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Does Intramuscular Injection of Human Chorionic Gonadotropin before Frozen Embryo Transfer Improve Intracytoplasmic Sperm Injection Outcomes?

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ABSTRACT

Background: Evidences reported the advantageous impact of human chorionic gonadotropin (hCG) in process of embryo implantation via activation of LH/hCG receptors expressed in endometrium.

Objective: The current context investigated whether intramuscular (IM) administration of hCG on the frozen embryo transfer (ET) day would improve the pregnancy outcomes of intracytoplasmic sperm injection (ICSI) cycles.

Methods: A total of 176 artificially prepared frozen-thawed embryo transfer (FET) cycles were randomly categorized into two equal groups: (A) hCG -treated group (in which intramuscular administration of 10,000 IU hCG on the morning of frozen embryo transfer day was done) and (B) control group (underwent routine FET protocol without hCG intramuscular administration. Our primary outcome was clinical pregnancy rate (CPR) that compared between the two groups, secondary outcomes were ongoing pregnancy and first trimester miscarriage rates.

Results: Basic and clinical patient characteristics were matched between groups. In terms of CPR, it was significantly superior in patients received IM hCG prior to frozen embryo transfer (FET) compared to control group (70.5% versus 52.3%, P value = 0.013). Regarding ongoing pregnancy rate (OPR), it was improved significantly in hCG group compared to control group (50% versus 28.4%, P value = 0.003). Miscarriage rates were similar among study groups. Univariate analysis revealed that age at the index ICSI cycle, serum progesterone on pregnancy test day (15 days after FET), number/quality of embryos transferred and hCG injection on the embryo transfer day were significant predictors of clinical pregnancy in frozen-thawed cycles. In the multivariate analysis, the intramuscular administration of hCG remained a significant predictor of clinical pregnancy after FET (adjusted odds ratio (AOR) 2.22; P = 0.014).

Conclusions: Intramuscular hCG administration on the frozen embryo transfer day significantly raise the rates of both clinical & ongoing pregnancy.

Keywords: Human chorionic gonadotropin, FET, Pregnancy rate.

INTRODUCTION

Successful embryo implantation requires a synchronized embryo-decidua cross-talking through biochemical messages between the blastocyst and the decidua [1]. HCG produced by blastocyst before implantation is one of such molecules [2]. Evidences documented a favorable impact of hCG on embryo implantation (acting via LH/hCG receptors, which are expressed and functionally active in human endometrium) [3, 4].

Human chorionic gonadotropin enhances micro-vascularization and angiogenesis needed in preparing a receptive endometrium through decrease in intrauterine macrophage colony stimulating factor, stimulation of vascular endothelial growth factor (VEGF) [5], and amplification of endometrial response to interleukin [6].

Human chorionic gonadotropin has important roles in prolonging the implantation window by suppression of insulin-like growth factor binding protein-1 (IGFBP-1) in the endometrium [7]. Additionally, hCG induces trophoblast proliferation and invasiveness [8], regulates the immunologic tolerance at maternal-foetal interface and suppress myometrial contractile activity [9].

Several controlled trials confirmed the clinical potency of intrauterine hCG administration before ET in the improvement of clinical outcomes of ART strategies [14–16].

Objective: The current study investigated whether the intramuscular administration of hCG would improve clinical & ongoing pregnancy outcomes in the artificially prepared FET cycles.

PATIENTS AND METHODS

A randomized controlled trial comprised 176 FET cycles that was conducted at a specialized fertility and gynecology center through the period from January 2021 to January 2023.

Inclusion criteria: Women aged 23-43 years (at the index stimulated ICSI cycle), underwent hormone replacement FET, endometrial thickness more than 8 mm at the day of progesterone initiation in frozen cycle and ≥ one top-quality embryo transferred were comprised in our study.

Exclusion criteria: Cases were ruled out when they were diagnosed with endometriosis, fresh ET cycles, FET cycles other than hormonal replacement therapy and refusal to engage in the study.

