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Impact of Intrauterine Injection of Human Chorionic Gonadotropin at Embryo Transfer on Intra Cytoplasmic Sperm Injection Outcome

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ABSTRACT

Background: for successful embryonic implantation, a healthy embryo at blastocyst stage and a functional endometrium ready to receive it are basic requirements. There is growing research evidence that reveals the importance of embryonic endometrial synchrony for the accomplishment of a successful conception.

Objectives: The aim of this research study is to assess the impact of hCG intrauterine injection procedure before embryonic transfer on enhancing pregnancy and implantation rates.

Patients and methods: a prospective randomized clinical research trial that was conducted on 600 cases undergoing embryo transfer via an ICSI program at the ART unit of the International Islamic Centre for Population Studies and Research (IICPSR), Al Azhar University. Cases were categorised randomly into 2 research groups. In the first group (study research group), intrauterine of 500 IU HCG were injected before the embryonic transfer procedure. The second group (control research group), has gone through embryonic transfer without prior injection of hCG.

Results: chemical and clinical pregnancy rates were statistically significantly more frequent among research study group (HCG intra uterine injection group). In addition multiple pregnancy was statistically significantly higher within research study group. Implantation Rate was statistically significantly higher among study group. Interestingly no statistical significant difference between study and control groups as regards early and late ovarian hyper-stimulation.

Conclusion: there is a possible role of HCG in enhancing and improving endometrial receptivity and increasing implantation and pregnancy rates. Future research efforts should consider racial, ethnic and genetic differences in response to HCG intrauterine injection.

INTRODUCTION

Human chorionic gonadotropin (HCG) is a hormone produced by the placenta after embryonic implantation. HCG is a glycoprotein hormone formed of 237 amino acids with a molecular weight of 36.7 k Daltons, around 14.5 α subunit-hCG and 22.2 k Daltons β-subunit HCG. It permits the corpus luteum to secrete progesterone hormone during the first gestational trimester [(1-5)].

HCG injection is widely implemented for final maturation induction in fertility management protocols. In the presence of one or more mature ovarian follicles, ovulation could be triggered by the administration of HCG. HCG is a multifaceted hormone with a very wide range of actions. HCG determines fetal fate by regulating maternal innate and adaptive immune responses, allowing the acceptance of the foreign fetal antigens [(6-8)].

Various research studies have focused on the correlation between endometrial receptivity and infertility. Even though the blastocyst can implant in various human tissues, astonishingly in the endometrium, this physiological phenomenon can only happen during a self-limited period of time (implantation window) [(9-11)].

HCG has a cornerstone role in cellular physiological differentiation / proliferation and could trigger apoptosis. HCG is one of the key molecules during the process of implantation, it effectively modulates several metabolic pathways within the decidua, contributing to endometrial receptivity [(12-16)].

The hypothesis that infertile women undergoing IVF/ICSI may benefit from the intrauterine infusion of HCG before embryo transfer was based on the findings of the previous studies which reported that hCG is secreted in human cleavage-stage embryos and at the time of implantation. It has been shown to enhance the endometrial receptivity in both human and non-human primates. After the first report of benefit effect of the intrauterine hCG (IU-hCG) infusion on pregnancy outcome by Mansour *et al.* [(20)], several research studies have been conducted to evaluate this intervention on ART outcomes. Some studies found significant improvement on pregnancy outcomes while some other did not. In a recent Cochrane review, researchers concluded that administration of IU-hCG in a dose of 500 IU or greater in fresh cleavage-stage embryo transfer cycles is associated with promising pregnancy outcomes [(17-20)].

AIM OF THE WORK

The aim of this research study is to assess the impact of hCG intrauterine injection procedure before embryonic transfer on enhancing pregnancy and implantation rates.

PATIENTS AND METHODS

A prospective randomized clinical trial that was conducted on 600 cases undergoing embryo transfer via an ICSI program at the ART unit of the International Islamic Centre for Population Studies and Research (IICPSR), Al-Azhar University.

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Inclusion research criteria: Cases' age 20-35 years old, the body mass index (BMI) ≤ 27kg/m^2, normal basal (day 3) FSH, LH and E2 serum levels and normal prolactin serum level.

