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*Original Article*

# Effects of a low hCG dose of 2000 IU on clinical outcomes of high-responder women undergoing IVF/ICSI

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**Abstract:** Purpose: Ovarian hyperstimulation syndrome (OHSS) is a major complication of ovarian hyperstimulation with exogenous gonadotropins. Human chorionic gonadotrophin (hCG) used for final oocyte maturation is believed to be responsible for triggering OHSS. To date, no consensus has been reached regarding the optimal dose of hCG for final oocyte maturation. This retrospective study was designed to determine whether the incidence of OHSS could be reduced without compromising the outcome of IVF/ICSI cycles by lowering the dose of hCG to 2000 IU. Methods: A total of 290 infertile high-responders undergoing their first IVF/ICSI treatment using the long protocol between January 2012 and May 2014 were divided into three groups according to the hCG dose (2000, 4000, and 10,000 IU) administered to trigger final oocyte maturation. The rates of oocyte recovery, oocyte maturation, fertilization, clinical pregnancy and the incidence of OHSS were compared between the groups. Results: The oocyte recovery rate increased obviously with increasing hCG dosage. The rates of oocyte maturation and clinical pregnancy rate were similar among the groups. The lowest fertilization rate was observed in the 10,000 IU hCG group. The incidence of OHSS (moderate and severe) was lower but not significantly in the 2000 IU hCG group (2.7%, 6.1% and 11.8% for the 2000, 4000 and 10,000 IU hCG groups, respectively; P=0.07). Conclusion: A dose of 2000 IU hCG may be effective for reducing the risk of OHSS without compromising the pregnancy rate in high-responder patients treated with the mid-luteal long protocol.

**Keywords:** In Vitro fertilization, human chorionic gonadotropin, oocyte recovery rate, embryo transfer, pregnancy rate, ovarian hyperstimulation syndrome

## Introduction

A recent report by the European Society of Human Reproduction and Embryology stated that ovarian hyperstimulation syndrome (OHSS) is still a major complication to ovarian hyperstimulation with exogenous gonadotropins [1]. Severe OHSS is a rare but potentially fatal condition associated with conventional *in vitro* fertilization (IVF) treatment, especially in high-responder patients who experience an exaggerated response to exogenous follicle-stimulating hormone (FSH).

Human chorionic gonadotrophin (hCG), a natural analogue of luteinizing hormone (LH), is used for final oocyte maturation in assisted conception cycles. Compared to LH, it has a longer circulating half-life (>24 h versus 60 min for LH), and as a result, it persists well after ovulation for up to 6 days [2]. Although hCG administration is an indispensable part of the IVF process, it is believed to be responsible for triggering OHSS in high-risk patients [3]. One strategy to decrease its occurrence is to lower the hCG dose administered.

To date, no consensus has been reached regarding the optimal dose of hCG for final oocyte maturation. Studies in animals have shown that the hCG dose required for oocyte maturation may be of less amplitude or duration than the one required for ovulation [4-6]. The lowest hCG dose reported for the purpose of lowering the OHSS in high-risk patients was 2500 IU [3, 7]. The dose of 2500 IU hCG was found to not adversely affect the probability of pregnancy but to effectively prevent OHSS; however, the numbers of patients in the two studies were small (n=23 and n=21). In addi-

tion, Kolibianakis reported decreases in the number of oocytes retrieved, the number of MII oocytes, and fertilization rates as well as in increased cancellation rate among patients who received 2500 IU hCG compared to 5000 or 10,000 IU hCG [7]. It remains unknown whether a further decrease in the dose of HCG (2000 IU) could further reduce the incidence of OHSS and whether a dose of 2000 IU hCG would impair the outcomes of IVF/ICSI cycles.

Although the use of GnRH agonist for final oocyte maturation rather than hCG resulted in a lower incidence of OHSS, it can only be used in a GnRH antagonist protocol and is associated with a lower rate of live births, a lower rate of ongoing pregnancy, and a higher rate of early miscarriage [8]. Even though intensifying luteal-phase support (LPS) resulted in improved pregnancy outcomes [9], for high-responder patients who have been using the GnRH analogue long protocol, lowering the hCG dose is still a valid approach to reduce the OHSS.

