Reproductive Medicine; *Endocrinology & Infertility*

Comparison of the Different Type and the Different Routes of Administration of Human Chorionic Gonadotropin During Trigger of Ovulation in ICSI Cycles and the Relation with Body Mass Index

Özlem MORALOĞLU[1], Sevtap KILIÇ[1], Gülnur ÖZAKŞİT[1], Beril YÜKSEL[2], Ayça IŞIK[1]
Leyla MOLLAMAHMUTOĞLU[1]

Ankara, Turkey

OBJECTIVE: To confirm that HcG levels in follicular fluid and serum after intramuscular (IM) or subcutaneous(SC) administration of purified (p)HcG and SC administration of recombinant(r)HcG and their associations with body mass index (BMI) and oocytes maturation in ICSI cyle.
STUDY DESIGN: We used pHcG or rHcG for triggering ovulation of all patients . Group1(n:56) received IMpHcG and Group2(n:57) received SCpHcG and Group3(n:47) received rHcG . Serum and follicular fluid HcG levels were measured on the day of oocytes retrieved as primary outcome.
RESULT: There was a significant difference on serum and follicular fluid HcG levels among three groups. No correlation was found between BMI and oocytes maturation for Group1 and Group2. But there was a significantly negative correlation between BMI and oocytes maturation in Group3.
CONCLUSION: When comparing the different administration routes of HcG , the S.C. route of HcG seems to be safer and useful for obese patients.

Key Word: Body mass index, Oosyte maturation, Human chorionic gonadotropin

Gynecol Obstet Reprod Med;14:3 (172 - 178)

Introduction

The mid-cycle luteinizing hormone (LH) surge is necessary for maturation of the oocyte and initiation of follicular luteinization. During IVF, a supra-physiological dose (10.000 IU) of HcG is given to mimic the effects of the natural LH surge. The administration of HcG at the end of a stimulation phase of gonadotrophins triggers the maturation of the cumulus-oocyte complex and allows the resumption of meiosis in the oocyte. Decreased periovulatory HcG concentrations have been shown to be associated with diminished fertilization rates.[1] The purified form of HcG is usually given via the intramuscular (I.M.) route, but subcutaneous (S.C.) administration has also been described.[2]

In fact, S.C. administration of HcG has been used in both Europe and North America [3] during ovulation induction and IVF treatment. The SC route of administration, allowing self-administration by the patient, had previously been chosen.[3] This is especially relevant when HcG administration, which is usually timed, is to be given late at night and nursing staff must be available outside normal office hours to administer the I.M. injection.

Recombinant HcG (rHcG) has been manufactured by transfecting non-human cell lines (Chinese hamster ovary cells) with genetic material capable of replicating identical amino acid sequences to the human compound and developed as a pharmaceutical product named Ovidrel® (Choriogonadotropin alfa; Serono Inc., São Paulo, Brazil). In limited, unpublished clinical studies, the half-life of r-HcG was estimated as ~ 30h.[1] The efficacy of 250µg r-HcG has been reported, and r-HcG has been shown to be well tolerated in the induction of final maturation in women undergoing ART.[2] Additionally, a dose of 250 µg r-HcG appears to be clinically equivalent to 10.000 IU u-HcG in this group

Drug distribution and metabolism in the body are dependent on the amount of adipose tissue as well as the route of administration and rate of plasma clearance.[4] The pharmacokinetic behaviour of HcG may also be affected by obesity. In general, a larger distribution volume results in a lower serum concentration [5] BMI is used widely in epidemiological studies as a good indicator of adiposity.[6] In a prospective cohort study, Salha et al. concluded that high body mass index (BMI) is detrimental to the success of IVF treatment and has an important influence on the distribution and metabolism of HcG (vii).