Exclusion research criteria: Previous ICSI trials, male azospermia and cases having uterine abnormalities e.g submucous fibroid, intrauterine synechiae and endometrial polyps.

All cases underwent controlled ovarian hyperstimulation using long mid-luteal protocol. They have been monitored by serum E2 and trans-vaginal sonography from the day 6 of ovarian stimulation, then every other day till at least 5 or more follicles reached 18 mm in diameter.

riggering dose of hCG have been given then, oocyte retrieval was conducted via vaginal route under sonographic guidance under general anaesthesia 34-36 hours after hCG administration. Cases were categorised randomly into 2 research groups. In the first group (study research group), intrauterine injection of 500 IU hCG have been performed 7-10 minutes before the embryonic transfer procedure.

The second group (control research group), have gone through embryonic transfer without prior injection of hCG. Progesterone have been prescribed for 16 days for luteal support in the form of 100 mg daily intramuscular injection. Serum β-hCG was assayed 14 days after embryonic transfer to diagnose chemical pregnancy. When pregnancy test was positive, luteal support was continued till 8^{th} gestational week. The transvaginal sonography was performed after 3 weeks, for diagnosis of clinical pregnancy.

Research outcome measures:

The primary research outcome measures were the clinical pregnancy rate (PR) and the implantation rate (IR). The secondary research outcome measures were the first trimesteric miscarriage rate, multiple pregnancy rate and the occurrence of ovarian hyper-stimulation.

Ethical consideration and Written informed consent:

An approval of the study was obtained from Al- Azhar University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.
- Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.
- The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:
- Probability (P-value)

- P-value <0.05 was considered significant.
- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

