# Comparison of two doses of recombinant hcg for oocyte maturation in obese women (BMI ≥30) undergoing assisted reproductive techniques

## Porównanie dwóch dawek rekombinowanej hCH użytych do dojrzewania oocytów u kobiet otyłych (BMI⩾30) przechodzących techniki rozrodu wspomaganego

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### Abstract

***Objective:*** *To compare the efficacy of two doses (250 µg vs. 500 µg) of r-hCG for oocyte maturation in obese women with a body mass index (BMI) ⩾30 and undergoing assisted reproduction techniques.*

***Materıals and Methods:*** *A Prospective, randomized, clinical study of seventy two patients undergoing IVF/ intracytoplasmic sperm injection cycles with BMI ⩾30 kg/m². Patients with high BMI were randomized to receive either 250 µg or 500 µg rhCG. Blood and follicular fluid (FF) samples were collected on the day of oocyte pick-up (OPU). The outcome measures were serum and FF hCG levels on the day of OPU, number of oocytes retrieved per patient, number of mature oocytes retrieved, clinical pregnancy rates (PR).*

***Results:*** *Serum hCG levels were significantly lower in patients receiving 250 µg of r-hCG than in patients receiving 500 µg of r-hCG. However FF hCG levels, implantation rates, abortion rates, clinical PRs were not significantly different*

***Conclusions:*** *250 µg of r-hCG is sufficient and safe to trigger ovulation in women with BMI ⩾30.*

Key words: **oocyte maturation** / **recombinant hCG** / **obesity** / / **serum and follicular fluid** / **IVF** /

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## Streszczenie

***Cel pracy:*** *Porównanie skuteczności dwóch dawek (250µg vs. 500µg) rekombinowanej hCG użytej do dojrzewania oocytów u kobiet otyłych z indeksem masy ciała (BMI) ⩾30 i poddanych metodom rozrodu wspomaganego.*

***Materiał i metody:*** *Prospektywne, randomizowane badanie kliniczne przeprowadzono na grupie 72 pacjentek poddanych IVF/ docytoplazmatycznemu podaniu plemnika z BMI⩾30 kg/m2. Pacjentki randomizowano do otrzymania r-hCG w dawce 250µg lub 500µg. Krew oraz płyn pęcherzykowy (FF) pobierano w dniu pobrania oocytów (OPU). Mierzono poziom hCG w surowicy i płynie pęcherzykowym, liczbę oocytów uzyskanych na pacjentkę, liczbę dojrzałych oocytów, wskaźnik ciąż klinicznych.*

***Wyniki:*** *Poziom hCG w surowicy był istotnie niższy u pacjentek otrzymujących 250µg r-hCG niż u pacjentek otrzymujących 500µg r-hCG. Jednak poziom hCG w płynie pęcherzykowym, liczba implantacji, liczba poronień, wskaźnik ciąż klinicznych nie różniły się istotnie między grupami.*

***Wnioski:*** *Dawka 250µg r-hCG jest wystarczająca i bezpieczna dla wywołania owulacji u kobiet z BMI⩾30.*

Słowa kluczowe: **dojrzewanie oocytów** / **rekombinowana hCG** / **otyłość** / **surowica** / / **płyn pęcherzykowy** / **zapłodnienie pozaustrojowe** /

## Introduction

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. In overweight and obese women, reduced bioavailability of hCG and possibly lower concentrations into the follicle might reduce final oocyte maturation [1-7].

In women undergoing assisted reproduction techniques (ART), obesity has been associated with the need for higher doses of gonadotropins, increased cycle cancellation rates, and fewer oocytes retrieved [8]. Lower rates of embryo transfer, pregnancy, and live birth have also been reported, as have higher miscarriage rates [8, 9]. Studies indicating no negative effect of obesity on IVF therapy agree on the necessity of high-dose gonadotropine administration in obese patients [10-12]. We can predict that as obese people have fertility issues, assisted reproductive technology protocols will also differ for obese patients. It is not clear which dose of r-hCG, 250 µg or 500 µg, is an effective dose to induce final oocyte maturation in obese and highly obese patients. The aim of this study is to compare two doses of r-hCG in obese (BMI ≥30) women undergoing IVF treatment in terms of serum and FF hCG levels, total and metaphase II (MII) oocytes obtained, and clinical parameters achieved.

## Materials and methods

This prospective, randomized study was done between December 2008 and May 2010. The study was approved by the Institutional Review Board (IRB) of our hospital and written consent was obtained from all participants.