Randomization

One hundred seventy-six candidates fulfilled the inclusion criteria and randomly categorized into two equal groups. Group allocation was concealed by preparing one hundred seventy-six sealed opaque identical serially numbered envelopes (88 envelopes for hCG-treated group and the other 88 for control group) using computer generated randomization sheet

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by MedCalc © version 13. Each envelope contained a corresponding letter denoting the allocated group.

Index stimulated ICSI cycles, embryo quality assessment and cryopreservation:

In both groups, controlled ovarian stimulation was implemented according to long GnRH agonist, short agonist, antagonist or progestin primed protocols. Metaphase II oocytes were injected 3 to 4 hours after retrieval. Assessment of fertilization was done 16-18 hours following ICSI by the existence of 2 pronuclei and subsequent divisions were monitored daily till verification of embryos done either at day 3 (cleavage-stage) or at day 5 (blastocyst stage). Fresh transfer of embryos was not included in the current study. Cleavage-stage embryos were scored according to number/symmetry of blastomeres, fragmentation%, vacuolization and multinucleation (top-quality cleavage-stage embryo criteria were: having eight cells, less than 20% fragmentation and absence of multinucleation). Top-quality blastocyst was characterized by having blastocoel expanding whole blastocyst with a good inner cell mass and a trophectoderm [10].

Endometrial preparation, luteal support and embryo transfer:

Oral estradiol valerate (Progynova) was commenced on menstrual cycle day 1, in a fixed dose regimen (8 mg /day, 2 mg X 4 times daily). After 12 days of estradiol supplementation, endometrial thickness was measured by means of transvaginal sonography (TVS). Endometrial thickness more than 8 mm was regarded as an optimal thickness. If endometrial thickness was suboptimal (< 8 mm), incremental doses of estradiol valerate were applied (maximum of 12 mg/day) till endometrial thickness approached 8 mm. If the endometrial stripe thickness remained refractory following twenty days of E2 supplementation, cycle cancellation was done. When endometrium approached ideal thickness, E2 and progesterone values were measured (in cases when serum progesterone values were more than 1.5 ng/mL, cycle cancellation was done). On the following day, in both groups, luteal support was achieved with IM progesterone (100 mg/day) and progesterone vaginal pessaries (400 mg twice per day) together with E2 administration. Embryo transfer was conducted 3 or 4 days after progesterone initiation (cleavage stage) and on the sixth day of progesterone initiation (blastocyst stage). In the morning of ET day, participants allocated in the hCG treatment group administrated 10000IU hCG (Choriomon, IBSA) by IM injection. In the controls, embryo transfer was performed without hCG administration.

Hormonal assays:

Serum β-hCG and progesterone levels were analyzed 15 days following FET. If serum β-hCG was positive, a TVS examination was conducted 14 days later [to establish intrauterine location, detect the numbers of gestational sacs present and visualize foetal viability (if present)].

The primary outcome was CPR (one or more gestational sacs of intrauterine location visualized by ultrasound scan at 5–6 weeks gestation) [11].

Secondary outcomes were OPR and miscarriage rate. Ongoing pregnancy is a clinical pregnancy continuing beyond 12 weeks of gestational age. First trimester abortion or pregnancy loss rate was measured (the number of pregnancies lost prior twelve weeks of gestation divided by the number of women with a positive pregnancy test.

Sample size calculation:

On the basis of review of past literature **Deng *et al.*** [12] revealed a significant improvement in terms of CPR in hCG-treated group compared to controls (66.7% versus 44.6%, P value = 0.004). The least sample size was 158 subjects increased to 176 to avoid 10% dropout and divided into 2 equivalent groups. The power of study was 80% and CI was 95%.