[1]Dr.Zekai Tahir Burak Women Health and Educational Research Hospital, [2]Assisted Reproduction and Embryology Unit, Ankara

Address of Correspondence: *Sevtap Kılıç*
Süleyman bey Sok. 29/10
Maltepe, Ankara

Submitted for Publication: *25.09.2008*
Accepted for Publication: *29.09.2008*

Elkind-Hirsch et al. also reported that the highest levels of HcG were measured in women with the lowest BMI.[3]

Although the pharmacokinetics after either route have been compared, the results obtained were inconsistent. Studies so far have been inconclusive as to the effect of BMI on serum concentrations of HcG predict the outcome of IVF treatment in terms of biochemical and clinical pregnancy rates.[3,4,6,8,9,10]

The first aim of our study is to compare the effect of the different types (recombinant and purified HcG) and different administration routes of HcG (SC. and IM.) which is used for ovulation induction in an IVF cycle, on serum and follicular fluid hormone levels (HcG).The second aim is to evaluate the effect of these different types and different administration routes of HcG on IVF outcomes.The third aim is to look for the correlation between patient's body size with serum and follicular fluid hormone levels and oocyte maturations.

Planning of this study was based on to find answers for these questions:

1- Are there any differences in terms of serum and follicular fluid HcG levels between the IM or SC route of administration of purified HcG (Profasi- 10.000 IU) injection or SC recombinant HcG(Ovidrel-250 µg) injection which we use to stimulate ovulation in IVF patients?

2- Does the administration of HcG from different routes and different types have any effect on parameters such as; oocytes maturations, fertilization rates, embryo grades and pregnancy outcomes?

3- Is there any correlation between BMI with serum and follicular fluid HcG levels in terms of drug distribution?

4- Is S.C. administration of HcG more preferable in terms of patient compliance, easy application and has potential side-effects?

Material and Method

Subjects

One hundred and thirteen (160) women, 19 to 39 years of age, were recruited from the Zekai Tahir Burak Women's Hospital Assisted Reproduction Clinic undergoing for the first or second IVF cycles between April 2007 and June 2007. All patients infertility were attributable to association with tubal (included endometriosis) factor infertility, unexplained infertility, hormonal - anovulatuar disorder, sub-fertile male factor infertility. Ethical approval was obtained from the local Research Ethics Committee prior to the study and written informed consent was obtained from all participants.

The exclusion criteria's were;

older than 39 years old, severe male factor (<5 million total progressive motile spermatozoa per mililiter And allowing for TESE), have any endocrine and systemic disease (Hyperthyroidism or hypothyroidism, hyperprolactinemia, DM,hypertension etc), diminished ovarian reserve (FSH of >10 IU/mL), more than 3 previous failed IVF cycles or who had an IVF cycle canceled because of poor response to gonadotropins, Previous history of severe ovarian hyperstimulation syndrome (OHSS), Prior documentation of intolerance or allergy to any gonadotropins, uterine anomaly or uterine fibroids and hydrosalpinges.

Superovulation Protocol

All patients were pretreated with an oral contraceptive (Desogen; Organon, West Orange,NJ) for 18 days, starting day 3 of the cycle. Pituitary suppression with a GnRH-agonist (Lupron SC; TAP Pharmaceuticals, Inc, Deerfield, IL) was initiated on cycle day 18 at dose of 1 mg daily injected SC into the thigh or arm. On day 2 of menses a baseline ultrasound (US) was performed to document that the ovaries were quiescent. Documentation of pituitary down-regulation, as indicated by an estrodiol level of<50 pg/ml and an LH level of <5 IU/mL.

Stimulation cycle began using r- FSH (Gonal-F; Ares-Serono, Geneva, Switzerland) starting at dose of 225 IU/d, which was administered for three days. Following starting r-FSH Lupron, dose was decreased to 0, 5 mg daily.

Estradiol monitoring and US was performed according to standard IVF clinic practice.

