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To Which Patients Should Intrauterine HCG Injection Be Applied In In Vitro Fertilization?

İntrauterin HCG Enjeksiyonu IVF' te Hangi Hastalara Uygulanmalıdır?

Ali Sami GÜRBÜZ[1,4], Necati ÖZÇİMEN[2], Emel Ebru ÖZÇİMEN[3]

[1]Novafertil IVF Center, Konya, Turkey
[2]KTO Karatay University, Medicana Hospital IVF Center, Konya, Turkey
[3]Baskent University Hospital, Obstetrics and Gynecology Department, Konya, Turkey
[4]KTO Karatay University Hospital, Obstetrics and Gynecology Department, Konya, Turkey

ÖZ

Amaç: İn vitro fertilizasyon (IVF) tedavisinde embryo transferinden önce yapılan intrauterin human chorionic gonadothropin enjeksiyonlarının etkisinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Yüz atmış sekiz hasta çalışma grubuna alındı. 274 hasta ise kontrol grubuna dahil edildi. Hasta grupları kendi içinde de ikiye ayrıldı.

Grup 1: Birinci ve ikinci IVF denemesi olan çalışma grubu hastaları (N:126)

Grup 2: Birinci ve ikinci IVF denemesi olan control grubu hastaları (N:189)

Grup 3: Üç veya daha fazla IVF denemesi olan çalışma grubu hastaları (N:42)

Grup 4: Üç veya daha fazla IVF denemesi olan control grubu hastaları. (N:85).

Tüm hastalara taze embryo transferi (ET) yapıldı. Çalışma grubuna ET öncesi intrauterine hcg enjeksiyonu uygulandı.

Bulgular: Üç veya daha fazla sayıda IVF uygulaması yapılmış hastalarda gebelik , klinik gebelik ve implantasyon oranları intrauterine hCG enjekte edilenlerde belirgin düzeyde yüksek çıkmıştır. (p=0,03, p=0,02, p=0,02 sırasıyla)

Sonuç: Intrauterin hCG enjeksiyonu tekrarlayan IVF başarısızlığı olan hastalarda gebelik oranlarını iyileştirmektedir.

Anahtar Kelimeler: Intrauterin hCG, infertilite, tekrarlayan IVF başarısızlığı, hCG

ABSTRACT

Aim: To investigate the impact of the intrauterine injection of hCG before embryo transfer in in vitro fertilization (IVF) according to number of transfer.

Material And Methods: One hundred and sixty-eight (168) patients were included to the study group; 274 patients were included to the control group. The patients were divided into 2 study and 2 control groups.

Group 1: Study group patients in the first and the second IVF cycles. (N=126)

Group 2: Control group patients in the first and the second IVF cycles. (N=189)

Group 3: Study group patients in the third or more IVF cycles. (N=42)

Group 4: Control group patients in the third or more IVF cycles. (N=85)

Fresh IVF-embryo transfer (ET) was performed to all patients. Intrauterine hCG injection was performed before ET in the study groups.

Results: The pregnancy, clinical pregnancy and implantation rates were significantly increased in the intrauterine hCG injected patients with third or more IVF cycles (p= 0.03, p= 0.02, p= 0.02) respectively.

Conclusion: The intrauterine hCG injection improved the pregnancy rates in the patient with recurrent IVF failures.

Keywords: Intrauterine hCG, infertility, IVF, recurrent IVF failures, hCG

INTRODUCTION

Infertility is not being able to become pregnant after 12 months or more of regular intercourse without contraception. In developed countries 15% of the couples are infertile [1]. Today there is an increasing demand for in vitro fertilization (IVF). Successful implantation after IVF and embryo transfer (ET) depends on embryo quality and endometrial receptivity [2].

Implantation is a complex and important process [3]. Cyclic Adenosine Monophosphate (cAMP), relaxin, gonadotropin, prostaglandin E2 (PGE2) and glycoprotein hormones are substances which affect implantation. These substances are secreted from embryo or endometrium [4-9].

One of the important implantation factors is human Chorionic Gonadotropin (hCG) [10]. It is secreted by the embryo before implantation and later in the implantation stage of the human endometrium [10, 11]. Licht et al. conducted a study and used an intrauterine microdialysis system for the purpose of re-

Yazışma Adresi/Correspondence Address:
Ali Sami Gürbüz
Novafertil Tüp Bebek Merkezi Yeni Meram Yolu, No:75 Meram, Konya, Türkiye
Tel/Phone: 0 5335530442
E-mail: alisamigurbuz@hotmail.com

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leasing low hCG concentrations to the endometrium in the luteal phase. They reported that hCG acts before the implantation stage in an autocrine-juxtacrine way until its appearance in the serum [9].