This retrospective study was designed to determine whether the incidence of OHSS could be reduced further without compromising the outcome of IVF/ICSI cycles by lowering the ovulatory dose of hCG to 2000 IU. For this study, high-responder women undergoing IVF/ICSI cycles were followed to evaluate the effects of three different doses of hCG (2000, 4000, and 10,000 IU) on the probability of ongoing pregnancy and in particular to determine the effect of the 2000 IU dose of hCG on ovum recovery, OHSS incidence, and IVF/ICSI outcome with the GnRH analogue long protocol.

## Materials and methods

### *Patient population*

This study was approved by the Institutional Review Board of Liuzhou Municipal Maternity and Child Healthcare Hospital, and for this retrospective study, formal consent was not required.

Two hundred ninety patients who underwent IVF/ICSI at the Centre for Reproductive Medicine of Liuzhou Municipal Maternity and Child Healthcare Hospital between January 2012 and May 2014 were included in the study. The inclusion criteria were: >20 retrieved oocytes at aspiration, age <40 years, presence of both ovaries, absence of a history of endometriosis and hydrosalpinx detected at ultrasound, FSH levels <10 IU/L, and estradiol (E2) <80 pg/mL on day 3 of the menstrual cycle. All of the patients were entered their first IVF/ICSI cycle without a history of previous IVF/ICSI treatment and all transferred with fresh embryos. In addition, we selected only patients whose oocytes were retrieved by the same skilled doctor.

### *Ovarian stimulation*

All of the patients underwent controlled ovarian hyperstimulation (COH) using a gonadotropin-releasing hormone (GnRH) agonist (triptorelin acetate; Ferring AG, Switzerland) starting on day 20 of the menstrual cycle before the oocyte retrieval cycle. Recombinant FSH (Gonal-F; Serono, Switzerland) was given continuously on menstrual cycle days 5-8 when quiescent ovaries were exhibited. The initial gonadotropin dose was 112.5-150 IU/d, tailored according to each patient's age and body mass index (BMI) and adjusted when necessary based on the patient's ovarian response. Purified urine hCG (Lizhu, China) was administered when at least two follicles 18 mm in diameter or larger were present on ultrasound and serum E2 levels reached 300 pg/ml per follicle of diameter >14 mm. The hCG doses given were 2000, 4000, and 10,000 IU, depending on each patient's E2 concentration on the day of hCG administration. The patients with E2 concentration ≤3000 pg/ml were given 10,000 IU hCG, whereby ≥6000 pg/ml were given 2000 IU hCG, others with 4000 IU hCG.

### *Oocyte retrieval and fertilization*

Transvaginal ultrasound-guided oocyte retrieval was conducted 36-37 hours after hCG administration. Fertilization was carried out *in vitro*, by either conventional insemination or ICSI, depending on semen parameters. Successful fertilization was defined when two clear pro-nuclei were present after 16-18 hours of insemination.

### *Embryo culture and transfer*

The fertilized oocytes were cultured with G1.5 and G2.5 sequential media (Vitrolife, Kungsbacka, Sweden) at 37°C with 6% $CO_2$ and 5% $O_2$. On the 3rd day of fertilization, embryos were graded according to the number and regularity

**Table 1.** Baseline characteristics and stimulation data in groups of patients who received 2000, 4000, or 10,000 IU hCG for triggering final oocyte maturation

| Group | 2000 IU hCG (n=74) | 4000 IU hCG (n=131) | 10,000 IU hCG (n=85) | P[a] |
|---|---|---|---|---|
| Baseline parameters | | | | |
| Age (years) | 30.6±3.8 | 30.3±3.3 | 30.3±3.5 | 0.75 |
| BMI (kg/m$^2$) | 20.7±2.3 | 20.7±2.5 | 21.3±3.2 | 0.36 |
| Duration of infertility (years) | 4.7±3.1 | 4.3±2.9 | 4.5±3.2 | 0.71 |
| Basal serum FSH (IU/L) | 6.4±1.5 | 6.3±1.4 | 6.8±1.5 | 0.08 |
| Basal serum LH (IU/L) | 4.3±1.8 | 4.4±2.6 | 4.1±1.9 | 0.72 |
| Basal serum E2 (pg/ml) | 42.7±13.9 | 40.5±16.3 | 38.1±13.9 | 0.05 |
| AFC | 13.8±3.2 | 15.0±5.0 | 14.8±4.9 | 0.09 |
| Duration of stimulation (days) | 11.2±1.3 | 11.0±1.5 | 11.1±1.6 | 0.05 |
| Total Gn consumption (tube) | 23.2±7.1 | 21.2±7.4 | 22.9±7.9 | 0.12 |
| Percentage of IVF/ICSI cycles | | | | |
| IVF (%) | 79.7 | 73.3 | 78.8 | 0.50[b] |
| ICSI (%) | 20.3 | 26.7 | 21.2 | |
| Hormone levels on the day of hCG administration | | | | |
| E2 (pg/ml) | 6920.4±1717.3[c] | 4042.4±858.2[d] | 2510.0±407.1[e] | 0.00 |
| Progesterone (ng/ml) | 1.3±0.5 | 1.2±0.5 | 1.2±2.3 | 0.67 |
| Follicles ≥14 mm | 12.0±4.1[c] | 12.3±3.4[c] | 7.7±2.1[d] | 0.00 |