When a minimum of three follicles exceeded 16 mm in average diameter, with acceptable serum E2 concentrations, an ovulatory dose of purified HcG (Profasi,Serono) was given by IM or SC injection or recombinant HcG (.Ovidrel, Serono, 250 µg) by SC injection to all patients. Subjects were assigned; using computer-generated random numbers table, to receive pHcG (Profasi, Serono) administered either IM or SC and to receive rHcG (Ovidrel) administered SC on the day after the last dose of gonadotropins. Patients were randomized in the ratio 1:1:1 to receive a single dose of 10.000 IU of pHcG IM or SC or 250 µg of rHcG SC.Only patients who fulfilled the criteria for HcG administration were randomized. The SC injection of pHcG(Profasi) was self -administered in the lower abdomen with a 5/8- inch, 27- gauge needle. The volume of diluent was 1 ml for both the SC and IM injections. Vaginal oocyte retrieval was performed under ultrasound guidance 36 hours after HcG injection. The oocytes were than fertilized using ICSI technology. All serum samples and follicular fluids were collected on the oocytes retrieval time. Follicular fluid was collected from the first mature follicle aspirated, using media-free collection tubes. The cell number for each embryo and embryo qualities were documented. Embryos were transferred on day 3 after retrieval and a maximum of 3 embryos was replaced into the patient's uterus.

Luteal phase support was prescribed by vaginally beginning on the day after egg retrieval (Crinone %8; Serono Labrotories, Inc.,Norwell, MA-400mg/daily) and was continued until 14 days after retrieval when a quantitative measurement of the HcG level was obtained.

The data regarding to the number of oocytes obtained, serum HcG levels, follicular fluid HcG levels, fertilization rate, number of embryos transferred, pregnancy ratios (+HcG/ET) and HcG ratios (follicular fluid HcG/serum HcG) were recorded for each patient. A calculation of the body mass index was also carried out for each patient using her weight and height (kg/m²).

Hormone Assays

Serum and follicular fluid aspirates were stored at - 200C after centrifuged at 3000 RPM for 10 minutes .Estrodiol, FSH, Progesterone and HcG levels were measured using an automated, electrochemiluminesans (ECLIA,E-170,ROCHE) system. Its sensivity for HcG was <0, 1 mIU/ml, linear interassay precision of 0, 1-10.000mIU/ml.

Statistical Analyses

For comparison of differences in ages, BMI, hormone measurements, (serum HcG 36 hours after administration; serum estradiol levels on day of HcG), duration and total dose of r-FSH treatment, number of oocytes retrieved, and oocytes maturation in the three groups, we used One-way Annova and Tukey test because of the dissociations of groups were normal. A Spearmen's product-moment correlation tests were used to determine the relation-ship between BMI and serum or follicular fluid HcG concentrations. The x^2 test was used to assess the difference between presence and absence of outcome variables (e.g. pregnancy, ohss). A p value of <0, 05 was regarded as statistically significant.

Results

There were 56 women in the IM pHcG group (Group1), 57 women in the SC pHcG group (Group2) and 47 women in the SC rHcG group (Group3). In terms of infertility etiologies; the tubal factor constituted 10, 7%, male factor constituted 32,1%, hormonal- anovulatory disorder 1, 7% and unexplained infertility constituted 55, 3% of group 1. The distribution was 14%, 31, 5%, 1, 7% and 52, 6% respectively for group 2. Group 3 consisted of tubal factor 8,5%, male factor 42,5% and unexplained infertility 48,9%. Baseline and cycle characteristics between groups are depicted in Table1. The mean age and mean BMI were similar for all study groups (mean age: 30; mean BMI: 25, 5). All patients' body mass indexes were between 19 and 36. There were no significant differences between groups cycle length, number of antral follicles, total gonadotropin usage, peak E2 level before HcG injection, E2 and P values on HcG day, number of oocytes retrieved, number of mature oocytes (M2) and number of fertilized oocytes (2PN) Table 1.