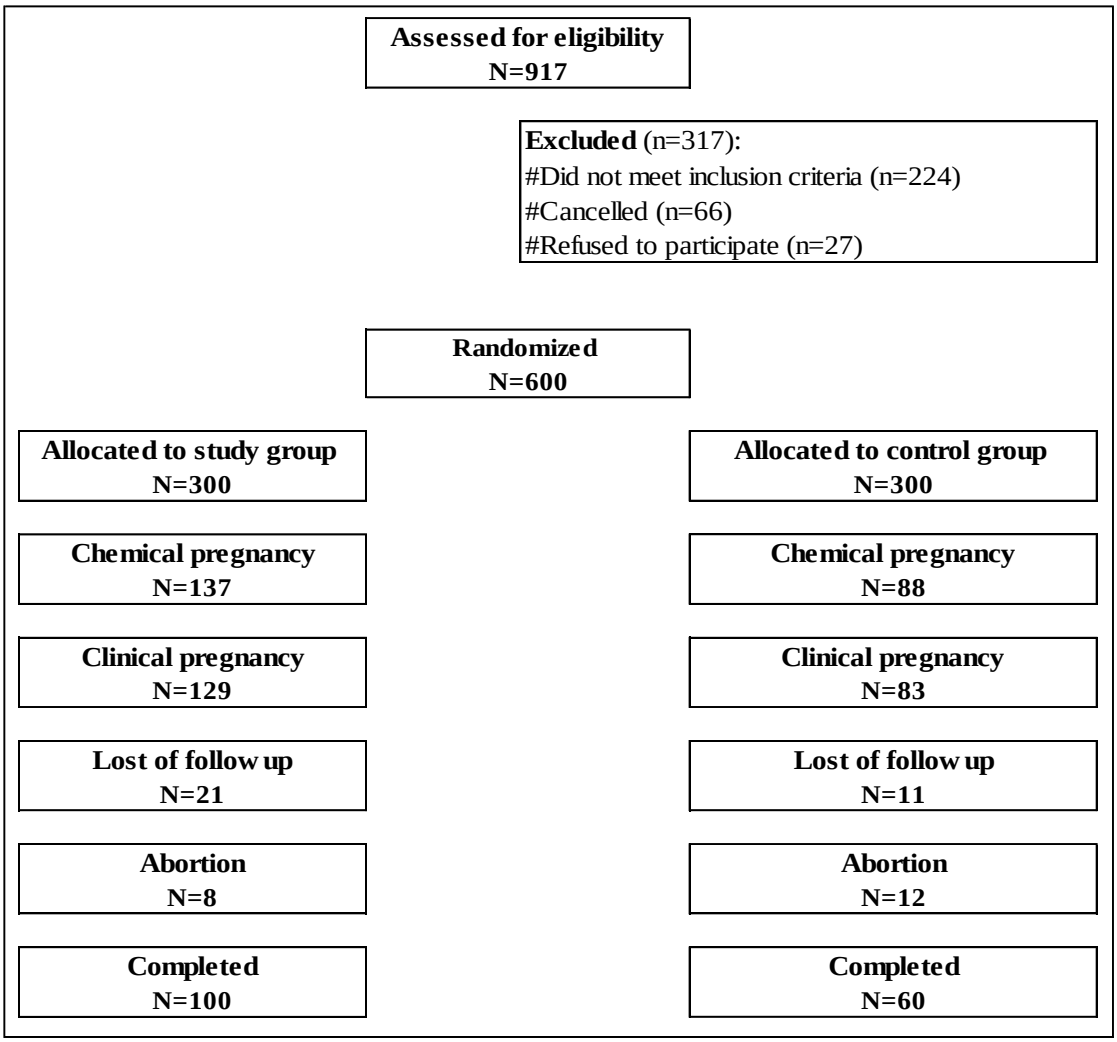

Figure (1): Study flow chart

Table (1): Demographic characteristics among the studied groups

Variables	Measures	Study (N=300)	Control (N=300)	P
Age (years)	Mean ± SD	29.5 ± 2.5	29.2 ± 2.4	^0.142
	Range	22.0–35.0	23.0–35.0	
BMI (kg/m2)	Mean ± SD	23.5 ± 1.3	23.4 ± 1.6	^0.456
	Range	20.0–27.0	19.5–26.8	
Duration of infertility (years)	Mean ± SD	7.3 ± 2.2	7.0 ± 2.1	^0.128
	Range	2.0–13.0	2.0–13.0	
Cause of infertility (n, %)	Male	153 (51.0%)	162 (54.0%)	#0.607
	Female	62 (20.7%)	57 (19.0%)	
	Combined	30 (10.0%)	22 (7.3%)	
	Unexplained	55 (18.3%)	59 (19.7%)	
Type of infertility (n, %)	Primary	224 (74.7%)	238 (79.3%)	#0.174
	Secondary	76 (25.3%)	62 (20.7%)	

^Independent t-test, #Chi square test

No statistical significant difference between study and control research groups regarding demographic characteristics (age, BMI, duration of infertility, cause and type of infertility) (P=0.142, 0.456, 0.128, 0.607 and 0.174 respectively) as shown in table (1).

Table (2): Basal hormonal profile among the studied groups

Variables	Measures	Study (N=300)	Control (N=300)	P
FSH (mIU/mL)	**Mean ± SD**	7.2 ± 0.9	7.3 ± 0.9	^0.358
	Range	4.0–9.2	4.7–9.4	
LH (mIU/mL)	**Mean ± SD**	5.7 ± 1.1	5.8 ± 1.1	^0.397
	Range	2.2–8.5	3.0–8.3	
Prolactin (ng/mL)	**Mean ± SD**	12.8 ± 2.3	12.6 ± 2.4	^0.266
	Range	7.3–19.0	6.8–19.4	
E2 (pg/mL)	**Primary**	56.9 ± 5.3	56.4 ± 5.6	^0.208
	Secondary	42.0–79.0	40.7–74.0	
TSH (µIU/mL)	**Mean ± SD**	2.11 ± 0.24	2.11 ± 0.24	^0.839
	Range	1.50–2.80	1.50–3.00	

^Independent t-test

Table (2) showed that there was no statistical significant difference between study and control research groups regarding basal hormonal profile (FSH, LH, prolactin, E_2 and TSH**)** (P=0.358, 0.397, 0.266, 0.208 and 0.839 respectively).

Table (3): Retrieved oocyte number and type among the studied groups

Variables	Measures	Study (N=300)	Control (N=300)	P
Retrieved oocytes	**Mean ± SD**	10.9 ± 1.4	10.9 ± 1.6	^0.978
	Range	7.0 – 14.0	6.0 – 15.0	
MII	**Mean ± SD**	9.0 ± 1.4	9.0 ± 1.5	^0.865
	Range	6.0 – 12.0	5.0 – 13.0	

^Independent t-test

No statistical significant difference between study and control research groups as regards number and type of oocytes retrieved (P =0.978 and 0.865 respectively) as shown in table (3).

