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Original Research Article

The effect of intrauterine human chorionic gonadotrophin injection before embryo transfer on the implantation and pregnancy rate in infertile patients: a retrospective study

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ABSTRACT

Background: It is known that human chorionic gonadotrophin (hCG) has an important function in angiogenesis and reduces the inflammatory response which in turn favour the implantation process. Human chorionic gonadotrophin is secreted early during the pregnancy, hence plays an important role. Objective of the study is to investigate the effect of intrauterine hCG injection before embryo transfer (ET) on pregnancy outcome in infertile couples.
Methods: 100 patients who have undergone embryo transfer from 01 October 2021, to 31 January 2022, in a study setting of ARC Fertility Centre, Saveetha Medical College and Hospital. After ensuring that the patients satisfy the inclusion and exclusion criteria, patients were divided into two groups. Group 1 received intrauterine hCG 5000 IU 3 to 4 hours before embryo transfer. Group 2 control group that did not receive intrauterine hCG. Every patient in either group had been called back on day 15 of embryo transfer to check serum beta hCG. If beta hCG shows a positive result, then primary outcome was positive. Every patient who is pregnancy positive had been called back after 15 days of positive result to see evidence of fetal heartbeat in early obstetrics scan. If fetal heartbeat is present, then secondary outcome was documented.
Results: There was significant paired difference in median values of endometrial thickness between day 2 and day on FET and it shows a positive effect on pregnancy results among the hCG group.
Conclusions: This study enabled us to determine the effect of intrauterine HCG in improving the success rate of *in vitro* fertilization (IVF).

Keywords: Assisted reproductive techniques, Endometrial receptivity, Human chorionic gonadotrophin, Embryo transfer, Frozen embryo transfer

INTRODUCTION

One in six to seven couples is laid low with infertility in world. Assisted reproductive techniques (ART) are currently in control of up to 7% of childbirths in developed countries.[1] In recent years, despite the key advances in numerous aspects of clinical and laboratory ART, pregnancy rate remains low.[2-4] Implantation is one in every of the crucial stages for ART achievement.[5,6] There are three major aspects to work out how successful ART will be including embryo quality, endometrial receptivity (ER) and embryo endometrium synchronization.[7] There are several factors that coordinate the complex process of implantation. The essential thing is human chorionic gonadotropin (hCG).[8,9] It mediates the complex molecular interactions between prepared uterus and a mature blastocyst. Unsuccessful implantation could be a major restrictive cause in assisted reproduction. It is anticipated that approximately 50-75% of pregnancy losses are due to implantation failure.[10] There seem to be three

components that are essential for establishing a receptive endometrium and initiating an embryo maternal crosstalk. These components include ovarian steroid hormones, local autocrine and paracrine signalling within the endometrium, and embryo-derived signals.

The most considerable human embryonic signal that has been known since long and the one among the earliest molecules that is secreted by the embryo before its implantation is hCG.[11] The embryo's mRNA is, indeed, activated when it is at the 6-8 cell stage, and therefore the blastocyst produces protein before its implantation.[12] hCG production by the syncytiotrophoblast increases after implantation. It is implicated in several actions that promote tolerance and angiogenesis at the maternal-fetal interface and has important physiological implications for successful pregnancy. To analyze the direct effects of hCG on the human endometrium for the primary time, an intrauterine micro dialysis instrument was created by Licht et al. The instrument was also used for measuring paracrine mediators within the cavum. The researchers showed that intrauterine administration of 500 IU of hCG/ml can cause a major inhibition of intrauterine insulin-like growth factor-binding protein 1 (IGFBP-1) and macrophage colony stimulating factor (M-CSF). Additionally, leukemia inhibitory factor (LIF), which could be a cytokine needed for implantation, vascular endothelial protein (VEGF), which could be a proangiogenic protein, and metalloproteinase-9 (MMP-9), which could be a regulator of tissue remodelling, were incited as significant factors.[13]

METHODS

It was a retrospective type of study. The study included 100 patients who have undergone embryo transfer from 01 October 2021, to 31 January 2022. The study was conducted at the ARC Fertility Centre, Saveetha Medical College, and Hospital.

Methodology

After obtaining ethical committee approval for the study, patient data, including maternal age, height, body mass index (BMI), weight, period of infertility, reasons for infertility, preconditions and previous IVF attempts and pregnancies, had been collected from the patient's database file.

After the ensuring that the patients satisfy the inclusion exclusion criteria, the patients were divided into two groups. Group 1 who received intrauterine hCG 5000 IU 3 to 4 hours before embryo transfer. Group 2 is the control group that did not receive intrauterine hCG.