Table 1:

Variable	Group1 I.M.pHcG (n:56)	Group2 S.C.pHcG (n:57)	Group3 rHcG (n:47)	(p)
Age (year)	30,0±5,4 (19–39)	30,6±4,7 (22–39)	28,7±4,5 (21–39)	0,175
BMI (Kg/m²)	25,4± 4,0 (19–36)	25,6±3,0 (20–33)	24,9±3,5 (17–34)	0,652
Duration of infertility(years)	7,8 ±5, 0 (2–22)	9,5± 5,2 (1,5–20)	7,9 ±4,2 (1,5–22)	0,135
Baseline E_2 levels (pg/ml)	45,7±18,6	41,2±12,7	47,3 ± 24,5	0,221
Baseline FSH levels (mIU/ml)	6,7±1,9	6,9±1,9	6,8 ±1,5	0,83
Total number of antral follicle count	9,4± 4,1	10,4±4,1	11,5± 5	**0,05**
Duration of r-FSH (days)	9,6 ±1,3	9,7± 1,7	9,8 ±1,5	0,72
Total dose of r-FSH (I.U.)	2349,9±1059,3	2266,4± 930,8	2215,5± 1221,1	0,81
Serum E_2 (pg/ml) day of HcG	2182,4 ± 913,2	2132,9± 818,8	2677,2± 1350,9	**0,01**
Serum P(pg/ml) day of HcG	6,7 ± 5,8	7,5 ± 6,5	8,2 ± 6,7	0,49
Total number of oocytes retrieved	10,2 ±5,2	9,2± 4,2	9,7± 4,5	0,50
Total number of M2 oocytes on day of oocyte retrieval	8 ± 4,8	7,4 ± 3,8	8,1 ± 4	0,63
Total number of 2PN on day of embryo	6 ±4	5,6± 3,7	5,1± 3,7	0,52

a- Data is presented as mean ± SD(range), p value of < 0, 05 was regarded as statistically significant

There was a significant difference among groups in terms of serum and follicular fluid HcG levels, HcG ratios (follicular HcG/serum HcG) with One-way Anova test (Table 2). We used Tukey test to find out which group was responsible for this difference. The lowest serum and follicular fluid HcG levels was found in Group3 (r-HcG group) (serum HcG:117,5 ±72,7; follicular fluid HcG: 60,5 ± 63,7; p:0,001) (Table-2).

We found a statistically significant difference between fertilization ratios of Group1 and Group3 (77% in Group1, 63, 5% in Group3; p:0,047) (Table 2). The pregnancy ratios (+HcG/ET) were 35, 7% in group1; 49, 1% in group2 and 25, 5% in Group3 (p:0, 04) (Table 3).

Table 2: Serum and Follicular Fluid HcG values and Fertilization Rates in Groups and mean differences

Parameter	Statisticsa	Group1 I.M.pHcG (n:56)	GGroup2 S.C.pHcG (n:57)	Group3 HcG (n:47)
Serum HcG on day of OPU	Mean±SEM Range	222,4±103,3 51,6- 500,7	261,8±144 77,6- 852,9	117,1±72,7 39,8-395,8
	Mean difference ± SEM (P value)	39,3± 21,1 (p:0,155)	144,7±22,1 (p:0,001)*	105,3±22,2 (p:0,001)*
Follicular fluid HcG on day of OPU	Mean±SEM Range	134,9±85,4 14 -341	153 ±107,2 34- 604	60,5± 63,7 10,2 -336
	Mean difference ± SEM (P value)	18,1 ± 16,6 (P:0,523)	92,5±17,4 (p:0,001)*	74,3±17,5 (p:0,001)*
HcG Ratio (follicular HcG/serum HcG)	Mean±SEM Range	0,58±0,24 0,21 -1,2	0,62± 0,32 0,13- 2	0,46±0,18 0,20 -0,91
	Mean difference ± SEM (P value)	0,04±0,04 (p:0,685)	0,16±0,05 (p:0,004)*	0,12±0,05 (p:0,043)*
Fertilization Ratios (%oocytes suitable for ICSI)	Mean±SEM Range	77±30,7 0-100	72,7±27,4 0-100	63,5 ±26,9 0-100
	Mean difference ± SEM (P value)	-4,3 ±5,3 (P:0,702)	9,1±5,6 (p:0,236)	13,4±5,6 (p:0,047)*

Table 3: Pregnancy ratios and outcomes of IVF in groups receiving p-HcG (I.M. or S.C.) and r-HcG.