Table (4): Chemical and clinical pregnancy rates among the studied groups

Pregnancy	Measures	Study (N=300)	Control (N=300)	P
Chemical (n, %)	**Positive**	137 (45.7%)	88 (29.3%)	**#<0.001***
	Negative	163 (54.3%)	212 (70.7%)	
Clinical (n, %)	**Positive**	129 (43.0%)	83 (27.7%)	**#<0.001***
	Negative	171 (57.0%)	217 (72.3%)	

#Chi square test, *Significant

Table (4) showed that chemical and clinical pregnancy rates were significantly more frequent among study research group (p value<0.001).

Table (5): Number of embryos transferred & Implantation Rate among the studied groups

Measures		Study (N=300)	Control (N=300)	P
Embro transfer (n)	**Mean ± SD**	2.3 ± 0.5	2.2 ± 0.5	^0.122
	Range	2.0-4.0	2.0-4.0	
Total sacs		157	89	
Total transferred embryos		689	671	
Implantation Rate		22.8%	13.3%	**#<0.001***

#Chi square test, *Significant, ^Independent t-test

Table (5) showed no significant difference between the number of embryos transferred to each case in study and control groups. Moreover table (5) showed that implantation rate was significantly higher among study group.

Table (6): Pregnancy number and multiple pregnancy rate among the studied groups

Pregnancy	Measures	Study (N=108)	Control (N=72)	P
Multiple	**Single**	71 (65.7%)	58 (80.6%)	**#0.031***

(n, %)	**Multiple**	37 (34.3%)	14 (19.4%)	
Number (n, %)	**Single**	71 (65.7%)	58 (80.6%)	&0.060
	Twin	28 (25.9%)	12 (16.7%)	
	Triplet	6 (5.6%)	1 (1.4%)	
	Quadriple	3 (2.8%)	1 (1.4%)	

#Chi square test, &Fisher's Exact test

Table (6) showed that multiple pregnancy was significantly higher among study research group (P=0.031). In addition, table (6) showed the distribution of the number of pregnancy sacs among the multiple pregnancy cases in both study and control groups.

Table (7): Pregnancy outcome among the studied groups

Outcome	Study (N=108)	Control (N=72)	P
Miscarriage	8 (7.4%)	12 (16.7%)	#0.053
Completed	100 (92.6%)	60 (83.3%)	

#Chi square test

Table (7) show that: 1st trimester miscarriage was non significantly less frequent among study research group **(P=0.053)**

Table (8): Hyperstimulation among the studied groups

Time	Study (N=300)	Control (N=300)	#P
Early	64 (21.3%)	72 (24.0%)	0.435
Late	6 (2.0%)	7 (2.3%)	0.779

#Chi square test, *Significant

Table (8) showed no significant difference between study and control research groups as regards early and late ovarian hyperstimulation **(P= 0.435 and 0.779)**

DISCUSSION

The following results were obtained in which there was no statistical significant difference between study and control research groups as regarding **demographic characteristics** (age, BMI, duration, cause and type of infertility. Additionally, there was no statistical significant difference between study and control research groups as regards **basal hormonal profile** (FSH, LH, Prolactin, E_2 and TSH). Also, no significant difference between study and control research groups as regards **number and type of oocytes retrieved** (total number of oocytes and MII nuclei oocytes). **Chemical and clinical pregnancy rates** were significantly more frequent among study research group (P value < 0.001). **Implantation rate** was significantly higher among study research group (P value < 0.001).