The following variables are noted during real cycle: day 2 baseline endometrial thickness, day 2 baseline estradiol (E2) level, day 2 progesterone (P) level, day 2 TSH, day 2 prolactin, day 2 HbA1C, endometrial thickness on day of transfer, endometrium and adnexa in ultrasound on day 2 thawing scan thickness, endowaves, vascularity, number of day 5 blastocyst transferred, quality of embryo transferred, and blood-stained catheter.

Every patient in either group had been called back on day 15 of embryo transfer to check serum beta hCG. If beta hCG shows a positive result, then primary outcome was positive. Every patient who is pregnancy positive had been called back after 15 days of positive result to see evidence of foetal heartbeat in early obstetrics scan. If foetal heartbeat is present, then secondary outcome was documented.

Analysis of results

The study groups were statistically analyzed for differences in the following outcome variables.

Primary outcome

The primary outcome of the study was positive pregnancy test (serum beta hCG).

Secondary outcome

Clinical pregnancy rate i.e., evidence of a heartbeat in follow-up ultrasound, a p value of <0.05 was considered statistically significant.

Inclusion criteria

The study included patients with age 20–40 years, no previous/one failure, no medical conditions, no endometrial (thin/thick/polypoidal endometrium) issues, no myometrial issues (myoma uterus/adenomyosis), no ovarian issues (endometriosis/endometrioma), no hydrosalphinx if hydrosalphinx is present, then include only after bilateral tubal disconnection, and good quality embryo.

Exclusion criteria

The study included patients with persistent inflammation of the reproductive tract including severe endometriosis, endometritis; uterine issues (structural anomaly, adenomyosis, myoma uterus); donors; multiple IVF failures; age >40 years; significant medical issues like DM, hypertension, autoimmune thyroiditis, severe hypo/hyperthyroidism, APLA, and SLE; thin endometrium <7 mm on the day of ET; thick endometrium >10 mm on the day of ET; and severe hydrosalphinx without disconnection.

Sample size, sampling technique and statistical analyses

Sample size of 100 patients (50 embryo transfers with intrauterine hCG administration 4 hours prior and 50 embryo transfer without intrauterine hCG) who have undergone IVF treatment from October 2021 – January

2022 at ARC Fertility Centre, Saveetha Medical College, and Hospital.

RESULTS

Total 100 research participants were included in this study, 50 in hCG group and 50 in control group who did not receive hCG. The mean age of the study group was 34.4 years (SD=5.2). The median age of both the study group and control group were almost same, 35 was the median age of study group who received intrauterine hCG (IQR=31 to 38) and 35.5 years for the group that did not receive intrauterine hCG (IQR=31 to 40). Also, there was no statistically significant difference between median years in age of both study groups (p=0.23). The 95% confidence interval for the mean and the mean values for each group are provided in Table 1.

Day 2 endometrial thickness

The mean day 2 endometrial thickness day 2 value of hCG group was 28 with standard deviation of 12.63 and for the group which did not get hCG the mean was 26.46 with standard deviation of 9.39. Also, there was no significant difference in median between two study groups with p value greater than 0.05 (p=0.733). The detailed statistics are mentioned in Table 2.

Day 2 progesterone

The mean of day 2 P4 (progesterone) of hCG group was 0.89 with standard deviation of 2.69 and for the group which did not get hCG the mean was 0.48 with standard deviation of 0.22. Also, there was no significant difference in median between two study groups with p value greater than 0.05 (p=0.4023). The detailed statistics are mentioned in Table 3.

Day 2 PRL (prolactin)

The mean of day 2 PRL of hCG group was 16.74 with standard deviation of 5.85 and for the group which did not get hCG the mean was 16.7 with standard deviation of 6.14. Also, there was no significant difference in median between two study groups with p value greater than 0.05 (p=0.9124). The detailed statistics are mentioned in Table 4.

Wilcoxon signed rank test

There was significant paired difference in median values of endothickness between day 2 and day on FET (Table 5). And it was statistically significant with p<0.0001. Also, the difference in median was significant in both the study groups with p<0.0001 (Table 6).

Chi square test of association

There was no significant association between pregnancy test result and the study group with p value greater than 0.05 (p=0.059, OR=0.5524). Among the hCG group out of 50 subjects 34 (68%) were test positive in pregnancy test and only 16 (32%) were having negative test result. In the study group of did not receive hCG, 23 (46%) were resulted in pregnancy test negative and only 27 (54%) were ended in test positive.

Table 1: Age (in years) among study and control group.

