15% of couples suffer from infertility worldwide which is defined as not occurrence of pregnancy after one year of regular unprotected sexual intercourse (1). Although the field of assisted reproductive technologies (ART) has seen considerable progress over the last couple of decades, the high rate of embryo implantation failure (> 50%) undermines the success of *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatments (2). Furthermore, the rate of other outcomes such as clinical pregnancy has been low after IVF/ICSI programs (3).

Hence, unwavering research efforts have been conducted to improve both the quality of

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embryos and receptivity of endometrium, which have been resulted in higher efficiency of the most critical step in IVF/ICSI cycles i.e., embryo transfer (ET) (4). With this goal in mind, human chorionic gonadotropin (hCG), a homologous isomer of luteinizing hormone, has been regarded as a key player in the process of embryo implantation in the early stages of pregnancy by improving embryo-womb cross-talk. This function emanates from autocrine and paracrine effects of hCG on the decidualization, vascularization, and tissue remodeling (5). human chorionic gonadotropin (hCG) also has a critical role in maternal-fetal immune tolerance via attracting regulatory T-cells and maintaining the balance between Th1 and Th2 effector cells, speeding up trophoblast invasion, apposition, and adhesion to the endometrium (6). This hormone that is produced and released by developing embryo, cytotrophoblast, and syncytiotrophoblast, initially stimulates the production of progesterone during the first trimester of gestation to support the maternal corpus luteum and ensure maintenance of ensuing pregnancy (7).

Instilled hCG inside the uterine cavity prior to ET is believed to exert its effect via two principal mechanisms comprising instigation of immune cells immigration into the uterus (8), and induction of a variety of cytokines in endometrium within the implantation window by modulating cytokine signaling transduction pathways (9). Considering the results of the two last meta-analyses of randomized controlled trials (RCTs) (10, 11), in infertile women undergoing assisted reproduction, the optimal IVF/ICSI outcomes including live birth rate (LBR), ongoing pregnancy rate (OPR), clinical pregnancy rate (CPR), and implantation rate (IR) can be obtained with local administration of 500 IU hCG via ET catheter during 15 minutes preceding ET, particularly at the cleavage stage. However, they proposed that because of incongruent and conflicting findings, more future confirmatory investigations are needed to reach a firm conclusion. In addition, to the best of our knowledge, except for one study that used intrauterine insemination (IUI) catheter for injection of hCG (12), in all the other studies in which hCG perfusion was performed within the 4-15-minute time frame before ET, the researchers utilized ET catheter for instillation.

Therefore, we decided to replicate this timing and dosage in a new clinical trial employing IUI catheter on the account of exploiting this type of catheter for intrauterine hCG injection is more cost-effective and easier to use than ET catheter. In this study, CPR, OPR, and LBR were considered as primary outcomes and IR, and biochemical pregnancy rate (BPR) were regarded as secondary outcomes. Also, the possible occurrence of complications, if any, such as miscarriage rate (MR), ectopic pregnancy (EP), intrauterine fetal demise (IUFD), and multiple pregnancy (MP) were recorded.

Materials and Methods

Subjects

This study was a randomized double-blind clinical trial in which infertile women who were referred to Shahid Beheshti infertility center in Isfahan, Iran from February to May 2021 were recruited. After complete history evaluation and physical assessment, we included only the patients under the age of 40 who were candidates for IVF/ICSI cycles with at least one high-quality embryo i.e., grade A or B. The exclusion criteria were infertility due to azoospermia, presence of hydrosalpinx, any endocrine dysfunction consisting of diabetes or thyroid disease, history of uterine surgery, severe endometriosis, operative hysteroscopy, recurrent pregnancy loss, and three or more repeated implantation failure (RIF). Also, because of the pandemic of Coronavirus disease 2019 (Covid-19), all the patients, their partners, and staff underwent triage questionnaire according to guidelines of the European Society of Human Reproduction and Embryology (ESHRE; https://www.eshre.eu/covid19). Only the triage-negative persons were included.