Patients with BMI ≥30 were accepted as eligible for the trial. Exclusion criteria included polycystic ovary syndrome (PCOS), a history of bad response to controlled ovarian hyperstimulation (COH), and a high basal FSH level >12 IU/mL. Patients underwent ovarian stimulation by standard midluteal phase GnRH agonist (Lucrin®; Abbott, USA) or GnRH antagonist (Cetrotide®; Merck Serono, Turkey) in flexible manner. Randomization was performed according to computer based randomization list and the subjects were divided into two groups; those who received 250 µg and those who recieved 500 µg hCG. The clinician was blinded to the hCG dose to be administered to the patients while referring the patients to get an appointment for the oocyte pick up (OPU). The nurse administered the hCG doses to the patients according to the dose indicated in the randomization envelope, which was given to the patient by the responsible nurse. Thus, the patients were not blinded to the dose of hCG.

GnRH agonist was started at a dose of 0.1 mg daily sc on luteal phase of preceeding cycle and continued with the same dose until down regulation was confirmed by onset of menstruation and serum E2 level <50 pg/mL, than dose halved. GnRH antagonist was initiated at a dose of 0.25 mg daily when leading follicle was 12 mm. Ovarian stimulation was administrated as recombinant FSH (rFSH; Gonal-F®; Merck Serono, Turkey). When two or more follicles had attained a minimum mean diameter of 18 mm, follicular maturation was achieved using either 250 µg of r-hCG subcutanously (Ovitrelle®; Merck Serono, Switzerland) or 500 µg of r-hCG subcutanously according to a computer-based randomization list.

Transvaginal ultrasound (TVUS)-guided oocyte retrieval was performed 36 hours after administration of r-hCG injection. After the retrieval, the oocytes were evaluated for maturity and intracytoplasmic sperm injection (ICSI) was used for fertilization. The embryos were cultured 3-5 days before the transfer. The day of transfer and the number of embryos transferred were decided by the physician based on patient and cycle characteristics.

Luteal phase supported by progesterone 100 mg amp. (Progynex™; Farmako, Turkey) im for 12 days folloved by progesterone gel intravaginally (Crinone® 8%; Merck Serono, Switzerland), if pregnancy occurs. Twelve days after the transfer, a serum β-hCG was measured. At 7 and 9 weeks a pelvic ultrasound was performed to monitor early pregnancy. Clinical pregnancy was defined as fetal heart beat seen by transvaginal ultrasonography. Progesteron supplementation was continued until week 10 of pregnancy.

**Table I.** Comparison of the baseline characteristics of patients receiving different doses of rhCG.

| | 250 µg hCG group (n=39) | 500 µg hCG group (n=33) | p |
|---|---|---|---|
| **Age (years)** | 31.4±4.5 | 31.7±4.7 | 0.472 |
| **BMI (kg/m²)** | 33.5±2.8 | 33.3±3.3 | 0.375 |
| **Previous trial** | 1.9±1.5 | 2.1±1.5 | 0.777 |
| **Protocol (Long / Short)** | %57.4/%42.6 | %48.0/%52.0 | 0.444 |
| **Total oocytes** | 13.1±5.5 | 13.2±5.0 | 0.949 |
| **MII** | 9.6±4.1 | 10.0±4.1 | 0.574 |
| **MII / Total oocytes** | 0.75±0.16 | 0.7±0.15 | 0.730 |
| **2PN** | 7.7±4.0 | 7.4±3.6 | 0.860 |
| **ET day** | 4.1±1.0 | 4.3±0.8 | 0.405 |
| **ET** | 2.1±0.8 | 2.1±0.7 | 0.883 |
| **Induction day** | 8.9±1.4 | 9.5±1.5 | 0.115 |
| **Serum $E_2$ on hCG day** | 2035.9±759.5 | 1966.3±886.6 | 0.721 |
| **Daily dose of gonadotropin** | 313.2±109.9 | 323.8±147.3 | 0.955 |
| **Total dose of gonadotropin** | 2823.7±1267.9 | 3019.3±1242.5 | 0.501 |

*Note:* Values are means±SD. BMI, body mass index; MII, metaphase II; 2PN, fertilization with two pronuclei

**Table II.** Comparison of cycle parameters of patients receiving different doses of rhCG.

| | 250 µg hCG group (n=39) | 500 µg hCG group (n=33) | p |
|---|---|---|---|
| **Serum hCG on OPU day** | 84.2±50.0 | 134.2±69.6 | **<0.001** |
| **FF hCG on OPU day** | 25.7±25.5 | 39.6±45.9 | 0.248 |
| **FF/ serum hCG on OPU day** | 0.70±1.43 | 0.67 ±1.35 | 0.675 |
| **Pregnancy rate** | %64.1 | %54.5 | 0.410 |
| **Clinical pregnancy rate** | %56.4 | %48.5 | 0.502 |
| **Abortion rate** | %25.0 | %20.0 | 1.000 |
| **Implantation rate** | 0.31±0.37 | 0.26±0.37 | 0.423 |
| **OHSS** | 1(2.5%) | 1(3.0%) | |

*Note:* Values are means±SD or percentages. OPU, oocyte pick-up; FF, follicular fluid; OHSS, ovarian hyperstimulation syndrome.