	Group1 I.M.pHcG (n:56)	Group2 S.SC.pHcG (n:57)	Group3 rHcG (n:47)	P-value[a]
Pregnant patients (+HcG/ET)	20(35,7%)	28(49,1%)	12(25,5%)	0,04*
Clinical Ohss (Ovarian Hyperstimulation Syndrome)	6(10,7%)	4(7%)	0(0%)	0,07
Clinical miscarriage	6(10,7%)	5(8,8%)	1(2,1%)	0,23
Extrauterin pregnancy	1(1,8%)	1(1,8%)	0(0%)	0,65

There were no significant differences among groups in terms of clinical ovarian hyperstimulation syndrome (OHSS) ratios, clinical miscarriage ratios and extrauterine pregnancy ratios (Table 3).

Similarity in mature oocytes count was observed in three groups. No correlation was found between BMI and oocytes maturation in Group1 and Group2 (r:-0, 05). There was significantly negative correlation between BMI and serum and follicular fluid HcG levels in group1 (r :- 0, 34, p : 0.009; r : - 0, 36, p : 0,006) higher than group2 (r :-0.12, p : 0.37; r :- 0.17, p: 0.20). No correlation was found between BMI and serum and follicular fluid HcG levels in Group 3. But there was significantly negative correlation between BMI and the mean number of mature oocytes in Group3 (r: -0,31,p:0,03) (Table-4).

Table 4: Correlations between Groups with Body Mass Index(BMI).

	BMI					
	Group1 I.M.pHcG (n:56)		Group2 S.C.pHcG (n:57)		Group3 rHcG (n:47)	
	(r)	(p)	(r)	(p)	(r)	(p)
Foll. HcG	- 0.36	0.06	- 0.12	0.37	- 0,20	0,16
Serum HcG	- 0.34	0.09	- 0.17	0.20	- 0, 21	0,14
M2 oocytes	- 0.10	0.44	- 0.11	0.41	- 0,31	0,03
2PN oocytes	- 0,15	0,24	-0,10	0,43	- 0,30	0,03
Fert. ratio	0.04	0.74	- 0.03	0.77	- 0,06	0,67
Pregn.ratio	- 0.05	0.68	- 0.19	0.88	- 0,09	0,50

Discussion

In natural menstrual cycle, spontaneous LH surge is effective in about 14-50 hours. After that follicle rupture, separation of oocytes cumulus cells, completion of meiotic maturation, mucinification of cumulus oophorus and progesterone production after follicular cell luteinization.[11] The

pharmacokinetic profile of follicle rupture by administrating exogenous HcG was reported to have a possibility in several studies.[12] Fisher et al. reported LH surge was delayed and its concentration was lower via IM route[13] Hence, it is obvious that the route effects the pharmacokinetic. Moreover, it is reported that the origin of the preparate -urinary or recombinant- also effects the number of mature oocyte number, luteal progesterone level and local tolerance.[14]

This study was planned to evaluate the relation between route of HcG administration, HcG origin and number of oocytes retrieved, number of mature oocytes (M2), number of fertilized oocytes, serum and follicular HCG values and BMI in normoresponder patients. Thus it differs from the literature in terms of preparate and administration route variability, evaluation of serum and follicular HcG levels and the relation of them with BMI. Moreover, this study is different from the literature as the groups are homogeneous for demographic and basal parameters like age, antral follicle count, BMI, basal and peak estradiol levels on HcG day and induction period which can effect the response of patients.