Parameters	Mean±standard deviation	95% confidence interval for mean	Median (IQR)	P value
Received intrauterine hCG	33.82±5.16	32.35-35.29	35 (31 to 38)	0.23
Did not receive intrauterine hCG	34.92±5.25	33.43-36.41	35.5 (31 to 40)	

*IQR- Inter quartile region, *Mann Whitney U test was done due to normality test was not satisfied with p<0.05 for both the groups; *p<0.05 is considered as statistically significant

Table 2: Day 2 endometrial thickness day 2 among study and control group.

Parameters	Mean±standard deviation	95% confidence interval for mean	Median (IQR)	P value
Received intrauterine hCG	28±12.63	24.41-31.59	30 (16 to 38)	0.733
Did not receive intrauterine hCG	26.46±9.39	23.79-29.12	22.9 (20 to 34)	

*IQR- Inter quartile region, *Mann Whitney U test was done due to normality test was not satisfied with p<0.05 for both the groups; *p<0.05 is considered as statistically significant

Table 3: Day 2 P4 (progesterone) among study and control group.

Parameters	Mean±standard deviation	95% confidence interval for mean	Median (IQR)	P value
Received intrauterine hCG	0.89±2.69	0.13-1.66	0.5 (0.33 to 0.70)	0.4023
Did not receive intrauterine hCG	0.48±0.22	0.42-0.55	0.5 (0.25 to 0.70)	

*IQR- Inter quartile region, *Mann Whitney U test was done due to normality test was not satisfied with p<0.05 for both the groups; *p<0.05 is considered as statistically significant

Table 4: Day 2 PRL (prolactin) among study and control group.

Parameters	Mean±standard deviation	95% confidence interval for mean	Median (IQR)	P value
Received intrauterine hCG	16.74±5.85	15.07-18.39	14.95-18.44	0.9124
Did not receive intrauterine hCG	16.7±6.14	16.9 (12.8 to 20.7)	16.3 (11.3 to 22.5)	

*IQR- Inter quartile region, *Mann Whitney U test was done due to normality test was not satisfied with p<0.05 for both the groups; *p<0.05 is considered as statistically significant

Table 5: Endometrial thickness on frozen embryo transfer day – day 2 endometrial thickness.

Mean difference±SD	95% CL mean	Median (IQR)	P value
4.18±0.96	3.98-4.37	4.2 (3.6 to 4.8)	<0.0001

*Paired t test is done to test whether there is significant difference in mean for the paired endothickness values of the study subjects, *p value<0.05 considered as statistically significant

Table 6: Endometrial thickness on frozen embryo transfer day – day 2 endometrial thickness.

Parameters	Mean difference±SD	95% CL mean	Median (IQR)	P value
Received intrauterine hCG	4.29±1.01	4.01-4.58	4.3 (3.9 to 5)	<0.0001
Did not receive intrauterine hCG	4.05±0.91	3.79-4.31	4.1 (3.6 to 4.7)	<0.0001

*Paired t test is done to test whether there is significant difference in mean for the paired endothickness values of the study subjects, *p value<0.05 considered as statistically significant

Table 7: Chi square association: pregnancy test.

Pregnancy test	Received intrauterine hCG (N)	Did not receive intrauterine hCG (N)	Total	Chi-square value	P value	Odds ratio	Relative risk (95% confidence interval)
Negative	16	23	39	2.06	0.059	0.5524	1.33 (0.91-1.95)
Positive	34	27	61				

*Chi square test is done to test whether there is significant association of the study group with the pregnancy test result; *p<0.05 is taken as statistically significant

Also, relative risk for the control group who did not get hCG to become pregnancy positive or negative was 1.33 (95% CI=0.91 to 1.95) when related to the hCG group. These results are representation in Table 7.

DISCUSSION

As female age advances the follicular pool within the ovaries decreases because of ovulation and physiological atresia. During this process oocytes with congenitally defective chromosomes do not seem to be recruited until the healthy oocytes are utilized. Hence, the defective oocytes start dominating the pool within the later reproductive years. Alternatively, as a component of the overall physiological aging of the body, chromosomal defects will be acquired secondary to prolonged exposure to the risks of accidental damage. Regardless of the mechanism, the available oocytes are poorer in quality with advancing age.[14]

No statistically significant difference between median of age of both study groups was observed in our study.

An analysis by the French National Register on IVF (FIVNAT) of all oocyte collections between 1989 and 1995 also reveals that women with no previous IVF pregnancy had lower success in response to ovarian stimulation, fertilization, and pregnancy rates, when compared with women with a previous failed IVF pregnancy (spontaneous abortion or ectopic pregnancy) or ongoing IVF pregnancy. Other investigators report similar findings.[15] The clinical pregnancy rate per oocyte collection decreased from 20.2% within the first try to 17.4% within the second, and to <13% after the sixth. This trend persisted regardless of the female age or subfertility diagnosis.[16]

There was no significant association between pregnancy test result and history of previous IVF failure in our study.