Considering power of study and level of significance equivalent to 90% and 5%, the

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sample size was calculated according to this formula: n= $(2\times(Z\alpha+Z\beta)^2/(ES)^2$ where Zα is value of Z at two-sided alpha error of 5% and Zβ is value of Z at power of 90% and ES is effect size (13). Thereby, the calculated number of participants was n= $(2\times (1.96+1.282)^2/ (0.65)^2$= 49.7(approximately 50). Thus, 100 liable women were randomly allocated into experimental (n= 50) and control (n= 50) groups. We tried to match the two groups in terms of age, body mass index (BMI), and anti-müllerian hormone (AMH). Randomization was carried out before the start of the treatment cycle by Random allocation software (14). This study was approved by the Ethics Committee of the Isfahan University of Medical Sciences (ethics code: IR.MUI.MED.REC.1400.414), followed the tenets of the declaration of Helsinki, and registered in https://fa.irct.ir/ (Registration ID: IRCT20160703028756N7). Written informed consent was obtained from all the participants.

Procedure

In the light of possible unknown effects of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) on IVF/ICSI cycles and outcomes, prior to initiation of the procedure, all the women underwent quantitative real-time PCR (qRT-PCR) test to ensure that they are free of Covid-19.

To prepare the desired dose of hCG, 5000 IU urine-derived hCG powder (Karma, BioScience GmbH, Germany) was dissolved in 0.4 ml EmbryoGlue® medium. Then, in the intervention group who were placed in a lithotomy position and their cervix was visualized through the insertion of Cusco's vaginal speculum, 40 µL of the prepared hCG (equivalent to 500 IU) was injected into the uterus via IUI catheter (Masstec Medical Co., Isfahan, Iran) within 15 minutes before ET. Also, cotton swabs were used to clean cervical mucus. To minimize the potential source of error, intrauterine flushing of hCG was carried out by an experienced gynecologist. For transfer of embryos, under abdominal ultrasonographic guidance with a full bladder and at a distance of 1-1.5 cm away from the uterine fundus, one to two 3-day post-fertilization (cleavage stage) fresh or vitrified/warmed embryos were transferred by Labotect catheter (Labotect GmbH, Labor-Technik-Gottingen, Germany) depending on patient age and the status of embryos. In the control group, the ET was conducted with the same procedure but without the prior intrauterine hCG instillation. Following ET, luteal phase support continued with 100 mg/day intramuscular progesterone injection (Aburaihan Co., Tehran, Iran). Biochemical pregnancy was confirmed if, after two weeks of ET, the serum value of βhCG was positive according to standard values of the laboratory. Vaginal ultrasound was leveraged one week later to confirm the presence of one or more gestational sacs which signifies the successful implantation. Clinical pregnancy (positive embryonic heart activity), ongoing pregnancy (continuation of pregnancy over than 12 weeks), live birth (parturition of a live fetus following 24 completed weeks of gestation), and miscarriage (fetal loss earlier than 20 completed weeks of gestation) were also surveyed.

Throughout the entirety of the study, the embryologist, the data analyzer, and all the patients were blinded to the allocation of the patients in two groups. Only the attending gynecologist who performed the procedure, followed up the outcomes, and collated the data was aware of the patient assignment.

Statistical analysis

Initially, the normal distribution of data was checked by the Kolmogorov–Smirnov test. An independent sample t-test was harnessed for continuously distributed data to compare between groups. For categorical data, the Chi-squared test was utilized. Continuous and categorical variables are represented as mean ±SD and frequencies and percentages, respectively. p-value≤ 0.05 was regarded statistically significant. All the analysis was accomplished using Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp).

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Results

The basic demographic and clinical characteristics of the patients are represented in Table 1. Characteristics were comparable between the two groups except for age and BMI. The CONSORT statement flow diagram is depicted in Fig 1. In both groups, no patient was lost to follow-up. Of particular note, none of the explored outcomes were significantly different between the two groups (Table 2). The p-values were adjusted for women's age and BMI leveraging logistic regression to assess the effect of potential confounding factors. However, this analysis demonstrated that the observed findings were independent of confounding factors (Table 2). Then, the intervention group was subdivided into fresh (n= 22) and frozen (n= 28) to evaluate the possible effect of the type of transferred embryo on the effectiveness of hCG infusion. The results delineated that type of transferred embryo does not influence ART outcomes (Table 3). In terms of adverse events, MR was not statistically different between intervention and control groups (p= 0.162; adjusted p= 0.135; Table 2) and between fresh and frozen subgroups in patients who received the hCG (p= 0.776; Table 3). Other adverse events were so few to permit the p-value to be calculated. Within this context, no cases of EP were seen, one twin pregnancy was recorded in the control group, and in control and intervention groups, respectively, one and two cases of IUFD were revealed.

