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A Prospective, Randomized, Double-Blind Study to Compare Two Doses of Recombinant Human Chorionic Gonadotropin in Inducing Final Oocyte Maturity and the Hormonal Profile during the Luteal Phase

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Abbreviations:

E2
: Estradiol

ET
: embryo transfer

hCG
: human chorionic gonadotropin

ICSI
: intracytoplasmic sperm injection

IVF
: *in vitro* fertilization

MII
: metaphase II

OHSS
: ovarian hyperstimulation syndrome
P
: progesterone
r-
: recombinant
TUGOR
: transvaginal ultrasound-guided oocyte retrieval
u-
: urinary
VEGF
: vascular endothelial growth factor

Context: Different doses of human chorionic gonadotropin (hCG) have been used in various *in vitro* fertilization (IVF) treatment protocols to achieve final oocyte maturation. There is as yet no agreement on the optimum dose required.

Objectives: The objective of this study was to compare the effectiveness of 250 and 500 μg recombinant hCG (r-hCG), which represented the lower and upper limits of the dose range, in inducing final oocyte maturation during IVF and intracytoplasmic sperm injection cycles.

Design: This was a prospective, randomized, double-blind study.

Setting: This study was performed at an IVF clinic in a teaching hospital.

Patients: Sixty patients with an indication for intracytoplasmic sperm injection were studied.

Intervention: The treatment dose used was 250 or 500 μg r-hCG.

Main Outcome Measures: The percentage of metaphase II oocytes retrieved per patient, as an indicator of oocyte maturation, and the hormone profiles of the treatment cycle starting from the day of hCG up to hCG+10 d were the main outcome measures.

Results: The percentage of metaphase II oocytes was similar in the two groups (89.3% *vs.* 86.0%; *P* = 0.326) despite higher serum and follicular fluid hCG levels on hCG+2 and hCG+4 d, as was the follicular fluid to serum hCG ratio in the 500-μg r-hCG group. Serum estradiol and progesterone levels were comparable initially, but became significantly higher in the 500-μg r-hCG group on hCG+10 d.

Conclusion: The two doses of r-hCG were equally effective in inducing final oocyte maturation. It remains unclear whether the higher midluteal estradiol and progesterone levels in the 500-μg r-hCG group confer any benefit. (*J Clin Endocrinol Metab* 90: 3933–3938, 2005)

DURING *IN VITRO* fertilization (IVF) treatment, human chorionic gonadotropin (hCG) is usually used as a surrogate LH surge to induce final oocyte maturation. Different doses of hCG have been used in various IVF treatment protocols to achieve this purpose. There is as yet no agreement on the optimum dose required. The first study that addressed this question reported a significantly lower successful oocyte recovery rate in patients who

received 2,000 IU urinary hCG (u-hCG) compared with patients who received either 5,000 or 10,000 IU u-hCG [1] . A later study showed comparable percentages of mature metaphase II (MII) oocytes in nonobese patients after 5,000 or 10,000 IU u-hCG given either im or sc in 36 IVF cycles [2] . These observations indicate that im administration of 5,000 IU u-hCG probably exceeds the minimum threshold for nonobese patients. It has since become an acceptable practice to administer 5,000–10,000 IU u-hCG.

Despite the use of u-hCG, there are situations in which no oocyte can be retrieved even after administration of a high dose of u-hCG. Apart from the different individual thresholds of serum hCG required to stimulate final oocyte maturation, the other commonly cited reason to explain the empty follicle syndrome is the failure to provide biologically active hCG, due to manufacture, packing, or shelf life of the drug [3] . Recombinant hCG (r-hCG) offers remarkable batch to batch consistency, high purity, and high specific activity [4] [5] . A pharmacokinetic study of u-hCG and r-hCG showed that the mean serum profile after administration of 250 μg r-hCG was similar to that seen after the administration of 5000 IU u-hCG [6] . Clinical studies have confirmed that treatment with 250 μg r-hCG leads to equivalent ovulation rates as 5000 IU u-hCG in anovulatory women undergoing ovulation induction [7] . r-hCG at a dose of 250 μg has also been shown to be similar to 5000 IU u-hCG in the number of oocytes retrieved per patient in IVF cycles [8] [9] . When a higher dose of r-hCG (500 μg) was compared with 250 μg r-hCG, the mean number of oocytes retrieved was the same, but the numbers of two pronuclear fertilized oocytes and cleaved embryos were significantly higher with 500 μg r-hCG [10] . The luteal phase serum progesterone (P) and hCG concentrations were also higher in patients receiving the higher dose of r-hCG [10] .