Note: Values are expressed as mean ± SEM. BMI, body mass index, AFC, antral follicle count. [a]One-way analysis of variance. [b]Exact chi-square for trend. [c,d,e]Means with different superscripts within each row differ significantly (P<0.05).

of blastomeres and the degree of embryonic fragmentation from Cummins's criteria [10]. To prevent having no embryos to transfer for a patient, one or two high quality embryos (grades 1 and 2) were transferred fresh or frozen by vitrification on the 3rd day. The rest of the D3 embryos were continuously cultured to the blastocyst stage. On day 5 or 6, the quality of blastocysts was scored according to the Gardner blastocyst grading system. At this stage, the blastocysts with >3BB grade were considered high quality, whereas the blastocysts with >3CC grade were transferred or frozen.

*Luteal supplementation*

All patients received intramuscular injections of 60 mg progesterone (P) in oil for luteal phase support starting the day of oocyte retrieval and continued until 9-10 weeks of gestation.

*Statistical analysis*

Statistical analyses were performed using the statistical package for the social sciences (SPSS) software package (ver. 13.0; SPSS Inc., Chicago, IL, USA). Normality was tested using the Kolmogorov-Smirnov test. Differences in data between groups were determined using one-way analysis of variance and chi-squared tests or Fisher's exact tests as appropriate. For all analyses, P<0.05 was defined as statistically significant.

## Results

The baseline characteristics and stimulation data for patients in all three hCG dosage groups are shown in **Table 1**. No statistically significant differences were observed among the groups in terms of age, BMI, duration of infertility, antral follicle count (AFC), and baseline serum FSH, LH, and E2 levels. In addition, no statistically significant differences were observed among the groups in the duration of stimulation, the total dosage of gonadotropin administered, and the P levels on the day of hCG administration. However, the average E2 level in the 2,000 IU hCG group was notably greater that those in the other groups, and the number of follicles >14 mm in diameter in the 10,000 IU hCG group was significantly lower than those in the 2000 IU and 4000 IU hCG groups on the day of hCG administration (**Table 1**).

**Table 2.** Outcome of ovarian stimulation in groups of patients who received 2000, 4000, or 10,000 IU hCG for triggering final oocyte maturation

| Group | 2000 IU hCG (n=74) | 4000 IU hCG (n=131) | 10,000 IU hCG (n=85) | P[j] |
|---|---|---|---|---|
| COCs retrieved | 24.9±5.6[k] | 23.7±3.1[k,l] | 23.1±3.8[l] | 0.02 |
| Oocyte recovery rate (%)[a] | 82.1[k] | 85.6[l] | 91.1[m] | 0.00 |
| MII in ICSI cycles (%)[b] | 86.7 | 86.7 | 85.4 | 0.79 |
| Fertilization rate (%)[c] | 79.0[k] | 76.7[k] | 71.6[l] | 0.00 |
| D3 embryos available[d] | 15 (12, 18.25)[k] | 14 (11, 16)[l] | 13 (9, 17)[m] | 0.01 |
| No. of high quality embryos on D3[e] | 8.0 (4, 12.25) | 7 (4, 10) | 6 (3, 12) | 0.13 |
| Rate of high quality embryos on D3 (%)[f] | 53.45±28.23 | 51.15±23.83 | 53.29±27.05 | 0.77 |
| D3 transfer | | | | |
| Embryos transferred | 2.00±0.00 | 1.92±0.28 | 1.83±0.42 | 0.64 |
| Implantation rate (%)[g] | 45.0 | 44 | 39.3 | 0.76 |
| Clinical pregnancy rate (%)[h] | 50.0 | 53.8 | 59.4 | 0.91 |
| Spontaneous abortion rate (%)[i] | 0 | 0 | 3.9 | 0.71 |
| D5 transfer | | | | |
| Embryos transferred | 1.10±0.30 | 1.19±0.46 | 1.21±0.21 | 0.79 |
| Implantation rate (%) | 60.0 | 51.1 | 55.3 | 0.40 |
| Clinical pregnancy rate (%) | 65.9 | 60.0 | 57.6 | 0.60 |
| Spontaneous abortion rate (%) | 3.7 | 9.5 | 5.9 | 0.41 |
| Rate of OHSS (%, n/N) | | | | |
| Moderate | 2.7 (2/74) | 6.1 (8/131) | 9.4 (8/85) | 0.23 |
| Severe | 0 (0/74) | 0 (0/131) | 2.4 (2/85) | 0.15 |