Multiple pregnancy rate was significantly higher among study research group (P= 0.031). 1st trimesteric miscarriage was non-significantly less frequent within research study group (P=0.053). Finally, no significant difference between study and control research groups regarding early and late ovarian **hyper-stimulation** (P=0.435 and 0.779 respectively)

In a similar research study investigating the influence of intrauterine hCG before embryo transfer (ET) on the clinical pregnancy and live birth rates, researchers reached to the same results as in the current trial. Where a total of 483 patients, all were having their first ICSI trial, were randomized into 2 groups, study group (n= 240) and control group (n= 243). The study group received 500 IU intrauterine hCG prior to ET and the control group received placebo. There was a statistically significant increase in implantation rate in the study group (23.6% versus 12.2% P-value <0.001) over the control group. Also, the pregnancy rate (54.6% versus 35.8%, P-value<0.001), clinical pregnancy rate (50% versus 32.1%, P-value<0.001), ongoing pregnancy rate (15.3% versus 9.2%, P-value<0.001) and live birth rate (14.3% versus 8.4%, P-value <0.001). They were all statistically significantly higher in the study group over the control group. They also had an increase in the multiple pregnancy rate in the study group (P-value< 0.05) with the occurrence of triplet pregnancy only in the study group. There was no difference in the rate of abortion between both groups [(8)].

Contradictory to our results, another research study had the aim to explore the factors that influence the outcome of intrauterine hCG infusion at the time of embryo transfer (ET), in particular, the effect of hCG infusions on fresh and frozen embryo transfers and whether prior recurrent implantation failure (RIF) impacts upon outcomes. The research was a case-control study based on a standardized database from a multi-site in vitro fertilization clinic. The

analysis contained 458 cases and 749 matched controls, with an intervention group of those given intrauterine hCG prior to ET and a control group of patients receiving no hCG infusion. Research outcomes were defined as clinical pregnancy and live birth rates. Two analyses were performed, the first separated frozen ETs (cases n = 224, controls n = 325) and fresh ETs (cases n =234, controls n = 424), with outcomes calculated in each group. The second analysis divided patients into those with RIF (cases n = 149, controls n = 200) and those without (cases n = 309, controls n = 549). The research team obtained the following results in which fresh ETs demonstrated a 5.8% reduction (adjusted odds ratio (AOR) = 0.60, P= 0.041) in clinical pregnancy rates with the use of intrauterine hCG. In those without defined RIF, clinical pregnancy rates were reduced by 8.1% (AOR = 0.61, P= 0.023) and live birth rates by 7.2% (AOR = 0.56, P= 0.32) with intrauterine hCG use. There were no statistical significant differences in outcomes in frozen ETs and in the RIF cohort. Finally the research team concluded that intrauterine hCG at the time of ET not only seems to have no benefit, but rather a negative effect in fresh ETs and those without RIF [(18)].

Another research study previously conducted with the aim to investigate the effects of the intrauterine perfusion of hCG before a frozen-thawed ET in women with different implantation failure numbers. It was a retrospective analysis of patients undergoing frozen ET who received an intrauterine injection of hCG 1000 IU before embryo transfer. The groups included women with their first implantation failure (A group, n = 26), second implantation failure (B group, n = 122) and three or more failures (C group, n = 77). Corresponding control groups (no infusion) were also included. The pregnancy rates were compared among these groups. The research team concluded that conception rates decreased with the number of transplant failures. The intrauterine administration of hCG before frozen ET significantly improved the pregnancy rates, especially after one and three or more implantation failures [(21)]**.** These results are consistent with our results.

CONCLUSION

The current research study denoted a possible role of hCG in enhancing and improving endometrial receptivity and thus improving pregnancy rate and implantation rate.

The results also states that intra uterine injection of hCG did not increase risk of ovarian hyper-stimulation syndrome, neither the possibility of affecting 1st trimesteric miscarriage rate. But also, results rose up the issue of increasing the multiple pregnancy rate which is an essential concern in the practice of ART. This could be of possible benefit in the nowadays idea of single embryo transfer.

RECOMMENDATIONS

From the previous results and conclusions it is recommended to use hCG intrauterine injection before ET during ICSI procedure for achieving better pregnancy and implantation rates. This will be of special benefit in patients with low implantation rates such as cases of recurrent implantation failure and elder females and in cases when there is only a single embryo to be transferred.

However, wider multicentre research studies are required to elucidate molecular and cellular impact of hCG on endometrial receptivity and the most adequate dosages that could be injected intra uterine to improve endometrial receptivity function without any deleterious effect on the process of implantation or ongoing pregnancies.

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