Ethical approval: Local Ethics Committee of Menoufia University approved the study design and written informed consents were obtained from entire subjects following explanation of the purposes, procedures, and nature of the present study. The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis

Data were gathered, processed and statistically analysed by utilizing an IBM special software with SPSS version 22 (Inc., Chicago, USA). Quantitative data were expressed in the form of mean ± SD and range. Qualitative data were expressed in the form of numbers and percentages. Shapiro- Wilk test was used to assess data distribution. Chi-square test (χ^2) was utilized to assess the correlation between two qualitative variables. Mann Whitney test was utilized to compare between 2 groups having quantitative variables not normally distributed. Logistic regression for detection of independent predictors of outcome. P value of ≤ 0.05 was considered statistically significant.

RESULTS

One hundred ninety candidates were assessed for eligibility to participate in the current study. Fourteen patients were excluded (of these, 10 did not meet the inclusion criteria and 4 declined to participate). So, one hundred seventy-six participants were available for

random allocation into two equal groups (88 in hCG treated group and 88 in control group) .All participants completed the trial and ready for analysis, as shown in CONSORT flow chart **(figure 1)**.

Basic and clinical patient characteristics of the index stimulated ICSI cycle were matched between the two groups. No significant differences were noticed between groups as regards age, BMI, cause/duration of subfertility and number of MII oocytes (Table 1).

Table (1): Basic and clinical characteristics of the index stimulated ICSI cycles (N=176)

Studied variables	hCG group (N=88)	Control group (N=88)	Test of sig	P value
Age / years				
Mean ±SD	28.9±5.69	28.9±5.53	U	0.995
Median(IQR)	29.0(23.0 – 33.0)	29.0(24.0 – 33.0)	0.006	
BMI				
Mean ±SD	25.1±3.18	25.0±2.61	U	0.700
Median(IQR)	26.0(22.0 – 28.0)	25.0(23.0 – 27.0)	0.385	
Duration of subfertility (years)				
Mean ±SD	4.31±1.47	4.50±1.57	U	
Median(IQR)	4.00(3.00 – 5.00)	4.00(3.00 – 5.00)	0.740	0.459
Cause of subfertility	**N (%)**	**N (%)**	χ^2	
Anovulation	16(18.2)	7(8.00)	7.39	0.286
Male factor	12(13.6)	15(17.0)		
Tubal factor	8(9.10)	13(14.8)		
Endometriosis	5(5.70)	5(5.70)		
Mixed	8(9.10)	8(9.10)		
Unexplained	33(37.5)	38(43.2)		
Others	6(6.80)	2(2.30)		
Number of oocytes retrieved				
Mean ±SD	21.7±12.8	20.6±11.4	U	0.679
Median (IQR)	20.0(11.2 – 30.7)	19.0(11.7 – 29.0)	0.414	
Number of MII oocytes				
Mean ±SD	14.1±9.39	12.8±8.61	U	0.400
Median (IQR)	12.5(6.00 - 20.0)	11.0(6.00 – 17.5)	0.841	

N: number %: percentage SD: Standard deviation IQR: Inter quartile range U: Mann Whitney test χ^2: Chi square test.

Regarding the clinical characteristics of frozen-thawed cycles in both groups, it were similar in endometrial thickness, duration of estradiol administration, number of transferred embryos and top-quality embryos transferred (Table 2).

Table (2): Frozen embryo transfer cycle characteristics (N=176)

Studied variables	hCG group (N=88)	Control group (N=88)	Test of sig.	P value
Endometrial thickness (mm)			U	
Mean ±SD	9.73±0.98	9.84±1.30	0.557	0.578
Median (IQR)	10.0(9.00 – 10.0)	10.0(9.00 – 11.0)		
Duration of estradiol administration (days)			U 0.265	0.791
Mean ±SD	14.2±1.27	14.3±1.68		
Median (IQR)	14.0(13.0 - 15.0)	14.0(13.0 – 15.0)		
Number of embryos transferred				
Mean ±SD	2.31±0.46	2.29±0.45	U	
Median (IQR)	2.00(2.00 - 3.00)	2.00(2.00 – 3.00)	0.326	0.744
Quality of embryos transferred	**N (%)**	**N (%)**	χ^2	
Grade A	81(92.0)	80(90.9)		
Grade B	7(8.00)	8(9.10)	0.073	0.787

N: number %: percentage SD: Standard deviation IQR: Inter quartile range U: Mann Whitney test χ^2: Chi square test.