Patients underwent pituitary desensitization using along GnRH agonist protocol, followed by stimulation with rFSH, prior to receiving a single dose HCG via different route. The surrogate LH surge of administered HcG plays a crucial role in intrafollicular events that should have been completed before the time of oocyte retrieval, such as softening of the connective tissue elements of the follicle which facilitates the detachment of the oocyte-cumulus complex from the follicle wall.[15] It is now well accepted that decreased HCG bioavailability can effect to oocyte maturation and follicular rupture.[16]

Weissman et al., studying the pharmacokinetics of a single dose of HcG IM versus SC, found a similar serum profile with the highest HcG concentrations achieved with a dose of 10.000 IU administered subcutaneously. They concluded that SC injection of HcG for induction of ovulation is a simple and safe method and might offer better patient tolerance of infertility treatment.[10] Similarly, we could not find any difference in serum samples of groups obtained 36 hours after similar injection. So we concluded that serum levels did not change with either rHcG or IM and SC pHcG. But, in their study investigating the relation between bioavailibity and obesity and IM administration, Carina et al. reported that the bioavaibility increased with IM route but decreased with obesity.[17] We also could not determine any difference among groups in terms of serum progesterone levels on HcG day, so we believe that the groups may also be homogeneous for granulose cell luteinization.

International Recombinant Human Chorionic Gonadotropin Study Group suggested that, both urinary HcG and recombinant HcG were effective in ovulation induction when administered SC and no statistically significant differences were observed between the treatment groups for the primary efficacy endpoints.[18] The primary endpoint was the number of oocytes retrieved. In our study we also evaluated other endpoint parameters like number of metaphase II (MII) oocytes and number of fertilized oocytes, but we could not find any difference among groups. Although there are several studies reporting a positive correlation between rHcG and oocytes maturation, we could not find any difference among groups in terms of MII oocytes and fertilization ratios.[19]

Nagata et al .concluded that the ratio of the level of HcG in the follicular fluid to the level of HcG found in the serum was a good marker for ovarian responsiveness and subsequent pregnancy. They reported that, the ratio of the level of HcG in serum must be at least 0,46 to be adequate for pregnancy.[20] In our study, we found that intrafollicular HcG levels were higher in SC HcG group than the levels in IM HcG and rHcG groups. The pregnancy ratio was higher in SC HcG group. These results concur well with Nagata et al., but they should be confirmed in larger sized groups to raise the statistical assurance.

Obese women have a larger volume of distribution than non-obese women, and this may lead to a lower serum concentration after drug administration. A strong negative correlation was observed between serum HcG levels and BMI in several studies.[5,21] Decreased periovulatory human chorionic gonadotrophin (HCG) concentrations have been shown to be associated with diminished fertilization rates. These data indicate that intra-follicular HcG concentration is inversely related to BMI, and may be related to a concurrent decrease in pregnancy rates.[22] Our results concur well with Carell et al. In our study we did not group the patients according to their BMI, but when all patients were evaluated, although it was not statistically significant, serum and intrafollicular HcG levels had a negative correlation with BMI.

There are several studies reporting alterations in pregnancy ratios with obesity.[23,24,25] These studies noted that obesity effected serum gonadotropin and steroid levels. We also observed a non significant negative correlation between BMI and serum-intrafollicular HcG levels and pregnancy ratios.

In conclusion, we believe SC pHcG preparates have the advantage of easier application and cost effectiveness. Moreover, we concluded that intrafollicular and serum levels of HcG at 250 mgr and 10000 IU dosages are not effective in the number of oocytes retrieved, number of MII oocytes and fertilization ratios. We believe, regardless of administration route, there is a slight negative correlation between serum and intrafollicular HcG levels.

Rekombinant veya Purifiye İnsan Korionik

Gonadotropinin ICSI Sikluslarında Ovulasyonu Tetiklemek Amacıyla Farklı Kullanım Yolları ile Body Mass Index İlişkisinin Karşılaştırılması

Çalışmamızda ICSI uygulamalarında (p) HcG'nın subkütan (SC) veya intramusküler (IM) kulanımındaki foliküler sıvı ve kan HcG düzeyleri ile recombinant(r)HcG nin subkütan uygulanımı ile elde edilen değerler ile body mass index (BMI) ve oosit maturasında ilişkiyi değerlendirmeyi amaçladık.