The primary outcome measure in these three studies [7] [8]

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[10] was the number of oocytes retrieved per patient as a function of follicular maturation. However, not all retrieved oocytes were mature. The proportion of MII oocytes may better reflect the effect of hCG on follicular maturation, because this indicates the resumption of meiosis. In this study we compared the effects of 250 and 500 μg r-hCG, which represented the lower and upper limits of the dose range, on the percentage of MII oocytes retrieved per patient during IVF and intracytoplasmic sperm injection (ICSI)/embryo transfer (ET) cycles as the primary outcome measure, with the hypothesis that the effects of the two different doses of r-hCG were similar. As a second objective, the hormone profiles of the treatment cycle starting from the day of hCG up to hCG+10 d were compared.

Patients and Methods

Patient recruitment

Infertile patients between 18–40 yr of age who were attending the Assisted Reproduction Unit at Department of Obstetrics and Gynecology, University of Hong Kong, for ICSI/ET treatment between August 2003 and March 2004 were recruited on the day of hCG administration. Inclusion criteria were 1) body mass index less than 28 kg/m^2 , 2) early follicular phase serum FSH level of 10 IU/liter or less, and 3) ICSI indicated because of severe semen abnormalities (<100,000 motile spermatozoa recovered after sperm preparation) or surgically retrieved spermatozoa from epididymis or testis in cases of

azoospermia. Patients were excluded if they had 1) a previous history of excessive or poor ovarian responses; 2) an excessive ovarian response, as defined by the presence of more than 15 follicles with an average diameter of 16 mm or greater; 3) a poor ovarian response, as defined by the presence of less than three follicles with an average diameter of 16 mm or greater; and 4) an indication for ICSI due to fertilization failure or less than 30% fertilization rate in previous IVF cycles. All patients gave written informed consent before participating in the study, which was approved by the joint institutional review board of University of Hong Kong and the Hospital Authority. Each patient was recruited to join the study only once.

IVF protocol

The details of the long protocol of ovarian stimulation regimen, gamete handling, ICSI, and assessment of oocyte/embryo quality at our center have been previously published [11] [12] . In brief, pituitary down-regulation was achieved with a short-acting GnRH analog (Suprecur, Hoechst, Frankfurt, Germany) starting from the luteal phase of the cycle preceding the treatment cycle. Ovarian stimulation was started in the early follicular phase if pituitary suppression was complete, as assessed by a basal serum estradiol (E2) level less than 220 pmol/liter and the absence of ovarian cysts on ultrasound scan. Human menopausal gonadotropin (Pergonal, Serono, Geneva, Switzerland) was given by im injection (300 IU daily for the first 2 d, followed by 150 IU daily). The ovarian response was monitored by ultrasound scan. r-hCG (Ovidrel, Serono, Bari, Italy) was given if the leading follicle was 18 mm in diameter or larger and there were at least three follicles more than 15 mm in diameter.

Patients were randomly allocated into either the 250 or 500 μg r-hCG group according to a randomization table, with the allocation group presealed in opaque envelops by a research nurse. The sequence of allocation was concealed until interventions were assigned by the research nurse after the patients were recruited by an attending doctor.

The drug was administered by a duty nurse who was not involved in the study. The clinicians and laboratory staff involved as well as the patient herself were blinded as to the dosage of r-hCG used. Transvaginal ultrasound-guided oocyte retrieval (TUGOR) was scheduled 36 h after the r-hCG injection. The local reaction to the injection sites was assessed on the day of TUGOR. Any local reaction, including itchiness, redness, swelling, bruising, and pain, was recorded, and severity was assessed as mild, moderate, or severe by the patients.

TUGOR was performed using a 16-gauge, double-channel needle (Cook IVF, Cook, Australia) under ultrasound guidance with a 5-MHz vaginal probe fitted with a needle guide. The double-channel needle allowed aspiration and flushing of follicles that were larger than 10 mm on both sides. Follicular fluid was collected from the first mature follicle aspirated on both sides using medium-free collection tubes. Each follicle was flushed once with culture medium, and the fluid from aspiration and flushing was examined by the embryologist (W.S.B.Y. or E.Y.L.L.). Denuded oocytes were cultured in G-1 version 3 medium for 2 h. MII oocytes were identified by the presence of a polar body. ICSI was performed on MII oocytes only. The injected oocyte was additionally cultured in a 15-μl droplet of G-1 version 3 medium under mineral oil in a CO_2 incubator. Fertilization was checked 14–18 h after the injection procedure. An oocyte was considered to be normally fertilized when two pronuclei were visible. When no pronucleus or only one pronucleus was visible, the oocyte was cultured for another 3–4 h and examined again. Normally cleaving embryos were replaced in the uterine cavity 48 h after the retrieval. Immediately before ET, embryos were examined for the number and regularity of blastomeres, and the degree of fragmentation.