Regarding CPR, it was significantly superior in patients received IM hCG prior to FET compared to control group (70.5% versus 52.3%, P value = 0.013). Regarding OPR, it was improved significantly in hCG group compared to controls (50% versus 28.4%, P value = 0.003). Miscarriage rates were similar in both groups (Table 3).

Table (3): ART outcomes of frozen embryo transfer among the studied groups (N=176)

Studied variables	hCG group (N=88)	Control group (N=88)	Test of sig.	P value
			$\chi 2$	
Clinical pregnancy	62(70.5%)	46(52.3%)	6.13	**0.013***
First trimester miscarriage	18	21	3.16	0.075
Ongoing pregnancy	44(50%)	25(28.4%)	8.60	**0.003***

*Significant χ^2: Chi square test

There were significantly higher levels of serum progesterone (estimated 15 days after FET) in hCG-treated group compared to control group (**Table 4**).

Table (4): Serum progesterone levels on pregnancy test day (ng/ml) among the studied groups (N=176)

Studied variables	hCG group (N=88)	Control group (N=88)	Test of sig.	P value
Serum progesterone on pregnancy test day (ng/ml) Median (IQR)	75.0(18.0 – 100)	19.0(13.2– 33.7)	U 6.70	**0.001****

Median and IQR: Non-parametric test. SD: Standard deviation IQR: Inter quartile range ** highly significant U: Mann Whitney test.

The univariate analysis revealed that age at index ICSI cycle (OR 0.072, 95% CI 1.01 – 1.13; P = 0.012), serum progesterone on pregnancy test day (OR 0.275, 95% CI 0.140 – 0.538; P = 0.001), number of transferred embryos (OR 0.904, 95% CI 1.28 - 4.75; P=0.007), top quality of embryos transferred (OR 1.61, 95% CI 1.52 – 16.4; P=0.008) and hCG injection on the embryo transfer day (OR 2.17, 95% CI 1.17 - 4.04; P = 0.014) were significant predictors of clinical pregnancy in FET cycles. In the multivariate analysis, top quality ET (AOR 1.64, 95% CI 1.54 – 5.17; P = 0.008), serum progesterone levels on pregnancy test day (AOR 0.306, 95% CI 0.167 – 0.561; P = 0.001) and hCG injection on the embryo transfer day (AOR 2.22, 95%CI 1.17-4.20; P=0.014) remained the significant predictors of the successful outcome (**Table 5**).

Table (5): Logistic regression analysis for predictors of clinical pregnancy among the studied groups

Studied variables	OR	P value	95%CI
			Lower – Upper
Univariate regression analysis			
Age (years)	0.072	**0.012***	1.01 – 1.13
BMI	0.081	0.137	0.975 – 1.21
Duration of subfertility (years)	0.009	0.934	0.812 – 1.21
Number of oocytes retrieved	0.014	0.291	0.961 – 1.01
Number of MII oocyte	0.012	0.485	0.954 – 1.02
Endometrial thickness (mm)	0.049	0.716	0.731 -1.24
Duration of estradiol administration (days)	0.026	0.804	0.837 -1.25
Number of embryos transferred	0.904	**0.007***	1.28 - 4.75
Quality of embryos transferred (Grade A)	1.61	**0.008***	1.52 – 16.4
Serum progesterone on pregnancy test day (ng/ml)	0.275	**0.001***	0.140 – 0.538
HCG injection on FET day	2.17	**0.014***	1.17 - 4.04
Multivariate regression analysis			
Age (years)	0.001	0.997	0.653 – 1.53
Number of embryos transferred	0.087	0.478	0.041 – 0.768
Quality of embryos transferred (Grade A)	1.64	**0.008***	1.54 – 5.17
Serum progesterone on pregnancy test day (ng/ml)	0.306	**0.001****	0.167 – 0.561
HCG injection on FET day	2.22	**0.014***	1.17 - 4.20