Table 1. Baseline demographic and clinical characteristic of the hCG-treated group and no-injection group.

Criteria		hCG-treated group (n= 50)	no-injection group (n= 50)	P value
Age, years		33.5±4.7	31.36±3.9	0.016
BMI, Kg/m^2		21.92±2.25	23±2.43	0.023
AMH, ng/ml		2.27±1.22	2.37±1.36	0.699
Number of transferred embryos	One	11 (22)	7 (14)	0.298
	Two	39 (78)	43 (86)	
Type of embryo	Fresh	22 (44)	20 (40)	0.685
	Frozen	28 (56)	30 (60)	
Causes of infertility	PCO	7	8	0.081
	Tubal factor	9	10	
	Unexplained	24	11	
	Male factor	10	21	

BMI: Body mass index; AMH: Anti-müllerian hormone; PCO: Polycystic ovary syndrome; Values represent mean ± SD or number (%).

Table 2. Pregnancy outcomes and complications of the hCG-treated group and no-injection group.

	hCG-treated group (n= 50)	no-injection group (n= 50)	p-value	Adjusted p-value
Biochemical pregnancies rate	13 (26)	9 (18)	0.334	0.171
Implantation rate*	12/89 (13.5)	8/93 (8.6)	0.317	0.175
Clinical pregnancies rate	11 (22)	7 (14)	0.298	0.245
Ongoing pregnancies rate	9 (18)	7 (14)	0.585	0.503
Live birth rate	7 (14)	6 (12)	0.766	0.705
Miscarriage rate	10 (20)	5 (10)	0.162	0.135

Values represent number (%); *: The only outcome that was calculated with the number of events divided by the number of embryos transferred for each patient.

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Table 3. Pregnancy outcomes and complications in the hCG-treated group who were subdivided in terms of fresh or frozen embryonic transfer.

Criteria	Fresh group (n= 22)	Frozen group (n= 28)	p-value
Biochemical pregnancies rate	7 (32)	6 (21.4)	0.406
Implantation rate	6 (27.3)	6 (21.4)	0.631
Clinical pregnancies rate	5 (22.7)	6 (21.4)	0.912
Ongoing pregnancies rate	4 (18.2)	5 (17.9)	0.976
Live birth rate	4 (18)	3 (10.7)	0.450
Miscarriage rate	4 (18)	6 (21.4)	0.776

Values represent number (%).