A maximum of two normally cleaved embryos were replaced into the uterine cavity 48 h

after the retrieval. Excess good quality embryos were frozen for subsequent transfer if the woman was not pregnant in that cycle. All fresh embryos were cryopreserved if serum E2 on the day of r-hCG injection exceeded 20,000 pmol/liter to reduce the risks of ovarian hyperstimulation syndrome (OHSS), which was graded into mild, moderate, and severe degrees [13] . The luteal phase was supported by P vaginal pessaries (Cyclogest, Cox Pharmaceuticals, Barnstaple, UK; 400 mg twice daily for 14 d starting from the day of ET). Blood was taken for serum E2, P, hCG, and vascular endothelial growth factor (VEGF) assays on the days of hCG, TUGOR (hCG+2 d), and ET (hCG+4 d) and 6 d after ET (hCG+10 d). A urinary pregnancy test was performed 20 d after administration of the ovulatory dose of r-hCG.

Only clinical pregnancies were considered and were defined by the presence of one or more gestational sacs or the histological confirmation of gestational product in miscarriages. Ongoing pregnancies were those pregnancies beyond 10–12 wk of gestation, at which stage the patients were referred out for antenatal care.

Serum E2, P, and hCG concentrations were measured using commercially available kits (Automated Chemiluminescence ACS-180 System, Bayer Corp., Tarrytown, NY). The sensitivity of the E2 assay was 10.0 pg/ml, and the intra- and interassay coefficients of variation were 8.1% and 8.7%, respectively. The sensitivity of the P assay was 0.11 ng/ml, and the intra- and interassay coefficients of variation for P were 5.0% and 7.8%, respectively. The sensitivity of the hCG assay was 2 IU/liter, and the intra- and interassay coefficients of variation for hCG were 1.8% and 4.9%, respectively. Serum VEGF was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine, R&D Systems, Inc., Oxon, UK), which was designed to measure $VEGF_{165}$ levels. The minimum detectable VEGF concentration by the assay was 9.0 pg/ml. The interassay coefficients of variation were 8.8%, 7.0%, and 6.2% at concentrations of 65, 250, and 1,003 pg/ml, respectively, whereas the intraassay coefficients of variation were 6.7%, 4.5%, and 5.1% at concentrations of 54, 235, and 910 pg/ml, respectively.

Statistical analysis

The primary outcome measure was the percentage of MII oocytes per patient. From our own statistics, the mean percentage of MII oocytes from 40 patients treated with IVF and ICSI was 87.0% with an SD of 13.8% [12] . It was assumed that 250 μg r-hCG was as effective as 500 μg r-hCG in inducing oocyte maturity if the percentage of MII oocytes per patient fell within 1 SD of the original proportion (*i.e.* 13.8%) when the patients were treated with 500 μg r-hCG. To fulfill such an assumption, the number of subjects required in each group was 24 to give a power of 90% at the 5% significance level. Hence, 30 patients were recruited for each group to allow dropout. The serum and follicular fluid hCG levels and their ratio; the serum E2, P, hCG, and VEGF levels on various days after r-hCG administration; outcomes of the IVF cycle; and side effects were secondary outcomes measures.

Continuous variables were expressed as the mean ± SD and were compared between the two groups using Student's *t* test. Categorical variables were compared using χ^2 test or Fisher's exact test where appropriate. Differences were considered significant at $P < 0.05$.

Results

Sixty patients with a mean age of 34.6 yr (range, 26–39 yr) were recruited. All patients received r-hCG injections and underwent TUGOR. The average duration of infertility was 5.6 yr, with a range of 1–14 yr. Forty-six (76%) patients had primary infertility, and 14 (23%) had secondary infertility. The characteristics of the patients at baseline and during IVF treatment are summarized in Table 1 . There were no differences between the two groups in terms of these prerandomization characteristics. No differences were observed in the number of follicles aspirated, the number of MII oocytes, and the percentage of MII oocytes to oocytes retrieved per patient between the two groups (Table 1).

Seven patients did not have ET because of threatened OHSS in five, no sperm from testicular biopsy in one, and failed fertilization in one. The overall clinical pregnancy rate was 20.0%/TUGOR cycle and 22.6%/ET cycle. There was no difference in the implantation and pregnancy rates between the two groups (Table 2).