Note: Values are expressed as mean ± SEM or rate. COCs, cumulus oocyte complexes; MII, metaphase 2. [a]Total number of oocytes recovered at follicle aspiration/total number of follicles aspirated from patients of each group. [b]Total number of oocytes at metaphase 2 stage when ICSI was performed/total number of oocytes recovered at follicle aspiration in ICSI cycles of each group. [c]Total number of oocytes fertilized/total number of oocytes inseminated in each group. [d]The number of embryos with a grade or better than 3 on day 3 after oocyte retrieval in each patient, median (interquartile range). [e]The number of embryos with a grade or better than 2 on day 3 after oocyte retrieval in each patient, median (interquartile range). [f]The number of embryos with a grade or better than 2 on day 3/the number of embryos with a grade or better than 3 on day 3 after oocyte retrieval in each patient. [g]The total number of gestational sacs present at 7 weeks of gestation/the total number of embryos transferred in each group. [h]The number of patients with ultrasound identification of an intrauterine gestational sac at 7 weeks gestation/the number of patients with embryo transferred in each group. [i]The number of patients who had spontaneous abortion before 28 weeks/the number of patients who had clinical pregnancy in each group. [j]Chi-square for trend or One-way analysis of variance. [k,l,m]Means with different superscripts within rows differ significantly (P<0.05).

The stimulation outcomes and clinical results for patients in the three groups are shown in **Table 2**. The oocyte recovery rate increased obviously with the increasing hCG doses (82.1%, 85.6% and 91.1% for the 2000, 4000 and 10,000 IU hCG groups, respectively; P<0.01), and the lowest fertilization rate was observed in the 10,000 IU hCG group. No statistically significant differences were observed in the proportion of metaphase 2 (MII) oocytes when ICSI was performed among the groups. The number of available embryos on D3 was notably highest in the 2000 IU hCG group, with no differences in the ratio of high quality embryos on D3 between the three groups. In addition, no significant differences were observed with respect to the rates of the implantation, clinical pregnancy, and spontaneous abortion for D3 embryos. Similarly, no significant differences in these outcomes were observed for D5 blastocyst transfer.

Severe OHSS only occurred in two patients (2/85) in the 10,000 IU group, and moderate OHSS occurred in two patients (2/74) in the 2000 IU group compared to eight patients (8/131) in the 4000 IU group and eight patients (8/85) in the 10,000 IU group. The incidence of OHSS (moderate and severe) was lower, but not significantly, in the 2000 IU hCG group (2.7%, 6.1%, and 11.8% for the 2000, 4000, and 10,000 IU hCG groups, respectively; P=0.07).

## Discussion

The results of the current study demonstrate that a decrease in the hCG dose used to trigger

final oocyte maturation to 2000 IU may be effective for reducing the risk of OHSS without compromising the clinical pregnancy rate in high-responder patients treated with the mid-luteal long protocol.

We did not observe a significant different in the OHSS incidence between the three hCG dosage groups, and this may be due to the low overall incidence of OHSS and/or simply to the small sample size in our study. Although the differences were not significant, despite the higher concentration of E2 and the greater number of follicles ≥14 mm on the day of hCG administration in the 2000 hCG group, the incidence of OHSS still appeared to be higher in the 4000 IU and 10000 IU hCG groups (2.7%, 6.1% and 11.8% for the 2000, 4000 and 10,000 IU hCG groups, respectively; P=0.07). Statistical confirmation of these findings in future larger studies may show that an hCG dose of 2000 IU can effectively reduce the incidence of OHSS.