Ovulasyonu tetiklemek amacıyla pHcG veya rHcG kullanıldı. Grup1 (n:56) IM pHcG, Grup2 (n:57) SC pHcG ve Group3 (n:47) rHcG uygulanan hastadan oluşmaktaydı. Serum ve folliküler sıvı HcG düzeyleri primer outcome olarak oosit toplama gününde ölçüldü.

Gruplar arasında serum ve folliküler sıvı HcG düzeylerinde önemli fark izlendi. Grup 1 ve Grup 2 için BMI ve oosit maturasyonları arasında korelasyon izlenmedi. Fakat grup 3 için BMI ve oosit maturasyonları arasında istatiksel anlamlı olarak negatif korelasyon izlendi.

HcG nin farklı kullanım yollarını karşılaştırdığımızda SC kullanımın obez hastalar için daha güvenli ve faydalı olduğu sonucuna ulaştık

Anahtar Kelimeler: Vücut kitle indeksi, Oosit maturasyonu, İnsan korionik gonodotropini

References

1. Trinchard-Lugan I, Khan A, Porchet HC: Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotropin in healthy male and female volunteers, Reprod Biomed Online 2002; 4:106-15
2. Chang P, Kenley S, Burns T, Denton G, Currie K, DeVane G, O'Dea L: Recombinans human chorionic gonadotropin (rHcG) in assisted reproductive technology: Result of a clinical trial comparing two doses of rHcG (Ovidrel) to urinary hCG (Profasi) for induction of final follicular maturation in in vitro fertilization-embryo transfer, Fertil Steril 2001;76:67-74
3. Elkind-Hirsch KE, Bello S, Esparcia L, Phillips K, Sheiko A, McNichol M.Serum human chorionic gonadotropin levels are correlated with body mass index rather than route of administration in women undergoing in vitro fertilization--embryo transfer using human menopausal gonadotropin and intracytoplasmic sperm injection.Fertil Steril. 2001,75 (4) :700-4.
4. Petros Stefanis, Sangeeta Das, Emil Barsoum-Derinas, Charles Kingsland, Iwan Lewis-Jones, Rafet Gazvani. Relationship between serum human Chorionic honadotrophin levels and body mass index in women undergoing in vitro fertilisation cycles. European Journal of Obtetrics and Gynecology and Reproductive Biology. 2007; 132: 204-8.
5. Dobbs, KE., Domesic DA., Domesic, JA. and Shapiro, S.S., Differences in serum follicle-stimulating hormone uptake after intramuscular and subcutaneous human menopausal gonadotropin injection. Fertil. Steril. 1994; 62: 983-7.
6. James R. Stelling, Emily T. Chapman, David Frankfurter, Doria H. Haris, Selwyn P. Oskowitz and Richard H. Reindollar. Subcutaneous versus intramuscular admintration of human chorionic gonadotropin during an in vitro fertilization cycle. Fertility and strerility. 2003; 79: 881-4.
7. Osama Salha, Tunde Dada ana Vinay Sharma. Influence of body mass index and self-adminstration of hCG on the outcome of IVF cycles: a prospective cohort study. Human Fertility. 2001; 4: 37-42.
8. Matts Wikland, Jan Borg, Ann-Sofi Forsberg, Ann-Helen Jakobsson, Peter Svalander and Urban Waldenström. Human chorionic honadotrophin self-adminstred by the subcutaneous route to induce oocyte maturation in an in-vitro fertilization ana embryo transfer programme. Human Reproduction. 1995; 10: 1667-70.
9. Sills ES, Drews, CD., Perloe, M., Kaplan, C.R. and Tucker, M.J. Periovulatory serum human Chorionic gonadotropin (hCG) consentration following subcutaneous and intramuscular nonrecombinant hCG use during ovulation induction: a prospective, randomized trial. Fertility and Sterility. 2001; 76: 397-9.
10. Weissman, A, Lurie, S, Zalel Y, Goldchmid, R and Shoham, Z. Human Chorionic gonadotropin: pharmacokinetics of subcutaneous adminstration. Gynecol. Endocrinol. 1996; 10: 1667-70.
11 Al-Inany H, Aboulghar MA, Mansour RT, Proctor M. Recombinant versus urinary gonadotrophins for triggering ovulation in assisted conception Hum Reprod. 2005, 20 (8) :2061-73
12. Wener S.,Glowania H-J,Hengst W,Happ J. Pharmacodynamics and pharmocokinetics after subcutaneous and intramuscular injection of human chorionic gonadotropin Fertility Sterility 1991;56 (2), 225-9
13. Fischer, R.A., Nakajama, S.T., Gibson, M. and Brumsted, J.R. (1993) Ovulation after intravenous and intramuscular human chorionic gonadotrophin. Fertil. Steril., 60, 418-22
14. Induction of final follicular maturation and early luteinization in women undergoing ovulation induction for assisted reproduction treatment-recombinant HCG versus urinary HCG. The European Recombinant Human Chorionic Gonadotrophin Study Group Human Reproduction, 2000 15 (7) 1446-51
15. Meniru, G.I. and Craft, I.L. (1997) Evidence from a salvaged treatment cycle supports an aetiology for the empty follicle syndrome that is related to terminal follicular developmental events. Hum. Reprod., 12, 2385-2387
16. F. Zegers-Hochschild, E. Fernández, A. Mackenna, C.