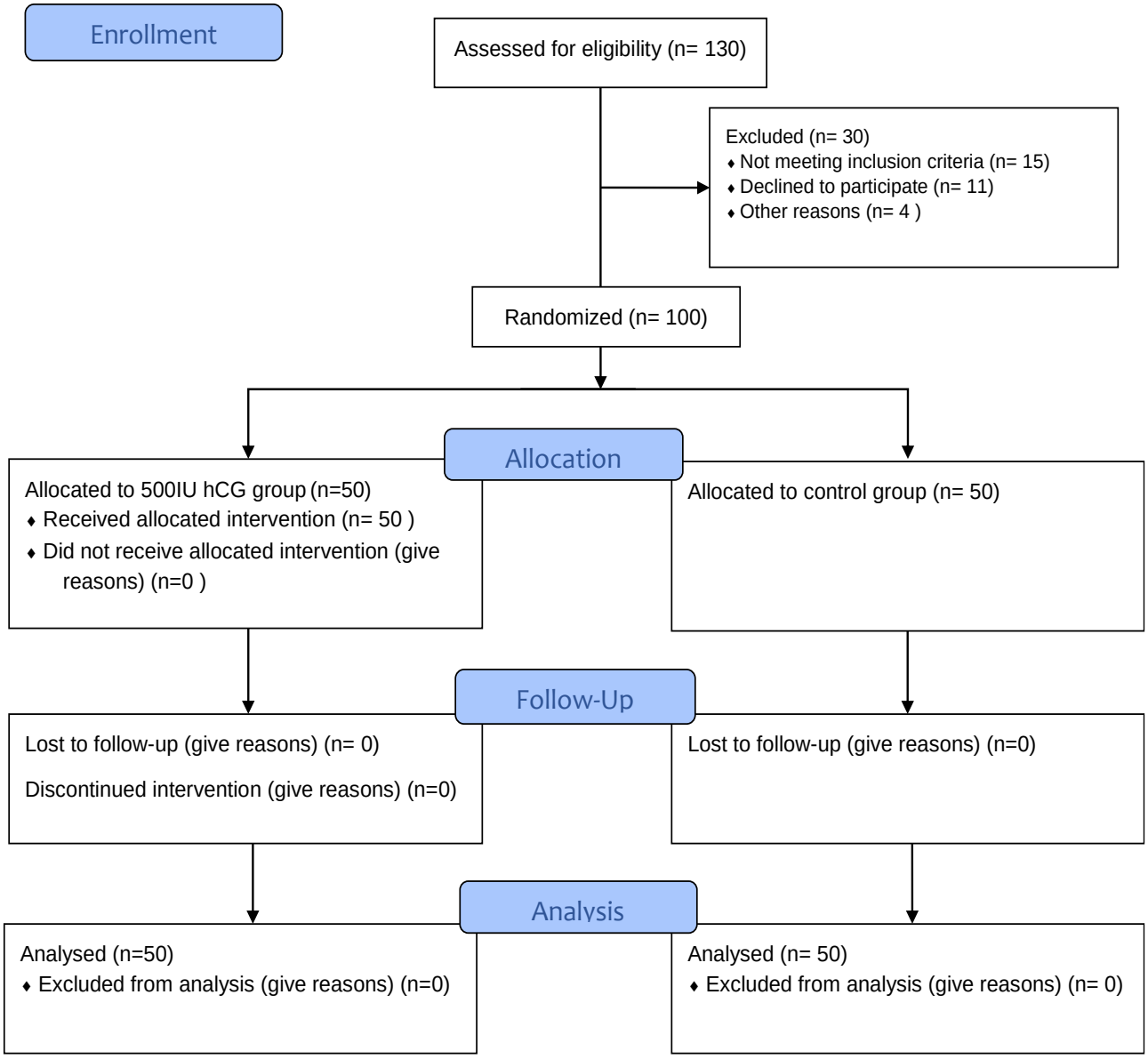

Fig. 1. Flow diagram of participants throughout the trial.

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Discussion

Regardless of woman age and the cause of infertility, the success rate of IVF/ICSI has been reported to be around 30%, of which 50-75% have been attributed to implantation failure (15). In this regard, several modalities have been propounded for improving embryo-decidua communication subsuming, among others, endometrial scratching, prescription of aspirin, heparin, and corticosteroids, and administration of hCG and autologous peripheral blood mononuclear cells into the uterine cavity (16).

As a unique heterodimeric placental glycoprotein hormone, hCG is secreted by cytotrophoblast to guarantee successful implantation via intensifying endometrial receptivity and facilitating a well-timed embryo-endometrium dialogue. Intrauterine infusion of hCG before ET, as an unconventional treatment option for infertility, is postulated to provoke the activation of vascular endothelial growth factor (VEGF), leukemia-inhibitory factor (LIF), and matrix metalloproteinase 9 (MMP-9), whereas prevents macrophage colony-stimulating factor (M-CSF) and insulin-like growth factor-binding protein 1 (IGFBP-1) (17). According to the last meta-analysis studies, it can be concluded that among different time- and dose-response relationships between intrauterine hCG injection and IVF/ICSI-ET outcomes, the 500 IU hCG during 15 minutes before ET yields the best outcomes (10, 11, 18). However, the majority of the studies have utilized expensive ET catheters. This encouraged us to investigate the application of intracavity instillation of hCG via IUI catheter, as a cheaper catheter.