Hormonal profiles from the day of ovulatory dose of r-hCG

Graphical representation of the changes in the serum E2, P, hCG, and VEGF levels over the treatment cycle is shown in Fig. 1 . Serum E2 and P levels were comparable on hCG, hCG+2, and hCG+4 d, but the levels became significantly higher in the group with 500 μg r-hCG on hCG+10 d ($P = 0.039$ and $P < 0.001$, respectively). In the 500 μg r-hCG group, the serum and follicular fluid hCG levels on hCG+2 d as well as the follicular fluid to serum hCG ratio were significantly higher (Table 1). The serum hCG level remained higher in the 500 μg group on hCG+4 d. The difference disappeared on hCG+10 d, when the serum hCG level approached zero. The serum VEGF levels showed a consistent rise starting from the hCG day, but the levels were similar in the two groups. It appeared that the serum VEGF level on hCG+10 d was

TABLE 1 -- Patient characteristics at baseline and during the IVF cycle

	250 μg r-hCG	**500 μg r-hCG**	***P***
Age (yr)	33.9 ± 4.1	34.6 ± 3.4	0.475
BMI (kg/m^2)	21.4 ± 2.6	22.1 ± 2.7	0.301
Type of infertility			0.222 [a]
Primary	25 (83)	21 (70)	
Secondary	5 (17)	9 (30)	
Duration of infertility (yr)	5.7 ± 3.0	5.3 ± 2.9	0.547
Duration of stimulation (d)	11.0 ± 1.8	11.5 ± 2.2	0.282
Total dosage of hMG (IU)	1958 ± 0.432	2108 ± 586	0.263
No. of oocytes retrieved	12.1 ± 5.6	10.9 ± 5.0	0.385
No. of MII	10.8 ± 5.2	9.6 ± 4.9	0.355
Percentage of MII/oocytes retrieved per patient	89.3 ± 10.3	86.0 ± 14.4	0.326
No. of oocytes fertilized (%)	7.9 ± 4.9 (73)	7.7 ± 4.3 (80)	0.867
No. of embryos cleaved (%)	7.6 ± 4.9 (96)	7.6 ± 4.2 (99)	1.000
No. of embryos transferred	1.6 ± 0.8	1.7 ± 0.6	0.367
No. of embryos frozen	4.1 ± 3.8	4.7 ± 4.0	0.533
No. of patients with no ET	4	3	1.000 [b]
Serum hCG (IU/liter)	100.1 ± 25.6	197.0 ± 52.9	<0.001
Follicular fluid hCG (IU/liter)	39.2 ± 21.0	95.4 ± 44.8	<0.001

Follicular fluid/Serum hCG ratio	0.38 ± 0.16	0.46 ± 0.14	0.029

Data are expressed as mean ± SD, except for type of infertility which was expressed as number (%). Comparisons between the two groups were performed using Student's *t* test unless otherwise specified. hMG, Human menopausal gonadotropin.

[a] χ^2 .

[b] Fisher's exact test.

TABLE 2 -- Treatment outcome

	250 µg r-hCG [no. (%)]	500 µg r-hCG [no. (%)]	*P*
Pregnancy outcome			
Ectopic pregnancy	1	0	0.491 [a]
Ongoing pregnancy	6	6	1.000 [b]
Implantation rate, ongoing pregnancy rate	7/45 (15.6)	7/52 (13.5)	0.998 [b]
Per TUGOR cycle	6/30 (20)	6/30 (20)	1.000 [b]
Per ET cycle	6/26 (23)	6/22 (26)	0.684 [b]

[a] By Fisher's exact test.

[b] By χ^2 test.

slightly higher in the 500 µg group, but this was not statistically significant (by Student's *t* test, *P* = 0.536).

The mean serum hCG levels on hCG day were 146.2 and 149.3 IU/liter for the pregnant and nonpregnant groups, respectively. The corresponding figures for follicular fluid hCG levels were 75.8 and 64.4 IU/liter. The mean follicular fluid/serum hCG ratio was also similar for the nonpregnant (0.48) and pregnant (0.40) groups. There was no cutoff for serum hCG, follicular hCG, or their ratio to achieve pregnancy. The lowest serum and follicular fluid hCG levels and their ratio associated with pregnancy were 62 IU/liter, 17 IU/liter, and 0.27, respectively. All these values were from the same patient in the 500 µg group. Six of the 14 pregnant patients had follicular fluid hCG levels less than 50 IU/liter.