In experimental models it has been demonstrated that hCG can affect endometrial functions [12-18]. It was also showed that recombinant hCG stimulated the secretion of six analytes (VEGF, LIF, IL-11, GMCSF, CXL 10 and FGF2) which act on implantation. hCG is a potent attractor of neutrophils, monocytes and lymphocytes which are known as inflammatory cells [19]. These effects of hCG play role on the regulation of embryo implantation [19, 20]. It has been reported that intrauterine hCG injection was effective before cleavage-stage ET [20, 21, 22]. However, there are also some other studies reporting that there were no useful effects before blastocyst transfer [23, 24].

Our aim is to show whether intrauterine injection of hCG before embryo transfer increases the pregnancy rates in the IVF patients or not.

MATERIAL AND METHODS

This retrospective study was planned to research if the intrauterine injection of hCG improved the implantation rate or not.

The patients' files were evaluated by two doctors. Two hundred and sixteen patients, who received intrauterine hCG between January 2015 - December 2016 in Novafertil IVF center, were enrolled in this study. Forty-eight patients who had systemic disease, azoospermia, uterine abnormality, leiomyoma, hydrosalpinx and who had known etiologies of recurrent failures of implantation, chromosomal abnormalities, presence of antiphospholipid antibodies or hereditary thrombophilia were excluded from the study. One hundred and sixty-eight patients were included in the study. The control group consisted of 274 patients who had IVF-ICSI at the same time. The control group was chosen from among 1920 patients by 1/7 sampling method. Same exclusion criteria were used for choosing the patients for the control group. If a patient was excluded from the control group, the next patient was included in the control group. Written informed consent was obtained from all participants. The patients were separated into 4 groups according to the number of transfers and intrauterine hCG injection.

Group 1: Study group patients in the first and the second IVF cycles (N=126).

Group 2: Control group patients in the first and the second IVF cycles (N=189).

Group 3: Study group patients in the third or higher IVF cycles (N=42).

Group 4: Control group patients in the third or higher IVF cycles (N=85).

In the study groups, intrauterine hCG was injected. In the control group, no intrauterine hCG was injected. The study and control groups were analyzed according to implantation and pregnancy rates.

IVF Procedure

Ovarian stimulation was performed using by gonadothropins (Follitropin Beta, MSD Follitropin Alfa, Merck, hMG, Ferring) and GnRH antagonist (Cetrorelix; Cetrotide®, Merck Serono) according to the patients' basal Estradiol (E2) level, antral follicle counts, ages and previous treatment protocols. GnRH antagonist was injected according to flexible protocol during gonadotropin induced ovarian stimulation. All patients received recombinant FSH or human menopausal gonadotropin from cycle day 3 for ovarian stimulation until 3 dominant follicles reached a diameter of 17mm, followed by injection of 250 mcg recombinant hCG or 10000 IU urinary hCG 35 hours before oocyte retrieval. After oocyte retrieval, 8% progesterone gel (Crinone®, Merck Serono) was applied for luteal support. Embryo Transfer (ET) was performed on the 3rd day after oocyte pick up as follows.

In the study group, 20 minutes before ET, just like in embryo transfer, the patient was put in the lithotomy position and the cervix was visualized using speculum. The cervical mucus was wiped out using a sterile piece of gauze, then the mucus was removed by suction with a 1 ml injector.

The preparation of the intrauterine injection of hCG that is described by Mansour's Technique consisted of adding 1ml 5000 IU of hCG (Choriomon, IBSA) to 3 ml of culture media (G.2 plus ref. 10132, Vitrolife) (20). The hCG for intrauterine injection was prepared by adding 0.4 ml of tissue culture media that contained 500 IU of hCG. And then just like in embryo transfer, a soft catheter (Wallace®, Smiths Medical International Ltd.) was used for intrauterine injection of hCG. If the soft catheter could not pass the internal cervical os, a more rigid catheter was used to administer hCG. This procedure was observed by using transabdominal ultrasound. After waiting nearly 20 minutes to provide enough time to contact hCG and endometrial epithelium, embryo transfer was performed by the same method. All transfers were performed by the same two medical doctors who specialized in IVF. One or two embryos were transferred according to the patients' ages and previous IVF attempts.

In the control groups, the embryos were transferred without injection of intrauterine hCG. In the study and control groups, the embryo transfer was guided by an abdominal ultrasound with a full bladder. The cervix was visualized with a vaginal speculum and then mucus aspiration and cervical washing were done in the control group. This procedure was made during injection of intrauterine hCG in the study group, so it was not repeated. Soft catheters were used for embryo transfer. The patients rested for 30 minutes after ET. The pregnancy test was performed 12 days after ET or 15 days after oocyte retrieval; and ultrasound examination was performed 3 weeks after a positive pregnancy test to visualize the number and the location of gestational sacs and confirm fetal heart pulsations.