Fabres, E. Altieri and T. Lopez Endocrinology: The empty follicle syndrome: a pharmaceutical industry syndrome, 1995 Human Reproduction, 10 (9) 2262-5

17. Carina CW,Ernest H.Y,Maureen MY,Oi Shan T. Bioavaibility of HcG after intramuscular or subcutaneous injection in obese and non-obese women 2003 Human Reproduction 18 (11) 2294-7
18. The International Recombinant Human Chorionic Gonadotropin Study Group. Induction of ovulation in WHO group II anovulatory women undergoing follicular stimulation with recombinant human follicule- stimulating hormon: a comparison of recombinant human Chorionic gonadotropin (rHcG) and urinary HcG. Fertility and Sterility. 2001; 75: 1111-8.
19. James R. Stelling, Emily T. Chapman, David Frankfurter, Doria H. Haris, Selwyn P. Oskowitz and Richard H. Reindollar. Subcutaneous versus intramuscular admintration of human chorionic gonadotropin during an in vitro fertilization cycle. Fertility and strerility. 2003; 79: 881-4.
20. Nagata Y, Honjou K, Sonoda M, Sumii Y, Inoue Y, Kawarabayashi T. Pharmacokitetics of exogeneus gonadotropin and ovarian respons in in vitro fertilization. Fertility and Sterility. 1999; 72: 235-9
21. Karen E. Elkind-Hirsch, M.Sc, Sandra Bello, Lori Esparcia, Kathy Phillips, Alex Sheiko and Milton McNichol. Serum human chorionic gonadotropin levels are correlated with body mass index dahter than route of adminstriration in women undergoing in vitro fertilization-embryo transfer using human meopausal gonadotropin and intracytoplasmic sperm injection Fertility and Sterility, 2001; 75: 700-04.
22. Carrell DT, Jones KP, Peterson CM, Aoki V, Emery BR, Campbell BR.Body mass index is inversely related to intrafollicular HCG concentrations, embryo quality and IVF outcome. Reprod Biomed Online. 2001;3 (2) :109-111
23. Lashen H, Ledger W, Bernal AL, Barlow D. Extremes of body mass do not adversely affect the outcome of superovulation and in-vitro fertilization. Hum Reprod. 1999; 14 (3) :712-5
24. Dechaud H, Anahory T, Reyftmann L, Loup V, Hamamah S, Hedon B Obesity does not adversely affect results in patients who are undergoing in vitro fertilization and embryo transfer. Eur J Obstet Gynecol Reprod Biol. 2006; 127 (1) :88-93.
25. Spandorfer SD, Kump L, Goldschlag D, Brodkin T, Davis OK, Rosenwaks Z Obesity and in vitro fertilization: negative influences on outcome. J Reprod Med. 2004; 49 (12) :973-7