Blood samples were taken from the antecubital vein just before the oocyte retrieval for determination of hCG concentration using an automated system. FF aspirates were collected from several follicles and pooled samples were stored at -20ºC. Thawed aspirates of each patient's pooled FF were centriufigated to remove blood and granulosa cells (GC). Flushing was intentionally not performed to the first samples obtained from the follicular liquid. To analyze serum and each patient's pooled FF concentrations of hCG, an automated chemiluminescence immunoassay (Cobas; Roche Diagnostics, Indianapolis, IN) was used. The lower detection level of hCG was <0.1 mIU/mL. The primary end points were the mean number of mature oocytes retrieved per patient and the secondary end points were the serum and intrafollicular hCG levels on oocyte retrievel day, the mean number of oocytes retrieved, fertilization and pregnancy rates (PR).

All statistical analyses were performed using the SPSS 15.0 statistical package (SPSS Inc., Chicago, IL). Descriptive statistics were given as mean, median, SD, minimum, maximum for numeric variables, and also were given as number and percent for categorical variables. The difference between two groups was analyzed with Student t-test when quantitative variable differences met the normal distribution and with Mann-Whitney U-test when the normal distribution could not be applied. Differences between groups in categorical variables were determined by Pearson's chi-square and Fisher's exact test. Statistical significance was established if $p \leq 0.05$.