Several previous studies have suggested that a lower hCG trigger dosage does not compromise clinical pregnancy rates [3, 7, 11, 12], and the lowest hCG trigger dosage tested in PCOS patients was 2500 IU [3, 7]. In agreement with these previous studies, in our study, we also found that the oocyte recovery rate decreased significantly as the dose of hCG decreased (82.1% to 85.6% to 91.1% with 2000, 4000, and 10,000 IU hCG, respectively, P<0.01). However, the ratios of MII oocytes were similar between the three groups, with a significantly decreased fertilization rate in the 10,000 IU hCG group. The number of embryos available on day 3 was notably higher in the 4000 IU group with a similar number and ratio of high quality embryos.

It remains unknown whether the LH (or hCG) dose that can induce ovulation is similar to that required for oocyte maturation as well as whether the same doses result in oocyte nuclei maturation and cytoplasm maturation. Animal studies have suggested that the dose required to induce oocyte maturation may be lesser in magnitude or duration than that required to induce ovulation [4, 6, 13]. Bomsel-Helmreich *et al*. [4] administered single hCG doses of 5-100 IU to 40 does in oestrus and found that low doses of hCG (5-10 IU) initiated resumption of oocyte meiosis, but did not induce ovulation. In contrast, they found that increasing hCG doses progressively induced nuclear maturation and simultaneously initiated ovulation of some follicles and then most follicles. In rats, Peluso *et al*. [5] also demonstrated that only a small percentage of the gonadotropin surge is required to induce maximal P secretion and oocyte maturation, and greater than 85% of the surge appears to be required to induce follicular rupture. These results suggest that the nuclear response, resumption of meiosis, occurs with the lowest doses of hCG used, and that luteinization, which progresses simultaneously with nuclear maturation, also does not require the same level of hCG necessary for follicular rupture [6]. In humans and primates, blunted LH surges in natural cycles or after follicular stimulation induce luteinized unruptured follicle syndrome, and ovum retention despite the presence of meiotic oocytes [14, 15].

These findings may explain why the percentage of mature oocytes when ICSI were performed was similar among the three dosage groups in our study. If the dose of hCG was sufficient to induce COC disassociation from preovulatory follicles, the oocytes in COCs are mostly matured. The previous findings outlined above may also explain why we observed no differences in the clinical pregnancy rate with different hCG doses, just as other previous studies reported [11, 12], because the dose of hCG required to induce a maximal P secretion that progresses simultaneously with luteinization of granulosa and thecal cells is similar to the dose needed to induce oocyte mature.

Importantly, the sensitivity of follicles to hCG also varies due to differences in LH receptor content. When the hCG level is insufficient to cause COC disassociation from all preovulatory follicles, a cohort of follicles with more LH receptor or that are more sensitive to hCG may be retrieved first. These oocytes may have higher quantity than those that cannot be retrieved at the same hCG level, which may explain why we observed a higher fertilization rate of retrieved oocytes in the 2000 and 4000 IU hCG groups compared to the 10,000 IU hCG group.

Although hCG has been available commercially for more than 50 years [16], the lowest effective dose for IVF has not been established. Only mural granulosa cells express the LH receptor (Lhcgr), which allows these cells to respond to the LH surge [17]. Granulosa cells may detect

the minimum levels of LH and hCG required for activation of the ovulation-luteinization program based on the intensity of the signal [18]. Thus, a low dose of hCG may reduce the magnitude of the increase in cAMP, which would result in the recovery of fewer oocytes as we found in our study that the oocyte recovery rate decreased significantly as the dose of hCG decreased. However, the trend in the oocyte recovery rate and the number of COCs retrieved were not prominent (82.1%, 85.6%, and 91.1% and 24.9±5.6, 23.7±3.1, and 23.1±3.8 in the three groups, respectively) or statistically significant.

In conclusion, a dose of 2000 IU hCG for triggering final oocyte maturation may be effective for reducing the risk of OHSS without compromising the clinical pregnancy rate in high-responder patients treated with the mid-luteal long protocol. However, this is a retrospective analysis and the E2 levels on the day of hCG administration in each group did not match. Thus, we need further research to identify the lowest effective hCG dose in high-responder patients using the GnRHa long protocol.

## Disclosure of conflict of interest

None.

## Authors' contribution

WHM: Project development, Manuscript Writing; LHW: Data Collection; NT and JHW: Data Analyzing.

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