Clinical pregnancies and implantation rates were accepted as primary outcome. Clinical pregnancy was calculated as the division of the number of the patients who had fetal heart rate positive by the number of patients who had embryo transfer. The implantation rate was calculated as the division of the number of patients who had existence of gestational sac in ultrasound by the total number of transferred embryos.

Statistics

We used the SPSS version 17 to perform the statistical analysis. We characterized sociodemographic variables with descriptive statistics, using the mean with the standard deviation for the quantitative variables and proportions for the qualitative variables. Power analysis was performed to find enough number of patients.

The Chi-square test was used to compare differences in the proportions of both groups. The relative risks with 95% Confidence Intervals for the proportions were calculated. A $p<0.05$ was considered to be statistically significant. The study was retrospective; for this reason, no ethical committee approval was taken.

RESULTS

In this study, a total of 168 couples participated to the study group, and 274 patients were included to the control group: 126 patients in Group 1, and 189 patients in group 2; 42 patients in Group 3, and 85 patients in Group 4.

The demographic and clinical characteristics of patients are shown in Table 1 and Table 2.

There were no statistically significant differences between Group 1 and 2 regarding age, number of transfer, estradiol levels, number of oocyte retrieval, number of MII oocytes (p= 0.06, p= 0.26, p= 0.25, p= 0.53, p= 0.35), respectively. There were no statistically significant differences between Group 3 and 4 regarding the same parameters (p= 0.53, p= 0.06, p= 0.55, p= 0.46, p= 0.61), respectively. (Table 1 and Table 2).

Table 1: Patient characteristics in Group 1 and Group 2 which had 1or 2 IVF cycles

	Group 1 N=126	Group 2 N=189	P Value
Age *(year,)* (mean±SD)	30,1±4,1	28,3±4,2	0,06
Previous IVF treatment (mean±SD)	1,41±0,4	1,51±0,4	0,26
Estradiol value on trigger day *(pg/ml)*(mean±SD)	2323±453	2170±643	0,25
Cumulus oocyte complexes (mean±SD)	10,8±2,9	11,2±2,8	0,53
MII oocytes (mean±SD)	6,8±1,8	7,1±1,5	0,35

Chi-square test

Table 2: Patient characteristics in Group 3 and Group 4 which had 3 or more IVF cycles

	Group 3 N=42	Group 4 N=85	P Value
Age *(year)* (mean±SD)	32,8±4,86	33,6±4,89	0,53
Previous IVF treatment (mean±SD)	4±1,1	3,84±0,74	0,06
Estradiol value on trigger day *(pg/ml)* (mean±SD)	1990±321	1830±745	0,55
Cumulus oocyte complexes (mean±SD)	9,19±4,18	8,71±3,75	0,46
MII oocytes (mean±SD)	5,61±2,76	5±2,98	0,61

Chi-square test

There were no statistically significant differences between Group 1 and Group 2 regarding the number of fertilized oocytes, number of transferred embryo (p=0.24, p=0.41, respectively). There were also no statistically significant differences between Group 3 and Group 4 regarding the number of fertilized oocytes, number of transferred embryo (p= 0.26, p= 0.78, respectively). The differences were not statistically significant between Group 1 and Group 2 according to pregnancy, clinical pregnancy and implantation rates (p=0.51, p=0.85, p=0.63) (Table 3 and Table 4).

However there were statistically significant differences between Group 3 and Group 4 regarding the rates of pregnancy, clinical pregnancy and implantation rates (p=0.03, p=0.02, p=0.02, respectively) (Table 4).

Table 3: Fertilization, pregnancy and implantation rates in Group 1 and Group 2 which had 1 or 2 IVF cycles

	Group 1 N=126	Group2 N=189	P value
2PN oocytes (mean±SD)	5,3±2,9	5,8±3,1	0,24
Transferred embryos (mean±SD)	1,49±0,4	1,52±0,3	0,41
Pregnancy (n) Pregnancy rates (%)	76 60,3 %	110 58,2 %	0,51
Clinical pregnancy (n) Clinical pregnancy rates (%)	62 49,2%	92 48,6%	0,85
Implantation rates (%)	37,9%	34,1%	0,63

Chi-square test

Table 4: Fertilization, pregnancy and implantation rates in Group 3 and Group 4 which had 3 or more IVF cycles

	Group3 N=42	Group4 N=85	P value
2PN oocytes (mean±SD)	4,3±2,4	3,7±2,2	0,26
Transferred embryos (mean±SD)	1,85±0,2	1,8±0,3	0,78
Pregnancy (n) Pregnancy rates (%)	20 47,6%	19 22,3%	0,03
Clinical pregnancy (n) Clinical pregnancy rates (%)	15 35,7%	16 18,8%	0,02
Implantation rates (%)	25,7%	13,1%	0,02