Endometrial thickness as a predictor of IVF-ET outcome has been investigated by numerous studies with variable results. While some study groups found a significant correlation between thickness of the endometrium and pregnancy rate.[17] Others reported no such relationship is reported that there is an extensive overlap in the ranges of endometrial thickness present in pregnant and non-pregnant cycles.[18,19] A pooled comparison of the published data on 2665 cycles reports that ranges of mean endometrial thickness for conception and non-conception cycles are almost the same (8.6 to 11.8 and 8.6 to 11.9 respectively).[20] These opposing conclusions may in part be

due to the different techniques used, such as vaginal versus abdominal ultrasonography, different ovarian stimulation protocols, measurement errors in obtaining a standard sagittal view of the uterus or marked ovarian enlargement distorting the endometrial outline. Similarly, no correlation was found between implantation and therefore the mean cross-sectional area of the endometrium. Endometrial thickness, as a proxy measure of endometrial growth, is reported to be unrelated to endometrial pattern on the day of hCG injection. The published results on natural cycle FTER report no differences in the endometrial thickness between the pregnant and non-pregnant groups.[21]

In hormone replacement cycles for FER and oocyte donation, results are more variable. While some published non-significant differences in endometrial thickness between the pregnant and non-pregnant groups, Abdalla et. al (10.2±2.63 versus 8.6±3.49 mm), Alam et al and Shapiro et al (10.5±3.5 versus 9.6±4.2 mm) report significant differences.[17,22-24] Raga et al also found that a minimum volume of 2 ml is a prerequisite for a receptive endometrium and that no pregnancy was achieved when endometrial volume measured <1.2 ml.[25]

Schild et al found that mean values for endometrial volume assessed by three-dimensional ultrasonography, are non-significantly higher in conception versus non-conception cycles.[26] No conception was observed below an endometrial thickness of 6.9 mm and volume of 1.59 ml on the day of oocyte collection.

Beyond an endometrial volume of 2 ml, no relationship was apparent in terms of endometrial receptivity. Various values of endometrial thickness between 6 and 10 mm have been proposed as discriminatory between conception and non-conception cycles, albeit with a low specificity and low positive predictive value. However, recent evidence indicates that embryonic implantation is possible even when endometrial thickness is <4 mm. The main advantage of ultrasonographic assessment of the endometrial thickness is given as its high negative predictive value in cases where minimal endometrial thickness was not reached.

In our study, the mean of day 2 endometrial thickness of hCG group was 4.04 with standard deviation of 0.68 and for the group which did not get hCG the mean was 0.48 with standard deviation of 0.22. Also, there was significant difference in median between two study groups. There was significant paired difference in median values of endo thickness between day 2 and day on FET (as mentioned in Table 8). And it was statistically significant.

Frozen-thawed (FT) embryo transfer could be a procedure used for the storage and transfer of excess embryos obtained during in vitro fertilization (IVF)–intracytoplasmic sperm injection (ICSI) cycles. In recent years, improvements in laboratory conditions and limitations on the quantity of embryos to be transferred have led to a progressive increase in FT embryo transfer cycles. Another preferred practice to stop multiple pregnancies in IVF cycles is to transfer one embryo and freeze all surplus embryos. However, the simplest solution for endometrial preparation in these cycles continues to be a matter of debate.[27] The mean of thawing scan endometrial thickness of hCG group was 8.18 with standard deviation of 0.92 and for the group which did not get hCG the mean was 8.03 with standard deviation of 0.68. Also, there was no significant difference in median between two study groups in our study.

Though it shows a positive effect on pregnancy results among the hCG group, there was no significant association between clinical result and the study group with p value greater than 0.05 (p=0.307, OR=0.6047). Among the hCG group out of 50 subjects 33 (66%) were resulted in pregnancy test positive and only 17 (34%) were having negative test result. In the study group of which who did not received hCG 23 (46%) were resulted in pregnancy test negative and only 27 (54%) were ended in test positive.

CONCLUSION

Implantation is one of the essential steps for the success of ART. Their success depends on three main factors: embryo quality, endometrial receptivity (ER), and synchrony between embryo and endometrium. There are various factors that regulate the complex process of implantation. In this regard, one may refer to hCG as the most important factor. As a conclusion, it may be claimed that intrauterine injection of hCG before ET is of relative capability to improve the implantation and pregnancy rates in ET cycles. However, the findings of the study are in contradiction with some other corresponding pieces of research in the literature. Therefore, more studies need to be conducted in this regard.

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