OR: Odds ratio ** highly significant

DISCUSSION

In the current study, we explored whether intramuscular administration of human chorionic gonadotropin (hCG) on the FET day would improve the interaction between the embryo and the endometrium resulting in better implantation and so pregnancy rates.

Previous studies had investigated the potency of hCG intrauterine wash prior to ET with contradictory findings [13–18]. A cochrane systematic review documented a significant improvement in both clinical pregnancy and live birth rates with intra-cervical administration of 500 IU hCG before cleavage-stage ET [13]. Furthermore, a recent review of literature confirmed the beneficial impact of intrauterine hCG infusion before ET [19].

However, there are various concerns about hCG intrauterine wash that could influence the successful pregnancy outcomes. First, endometrial trauma caused by intrauterine infusion approach is not neglected. Second, it is of great importance to remember that the extra infusion fluid can dislodge embryos from its main location and impede the adherence of the embryo to the decidua [20].

In our setting, intramuscular route of hCG administration was simple/easy technique, devoid of operational diversity (for nurses) or added stress (for patients) and could be achieved freely without relying on the ET timing.

This study confirmed that better CPR/OPR were identified in FET cycles received 10,000 IU IM hCG compared to cycles lacking hCG administration. We also reported that significant predictors of successful outcome were: age at the index ICSI cycle, serum progesterone estimated on pregnancy test day, number of transferred embryos, top-quality of embryos transferred and use of hCG injection. After adjusting the confounding variables by multivariate analysis, the intramuscular administration of hCG on the embryo transfer day remained a significant predictor of successful outcome (AOR 2.22, P = 0.014). In agreement with our observations, a study comprised 200 FET cycles in which participants administrated hCG (5000 IU hCG IM on the day of FET then every 72 hours till 3 doses) and demonstrated a significant improvement in clinical pregnancy in hCG-treated group [21]. Also, **Deng and his colleagues** [12] observed a superiority in all pregnancy rates (live birth rate, CPR and OPR) when 10000 IU hCG was administrated intramuscular prior to secretory transformation. Furthermore, such superiority in pregnancy rates after hCG injection remained highly significant following the adjustment of the confounding variables by multivariate analysis.

In contrast to our findings, a randomized trial by **Shiotani and his colleagues** [22] reported that hCG administration did not augment the pregnancy rates in frozen-thawed cycles. Furthermore, **Ben-Meir and his colleagues** [23] administered 250 IU recombinant hCG 3 times (at progesterone initiation, the day of embryo transfer and six days thereafter) and observed no improvements in implantation or pregnancy rates in hCG treatment group. Also, **Lee and his colleagues** [24] in a double-blinded randomized trial comprised 450 frozen embryo transfer (FET) cycles revealed that luteal support by administrating 1500 IU hCG (on the day of FET and 6 days later) failed to improve the OPR in frozen thawed cycles and the only significant predictor of successful cycle outcome was the number of transferred embryos.

LIMITATIONS

Limitations were due to presence of confounding variables correlated also with positive pregnancy outcomes in frozen-thawed cycles but after adjusting the potential confounding variables, multivariate analysis showed that IM hCG administration on FET day remained a significant predictor of clinical pregnancy outcome.

In order to reinforce our observations regarding superior pregnancy rates associated with hCG administration, conduction of larger RCTs involving many institutions should be recommended before applying IM. hCG into clinical practice.

CONCLUSIONS

Intramuscular hCG administration on the frozen embryo transfer day significantly raise the rates of both clinical & ongoing pregnancy.

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