Side effects

The side effects of the administration of r-hCG are shown in Table 3 . Six patients (10%) had OHSS; in five, the OHSS was mild. One patient required hospital admission for fluid replacement. No other intervention was necessary to treat the OHSS. Local reactions at the injection site of r-hCG were uncommon, except for pain. Among the 43 patients (72%)

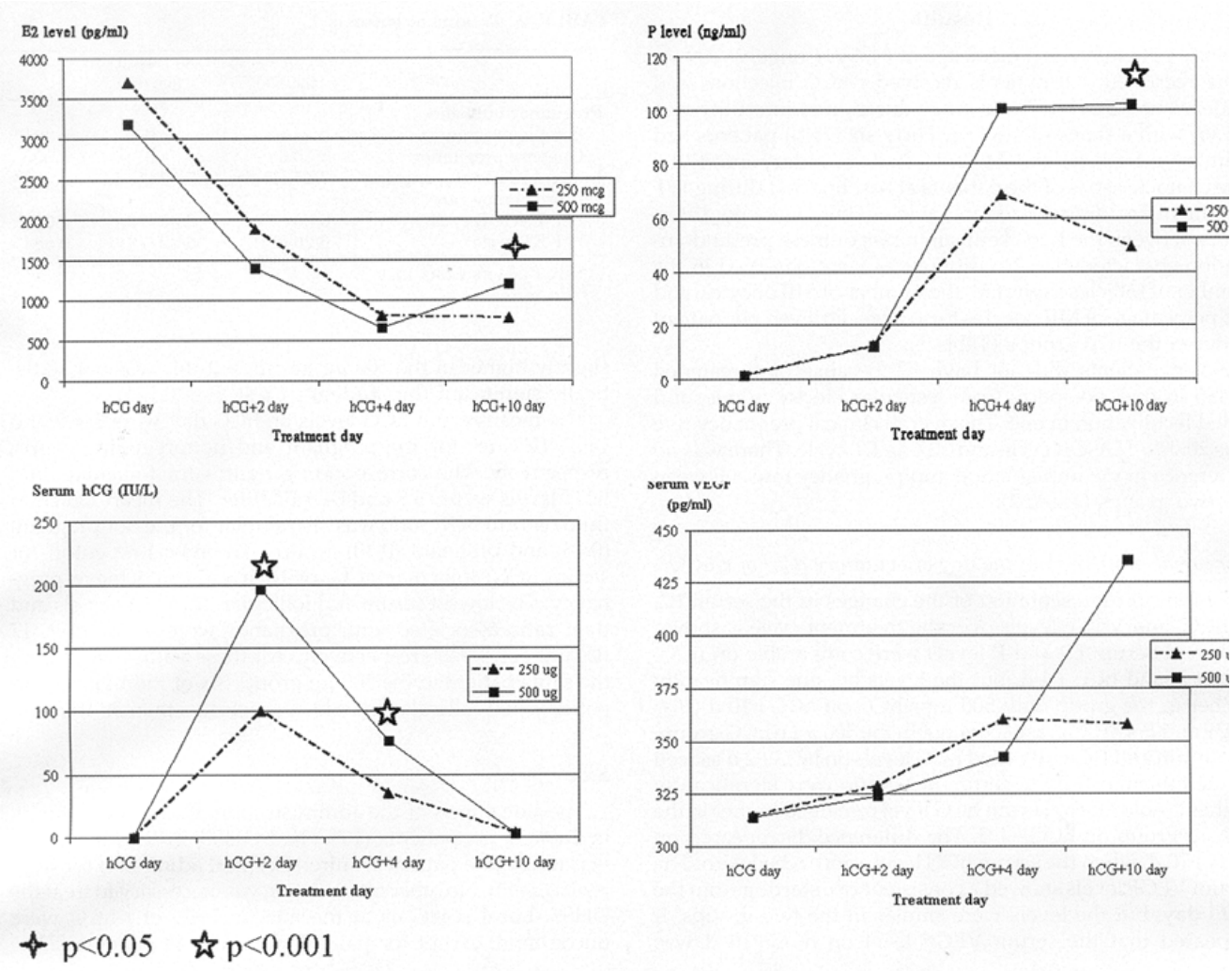

Figure 1. Serum E2, P, hCG, and VEGF levels on various treatment days.

who complained of pain from the injection site, 11 (18%) rated it as severe. However, the complaint was equally divided between the two groups.