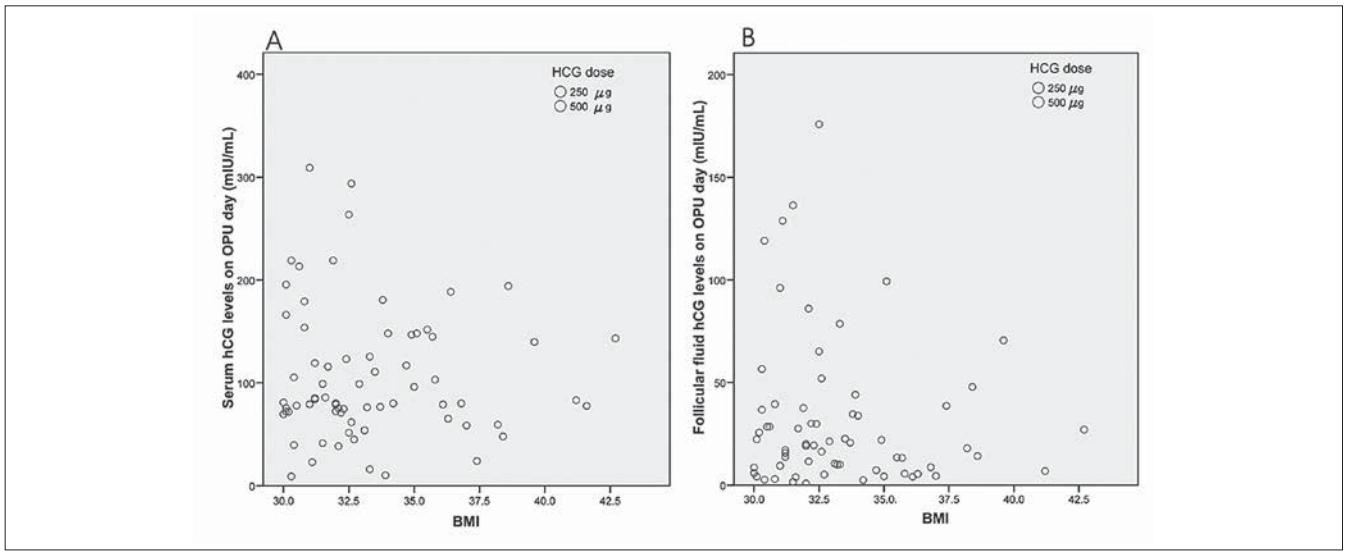


**Figure 1.** Distribution of serum and follicular fluid hCG levels on oocyte pickup day. BMI – body mass index.

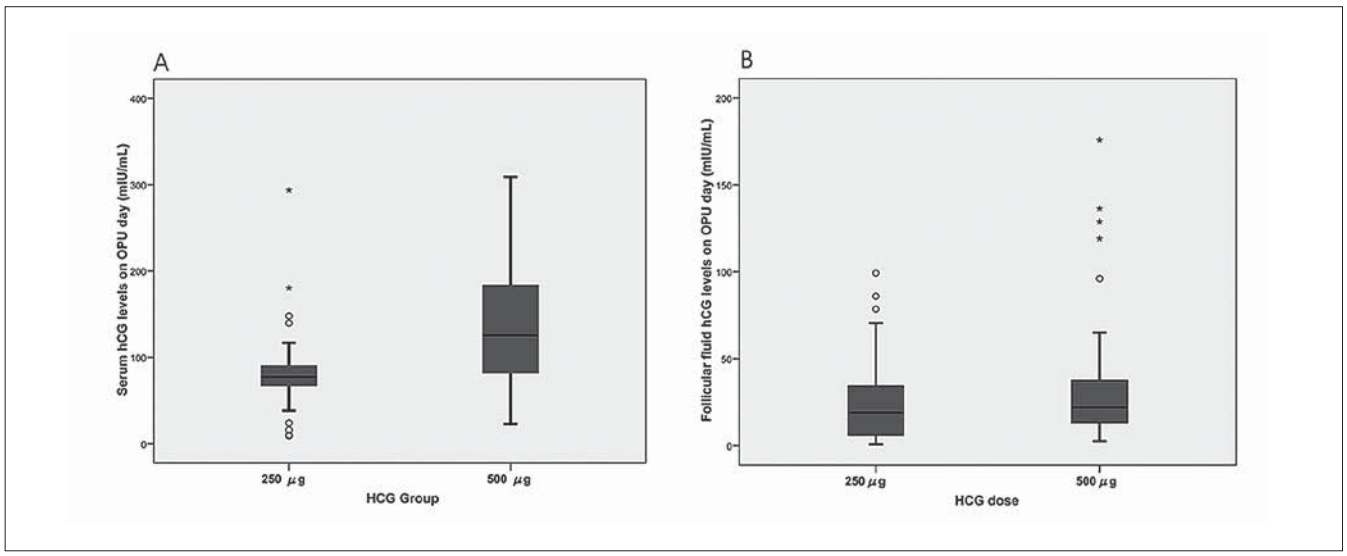


**Figure 2.** Serum and follicular fluid hCG levels on oocyte pickup day.

## Results

In the present study, 80 obese patients were included. The mean age was 31.6±4.6 years (min 22-max 46), mean BMI 33.4±3.0 (min 30-max 42.7). Patients were randomized in two groups and administered either 250 or 500 µg r-hCG. Eight patients with irregular follow-ups and uncomplete procedures were excluded from the study. As a result, 39 subjects were injected subcutanously with 250 µg of r-hCG and 33 cases received 500 µg of r-hCG subcutanously. Of the patients included in this study 53 have a BMI ranging between 30 and 34 and 19 have a BMI ≥35.

Baseline characteristics were comparable in the two groups (Table I). Thus, the two r-hCG groups were essentially identical with respect to age, BMI, number of previous trial, the type of protocols used. Also, the mean number of total and MII oocytes and their ratio, the mean number of fertilization with two pronuclei (2PN), and the mean number of embryos transferred were found not to be significantly different. Besides that, induction day, serum E2 on HCG day and the daily dose of gonadotropin were also not significantly different. Cycle parameters of the study groups are shown in Table II. Serum hCG levels were significantly lower in patients receiving 250 µg of r-hCG than in patients receiving 500 µg of r-hCG ($p<0.001$). The group administered 500 µg r-hCG had higher FF hCG levels, although both groups had comparable FF hCG levels on ovum pick-up day. The ratios of FF-to-serum hCG were identical in both groups. Distribution of serum and FF hCG levels on OPU day were shown in Fig.1 and Fig.2. The overall PR was 64.1% in the 250 µg r-hCG group and 54.5% in the 500 µg r-hCG group. There was no significant difference in the PRs ($p=0.410$). Clinical PRs were 56.4% in the 250 µg r-hCG group versus 48.5% in the 500 µg r-hCG group ($p=0.502$). Similarly, implantation rates and abortion rates were not

significantly different. The ratio of moderate and severe OHSS was not significant (2.5% vs 3.0% respectively).