Chi-square test

DISCUSSION

Previous investigators showed that hCG was produced by the blastocyst before its implantation [11, 12]. hCG has an important role for implantation. Implantation is a complex process. During implantation, high expression of trophinin and a mediator of cell adhesion is acquired by endometrial epithelial cells. hCG associated with Interleukin 1ß (IL-1ß) supplies the increase of apical cell adhesion [25]. Myometrial smooth cell proliferation is induced by hCG. hCG increases progesterone receptors [26, 27]. Uterine natural killer cells play a major role in the establishment of pregnancy [28]. hCG regulates the TH1/TH2 balance C3 and C4 A/B factors which modulate decidual immunity. hCG also initiates the endometrial angiogenesis at the implantation site [29, 30].

There are reports about hCG effect on endometrium. Licht et al. showed the direct effect of hCG on human endometrium. They also showed the inhibiting effect of hCG administration on IGF-binding protein I (IGFBFP-I) and M-CSF [31]. Banerjee et al. performed an experiment on nonhuman primate model. They infused hCG into the uterine cavity during the receptivity window. They showed that this infusion induced morphologic, biochemical, and molecular changes in the endometrium [32]. Based on these studies, we wanted to show if hCG injection to the endometrium before ET improves pregnancy rate or not. In our study, we demonstrated that intrauterine injection of 500 IU of hCG before ET improved the pregnancy rate in patients who had their third or more IVF procedures at a statistically significant level. The present study showed there was no statistically significant pregnancy and implantation rates in the patients who had their first or second IVF procedures.

Mansour et al. found no statistically significant difference between the study and control groups when they injected 100 IU of hCG or 200 IU of hCG. Then, they increased the hCG dose to 500 IU, and they demonstrated for the first time in the literature that injection of 500 IU of hCG before ET improved implantation and pregnancy rates in IVF/ICSI [20].

Similar to Mansour's study, we used 500IU of hCG for intrauterine injection. We found more pregnancy and implantation rates in the patients who had 3rd or more IVF cycles. When we evaluate the results, unlike the results of Mansour, while there were no differences in the patients who had the first 2 trials, applying hCG in patients with more trials contributed to pregnancy rates. The majority of the patients who had good endometrium and embryo in the first 2 trials could become pregnant. In those who were not pregnant, endometrial problems are considered. If the embryo quality is good and the pregnancy rates are low, endometrial receptivity disorders are considered. In other words, as the number of the trials increase in the patient, the possibility of a problem in endometrial receptivity will also increase. Administering hCG will contribute to the improvement of endometrial receptivity, which complies with our findings. This was a retrospective study, and we accepted that the number of IVF procedures increased the possibility of endometrial factor in IVF failure. In the first two IVF cycles, there may be unsuitable IVF protocols, unsuitable medicine for patients or existence of hydrosalpinx or endometrial polyp or quality of embryo, which effect the results of IVF procedure. When the patients reach the 3rd IVF procedure, these negative effects on results may be minimum, and the most important factor on implantation will be the endometrial factor.

Navali et al. applied intrauterine hCG right after oocyte retrieval and observed that there were increases at a rate of 2-fold in chemical pregnancy, clinical pregnancy and implantation rates when compared with the control group. They claimed that applying intrauterine hCG after OPU would be more suitable when compared with the application before ET. However, observation of low pregnancy rates in the control group when compared with the general patient population has made this study become controversial [33].

Mechanical endometrial injury (biopsy/scratch or hysteroscopy) in the cycle preceding ovarian stimulation for IVF has been proposed to improve implantation in women with unexplained recurrent implantation failure. Mechanical trauma to the endometrium also changes gene expression, and supplies growth hormone secretion. These changes make endometrium more receptive [34]. In the present study, the possible mechanical effect of the intrauterine hCG injection besides hCG's biochemical effects on endometrium could also help to improve pregnancy and implantation rates.

Fresh embryo transfers were included in the present study. Of course, fresh embryos secreted also hCG, but thawed embryos' hCG productions are decreased prior to the implantation because of vitrification process. Therefore, thawed embryo transfers may benefit from intrauterine hCG injection more than fresh embryo transfers. Santibanez et al. reported a significant increase in the clinical pregnancy rate in thawed embryos [21].

There are several limitations in this study. First of all, we could not test the embryos regarding to their quality; and secondly, the impact of the mechanical effect of hCG injection catheter on the endometrium was unknown.

The intrauterine injection of hCG before embryo transfer is a simple procedure and does not require extra time, extra training and extra clinical staff. It is not expensive either. The intrauterine injection of hCG before ET showed an increase in the implantation and pregnancy rates. However, based on our results, to increase IVF success, it will be better to use intrauterine hCG injection to the patients who have 3 or more IVF procedures. In this respect, more clinical prospective trials are needed.

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