TABLE 3 -- Side effects

	250 µg r-hCG [no. (%)]	**500 µg r-hCG [no. (%)]**	***P* value**
OHSS	4 (13)	2 (7)	0.671 [a]
Mild	3 (10)	2 (7)	
Moderate	1 (3)	0 (0)	
Local reactions at the injection site of hCG			
Itching	4 (13)	0 (0)	0.112 [a]
Redness	3 (10)	3 (10)	1.000 [a]
Swelling	1 (3)	4 (13)	0.353 [a]
Bruising	0 (0)	2 (7)	0.492 [a]
Pain at the injection site of hCG	22 (73)	21 (70)	0.774 [b]
Mild	12 (40)	8 (27)	
Moderate	5 (17)	7 (23)	
Severe	5 (17)	6 (20)	

[a] By Fisher's exact test.
[b] By χ^2 test.

Discussion

The midcycle LH surge promotes several periovulatory events, including disruption of the oocyte-cumulus oophorus cell contact and induction of follicular rupture, resumption of the oocyte's meiotic division [14] , cumulus oophorus mucinification, luteinization of the follicular granulose cells [15] , and, subsequently, secretion of P [16] . This chain of events can also be induced by hCG due to the similarity between the two glycoproteins, allowing hCG to bind to the same receptor as LH [17] . Three prospective, randomized studies have been conducted using r-hCG in IVF/ET cycles, comparing 250 μg r-hCG with 5,000 IU u-hCG [8] [9] or two different doses of r-hCG (250 and 500 μg) with 10,000 IU u-hCG [10] . The primary end point of all three studies was the number of oocytes retrieved per patient. Although this outcome parameter represents disruption of the oocytecumulus oophorus cell contact and hence the ease of aspiration, it is subjective to the experience of the surgeons to a certain degree. The percentage of MII oocytes is an indicator of resumption of meiosis and final maturation of the oocyte

and can be objectively assessed after the oocytes have been denuded before ICSI. We therefore chose to study this parameter as the primary end point in our study. There was no difference in the percentage of MII oocytes retrieved between the two groups, demonstrating equivalence in the two doses of r-hCG in inducing final oocyte maturation.

The serum and follicular fluid hCG levels were significantly higher in the group who received a higher dose of r-hCG. The follicular fluid to serum hCG ratio was also significantly higher in the latter group. These observations, however, did not translate into a higher number of oocytes retrieved, percentage of MII oocytes, or other cycle characteristics, including pregnancy outcome, although the sample size was too small to detect a small difference in the pregnancy rate. Oocyte maturation can be achieved with hCG doses of lesser amplitude than those required for follicular rupture [18] [19] [20] . In fact, a low serum hCG level before oocyte recovery (<100 IU/liter) was equally potent as the higher levels at initiating maximal oocyte maturity and achieving a similar percentage of MII oocytes recovered [21] . The lowest serum hCG level on the day of hCG administration was 37 IU/liter in our study. The percentage of MII in this particular patient was 86%. Although the critical blood level of hCG capable of producing a maturational effect on the oocyte could not be determined, this level seemed adequate.

A previous study has shown that the ratio of the level of hCG in follicular fluid to that in serum was a good marker for pregnancy [22] . It was suggested that a follicular fluid level of 62 IU/liter was the lowest level of hCG associated with pregnancy, and the ratio of the follicular fluid to serum hCG level must be at least 0.46 for pregnancy to occur. A later study did not find a significant difference in the follicular fluid to serum hCG levels for pregnant and nonpregnant patients, and the lowest ratio for pregnancy was 0.54 [23] . Our study possessed the same limitation as the study by Stelling *et al.* [23] , in that the sample size did not have adequate power to distinguish a small difference in pregnancy rate. However, we have

shown that a ratio as low as 0.27 could achieve pregnancy.

The serum E2 and P levels were significantly higher in the group with a higher dose of r-hCG on hCG+10 d, corresponding to the midluteal phase and, hence, an indirect evidence of corpus luteal function. A higher P level with 500 μg r-hCG has also been described in an earlier study [10] . Whether such a difference in the serum P level confers a more favorable luteal environment for implantation is unclear. When spontaneous cycles of normal fertile women were studied, significantly higher E2 concentrations were shown in serum [24] , saliva [25] , and urine [26] during the midluteal phase of pregnant cycles. Stewart *et al.* [24] suggested that the enhanced ovarian steroid secretion in pregnant cycles could be due to a gonadotropic stimulus such as hCG from the preimplantation embryo. In contrast to those in fertile subjects, midluteal E2 and P concentrations during spontaneous cycles were similar in pregnant and nonpregnant cycles of infertile patients [27] . The explanation for this difference between fertile and infertile women is unknown. There is little recent information in humans with regard to the importance of luteal E2 during stimulated cycles in patients undergoing IVF-ET. Luteal phase serum E2 and P concentrations were not related to the outcome of IVF treatment [28] [29] . In contrast, Gidley-Baird *et al.* [30] and Hutchinson-Williams *et al.* [31] found significantly higher E2 levels during the midluteal period in pregnant than in nonpregnant cycles. Different luteal supports, including no support, hCG, and P, were employed, and this may explain the different results observed in these studies. The comparable pregnancy rate found in our study did not preclude the benefit of higher E2 or P, because the study was not powered to detect this difference. The E2 level was also significantly higher in the 500 μg r-hCG group. Interestingly, the incidence of OHSS was higher in the 250 μg group, although the difference was not statistically significant. This paradoxical observation might be explained by the fact that patients at risk for OHSS were excluded from the study, and the number of patients with OHSS was small.