Our results, at odds with previous studies (10, 11), demonstrated that administration of hCG as an adjunct modality of ART does not significantly improve chances of implantation and subsequent clinical pregnancy in the case group than their counterparts in the control group (Table 1). Abdallah et al., similar to our study, employed an IUI catheter for administration of 500 IU hCG around the time of ET and did not notice any beneficial effect on CPR, OPR, and LBR (12). However, in contrast with the present study, they infused hCG into the uterus 4 minutes before ET, injected culture media as a placebo in the control group before ET, transferred both morula/blastocyst-stage and cleavage-stage embryos, defined live birth as delivery of live baby 28 weeks of pregnancy or over and miscarriage as the spontaneous loss of pregnancy before 28 weeks of gestation, enrolled larger sample size (case and placebo groups were 90 and 91, respectively), and two groups were totally comparable in terms of baseline and cycle characteristics. It can be speculated that the injection of hCG less than 5 minutes before ET was one of the reasons for the not improvement of IVF/ICSI outcomes in their study. Two possible scenarios could be ascribed to this event. On the one hand, the volume of hCG preparation that is introduced to the uterus very close to the planned ET may lead to an excess volume after ET, followed by floating and washing out the embryo that increases the chance of aberrant implantation (19). On the other hand, minor trauma introduced by an hCG-loaded catheter less than 5 minutes before actual ET can possibly contribute to low rates of IVF/ICSI outcomes (20). In this regard, Laokirkkiat and colleagues unraveled that hCG infusion with ET catheter 4 minutes before fresh or frozen-thawed ET can improve IR, but not CPR and LBR. This pinpoint that, irrespective of the time of hCG injection, an ET catheter is more effective than an IUI catheter for hCG instillation (21). We hypothesized that more length and less diameter of soft ET catheters permit the technicians to inject the substance more efficiently (22). Further to this, the IUI catheter has a straight shape while the ET catheter has a small curvature at the end that allows imitating the anatomy of the womb. What's more, the presence of a notch in the IUI catheter may possibly traumatize the endometrium and instigate mild uterine contractions, which in turn impairs

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implantation. Ultimately, contrarily to IUI catheters, ET catheters are qualified by quality control tests, namely Mouse Embryo Assay (MEA) and endotoxin assay (LAL) to prevent the embryotoxicity (23).

To eliminate the possible impact of embryo status as a potential confounder, in this study, only cleavage-stage embryos were transferred because researchers have unveiled that the transfer of embryos at the blastocyst stage does not provide enough time for the hCG to execute its beneficial effect on the endometrium before implantation (24). Although previous RCTs sparsely continued follow-up until live birth to record the most important primary outcome (11), we scheduled to uncover the LBR. In addition, by contrast with most previous RCTs (11), we reported the presence or absence of some complications including MR, EP, IUFD, and MP. Given the hypothesis that the vitrification process might cause damage to embryos (25), the outcomes of fresh and frozen subgroups were compared in our study; however, contrary to the results of Gao et al. (10), the fresh subgroup did not exhibit significantly superior pregnancy outcomes than the frozen subgroup (Table 3).

Nonetheless of these strengths, our work has some limitations that should be considered. These contain low sample size, not consideration of a placebo (infusion of plain culture media or saline without hCG) in the control arm (sham group) to address the plausible positive or negative influence of the infusion itself and to decrease the performance bias that can be stemmed from extra catheterization of the uterine cavity and subsequent local injury (26), and exclusion of RIF patients in whom the efficacy of hCG instillation has been indicated (27). Finally, some possible effects of Covid-19 on the results were ignored. For instance, regarding the false-negative rate of qRT-PCR, ruling out SARS-CoV-2 infection only by the qRT-PCR is not reliable (28). Besides, although all the females were qRT-PCR negative at the beginning of the procedure, they were not screened further In view of the plausible unknown effects of the Covid-19 on IVF/ICSI endpoints (29), this may underpower our findings. Additionally, Gacci et al showed that Covid-19 can impact sperm parameters for three months (30); while, in our center, collection of sperm is undertaken up to one month after recovery of men from SARS-CoV-2 infection. More importantly, emerging evidence has demonstrated that Covid-19 can negatively affect the quality of oocytes, function of the ovary, and endometrial receptivity and subsequently cause implantation failure (31). Therefore, the possible confounding effects of Covid-19 on our results should not be underestimated.

In aggregate, intrauterine injection of hCG preceding ET is not a complex and time-consuming procedure (32); although, the results of the current study and recent published RCT by Abdallah and colleagues (12) add uncertainties about the applicability of IUI catheters. Our results indicate that IUI catheter is not suitable for hCG injection and ET catheter cannot be substituted for this purpose. However, the effect of different hCG preparations in terms of source and brand on the results of the studies should also be noted (33).

Taken together, the findings of this study do not support the superiority of hCG injection via IUI catheter into the womb of subfertile women around the time of ET. Larger scale multicenter RCTs are required to establish a guideline and cement the application of intrauterine hCG injection prior to ET as adjuvant therapy of IVF/ICSI cycles in daily clinical ART practice.

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