When the pregnancy was achieved, the lowest and highest serum hCG levels (mIU/mL) were 15.98 and 293.70, respectively, in the 250 μg group, whereas 22.88 and 309.01, respectively, in the 500 μg group. On the other hand, the lowest and highest FF hCG levels were 7.3 and 99.26, respectively, in the 250 μg group, whereas 8.6 and 128.80, respectively, in the 500 μg group.

## Discussion

The optimum dose of r-hCG necessary for the final follicular maturation is commonly accepted as 250 μg [13]. In studies administering r-hCG or u-hCG, patients with an increased BMI (overweight and obese) were found to have decreased concentrations of serum hCG compared to the control group with a normal BMI, concluding that an increased gonadotropin dose was required to overcome follicular maturation [1].

In the present study assessing patients with a BMI ≥30, no difference in terms of obtained total or MII oocytes and cycle parameters was observed whether 250 μg or 500 μg of r-hCG was injected for the final maturation of follicles as previous reports. Although serum hCG levels were found to be markedly elevated in the 500 μg r-hCG group, there were not statistical significance in FF hCG levels. Cycle parameters, such as clinical pregnancy, implantation, were also found to be nonsignificant in both groups.

In a previous study of Kahraman et al. administration of two different doses of hCG (250 μg and 500 μg) were compared in a group of patients with BMI ≥26 and they reported that treatment with 250 μg r-hCG was as efficient as 500 μg [14]. Chan et al. compared two groups, administered 250 or 500 μg of r-hCG, and obtained similar results in both groups [15]. However, other studies stressed the diminished PR in obese patients undergoing assisted reproduction and the need of increasing hCG for IVF therapy [8, 9, 11, 12]. The bio-availabilityof hCG and BMI are reported to be negatively correlated and adiposity is recognized to possibly affect the intrafollicular microenvironment and thus oocyte maturation [16]. Detti et al. have administered three different doses of u-hCG and reported that (i) BMI may be used as a predictor for hCG concentration and that (ii) hCG dose titration should be carried out with respect to BMI [17]. Moreover, the negative correlation between BMI and serum hCG levels were reported as being independent from the administration procedure (intramuscularly or subcutaneously) [18]. However, other studies have been unable to find any negative impact of obesity on ART outcome. A common result of these studies is that obese patients require more gonadotropin [10-12]. Stefanis et al. measured serum hCG concentrations in patients undergoing IVF treatment after 12 or 36 hours of subcutaneous injection of 5000 IU hCG [19]. No correlation was found between serum hCG levels and the number of retrieved or fertilized oocytes. Moreover, no relation was established between BMI and hCG levels. In this study, we have administered r-hCG subcutaneously and found that serum hCG levels were higher in the 500 μg r-hCG group compared to the 250 μg r-hCG group (134.2±69.6 vs. 84.2±50.0, $p<0.001$). However, it did not affect the outcome for implantation and clinical PRs in the 250 μg r-hCG group and the 500 μg r-hCG group.

Ku et al. compared two groups with a BMI ≥24 and <24 in their study investigating the possible effects of BMI on IVF outcomes [20]. No differences were observed between both groups in peak E2 concentrations, endometrial thickness, number of retrieved oocytes and transferred embryos and cumulative embryo scores. They also reported that although a higher dose of gonadotropin was administered in the BMI ≥24 group, the implantation rate and clinical PR were higher in the BMI <24 group. Orvieto et al., compared obese (BMI ≥30) and non-obese (BMI <30) groups undergoing IVF [21]. Obese women required a longer stimulation and a higher dose of gonadotropin, nevertheless their PR was lower.

The limitation of our study is the lack of appropriate subject numbers. Assuming the difference of 5 % in clinical pregnancy rate and standard alpha and beta = 0.20 errors (to have 80% power) one would need more than 250 patients in each arm. Although the ultimate goal to obtain reliable results is to evaluate the sufficient number of patients given by the power analysis, finding patients with a high BMI matching the inclusion criteria of the study is a limiting factor. Thus only multicenter studies on large populations would have the sufficient power to answer this question

## Conclusions

Within the parameters of this prospective randomized study, we can conclude that 250 μg of r-hCG is sufficient and safe to trigger ovulation in women with BMI ≥30. No clinical or statistical advantage could be demonstrated for the higher dose of r-hCG in obese patients. Since low doses of r-hCG give similar results, additional doses will not be necessary, reducing the IVF therapy cost. However, increasing the number of obese patients enrolled in well-designed additional studies will augment the statistical power allowing us to draw a final conclusion.

**Conflict of interest**

We declare that we have no conflict of interest.

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