VEGF expression has been localized to the corpus luteum in humans [32] [33] . Because VEGF has a potent angiogenic property, its presence in the corpus luteum supports its role as a regulator of angiogenesis in this tissue. We observed a continuous rising concentration in serum VEGF in the luteal phase, consistent with previous studies [34] [35] . This increase is important in inducing adequate angiogenesis and development of the corpus luteum. In a small study, serum VEGF levels on d 11–14 after ET in patients who conceived after IVF were found to be higher than in those who did not [36] . This could suggest an augmentation of ovarian VEGF production by hCG around the onset of intrauterine gestation, not necessarily better corpus luteal function. We could not show a difference in serum VEGF levels between the two study groups. Several studies also suggested that VEGF may play an important role in the development of OHSS, because of its vascular permeability effect [34] [36] [37] [38] [39] [40] . We failed to demonstrate a difference in serum VEGF levels throughout the treatment cycle between patients who developed OHSS and those who did not (data not shown), probably because our patients were not at high risk of developing this complication, and the number of those who developed OHSS was too small.

In conclusion, we have shown that 250 μg r-hCG was as effective as 500 μg r-hCG in inducing final oocyte maturation. The serum and follicular fluid hCG levels and their ratio were higher in the group receiving 500 μg r-hCG. However, very low levels of serum and follicular fluid hCG were associated with pregnancy. The other differences were the midluteal phase serum E2 and P level in favor of the 500 μg r-hCG group. Whether this results in a better luteal environment for implantation needs additional study.

References

1. **Abdalla HI, Ah-Moye M, Brinsden P, Howe DL, Okonofua F, Craft I** 1987 The effect of the dose of human

chorionic gonadotropin and the type of

gonadotropin stimulation on oocyte recovery rates in an in vitro fertilization program. Fertil Steril 48:958–963 Abstract

2. **Wikland M, Borg J, Forsberg AS, Jakobsson AH, Svalander P, Waldenstrom U** 1995 Human chorionic gonadotrophin self-administered by the subcutaneous route to induce oocyte maturation in an in-vitro fertilization and embryo transfer programme. Hum Reprod 10:1667–1670 Abstract

3. **Zegers-Hochschild F, Fernandez E, Mackenna A, Fabres C, Altieri E, Lopez T** 1995 The empty follicle syndrome: a pharmaceutical industry syndrome. Hum Reprod 10:2262–2265 Abstract

4. **Fonjallaz P, Loumaye E** 2001 Recombinant hCG (OVIDREL) and recombinant interferon-β1a (REBIF) (no. 13 in a series of articles to promote a better understanding of the use of genetic engineering). J Biotechnol 87:279–281 Citation

5. **Hugues JN** 2001 Recombinant human follicle-stimulating hormone: a scientific step to clinical improvement. Reprod BioMed Online 2:54–64

6. **Trinchard-Lugan I, Khan A, Porchet HC, Munafo A** 2002 Pharmacokinetics and pharmacodynamics of recombinant human chorionic gonadotrophin in healthy male and female volunteers. Reprod BioMed Online 4:106–115

7. **International Recombinant Human Chorionic Gonadotropin Study Group** 2001 Induction of ovulation in World Health Organization group II anovulatory women undergoing follicular stimulation with recombinant human follicle-stimulating hormone: a comparison of recombinant human chorionic gonadotropin (rhCG) and urinary hCG. Fertil Steril 75:1111–1118 Abstract

8. **European Recombinant Human Chorionic Gonadotrophin Study Group** 2000 Induction of final follicular maturation and early luteinization in women undergoing ovulation induction for assisted reproduction treatment: recombinant HCG versus urinary HCG. Hum Reprod 15:1446–1551 Abstract

9. **Driscoll GL, Tyler JPP, Hangan JT, Fisher PR, Birdsall MA, Knight DC** 2000 A prospective, randomized, controlled, double-blind, double-dummy comparison of recombinant and urinary HCG for inducing oocyte maturation and follicular luteinization in ovarian stimulation. Hum Reprod 15:1305–1310 Abstract

10. **Chang P, Kenley S, Burns T, Denton G, Currie K, DeVane G, O'Dea L, U.S. Multicenter Study 7929 Investigator Group** 2001 Recombinant human chorionic gonadotropin (rhCG) in assisted reproductive technology: results 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 76:67–74 Abstract

11. **Ng EHY, Yeung WSB, Lau EYL, So WWK, Ho PC** 2000 High serum oestradiol levels in fresh IVF cycles do not impair implantation and pregnancy rates in subsequent FET cycles. Hum Reprod 15:250–255

12. **Ng EHY, Lau EYL, Yeung WSB, Ho PC** 2001 Human menopausal gonadotrophin is as good as recombinant human follicle stimulating hormone in terms of oocyte and embryo quality: a prospective randomised trial. Hum Reprod 16:319–325 Abstract

13. 1995 Guideline no. 5: management and prevention of ovarian hyperstimulation syndrome (OHSS). London: Royal College of Obstetricians and Gynaecologists

14. **Lawrence TS, Dekel N, Beers WH** 1980 Binding of human chorionic gonadotropin by rat cumuli oophori and granulosa cells: a comparative study. Endocrinology 106:1114–1118 Abstract

15. **Brailly S, Gougeon A, Milgrom E, Bomsel-Helmreich O, Papiernik E** 1981 Androgens and progestins in the human ovarian follicle: differences in the evolution of preovulatory, healthy nonovulatory, and atretic

follicles. J Clin Endocrinol Metab 53:128–134 Citation

16. **Hoff JD, Quigley ME, Yen SS** 1983 Hormonal dynamics at midcycle: a reevaluation. J Clin Endocrinol Metab 57:792–796 Abstract

17. **Pierce JG, Parsons TF** 1981 Glycoprotein hormones: structure and function. Annu Rev Biochem 50:465–495 Citation

18. **Bomsel-Helmreich O, Vu N Huyen L, Durand-Gasselin I** 1989 Effects of varying doses of HCG on the evolution of preovulatory rabbit follicles and oocytes. Hum Reprod 4:636–642 Abstract

19. **Peluso JJ** 1990 Role of the amplitude of the gonadotropin surge in the rat. Fertil Steril 53:150–154 Abstract

20. **Ishikawa J** 1992 Luteinizing hormone requirements for ovulation in the rat. Biol Reprod 46:1144–1150 Abstract

21. **al-Hassan S, Fishel S, Fleming S, Thornton S** 1998 Low plasma levels of hCG after 10,000-IU hCG injection do not reduce the number or maturation of oocytes recovered in patients undergoing assisted reproduction. J Assist Reprod Genet 15:583–586 Abstract

22. **Nagata Y, Honjou K, Sonoda M, Sumii Y, Inoue Y, Kawarabayashi T** 1999 Pharmacokinetics of exogenous gonadotropin and ovarian response in in vitro fertilization. Fertil Steril 72:235–239 Abstract

23. **Stelling JR, Chapman ET, Frankfurter D, Harris DH, Oskowitz SP, Reindollar RH** 2003 Subcutaneous versus intramuscular administration of human chorionic gonadotropin during an in vitro fertilization cycle. Fertil Steril 79: 881–885 Abstract

24. **Stewart DR, Overstreet JW, Nakajima ST, Lasley BL** 1993 Enhanced ovarian steroid secretion before implantation in early human pregnancy. J Clin Endocrinol Metab 76:1470–1476 Abstract

25. **Lipson SF, Ellison PT** 1996 Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. Hum Reprod 11:2090–2096 Abstract

26. **Baird DD, Wilcox AJ, Weinberg CR, Kamel F, McConnaughey DR, Musey PI, Collins DC** 1997 Preimplantation hormonal differences between the conception and non-conception menstrual cycles of 32 normal women. Hum Reprod 12:2607–2613 Abstract

27. **Laufer N, Navot D, Schenker J** 1982 The pattern of luteal phase plasma progesterone and estradiol in fertile cycles. Am J Obstet Gynecol 143:808–813 Abstract

28. **Nylund L, Beskow C, Carlström K, Fredricsson B, Gustafson O, Lunell NO, Pousette A, Rosenborg L, Slotte H, Akerlof E** 1990 The early luteal phase in successful and unsuccessful implantation after IVF-ET. Hum Reprod 5:40–42 Abstract

29. **Ng EHY, Yeung WSB, Lau EYL, So WWK, Ho PC** 2000 A rapid decline in serum oestradiol concentrations around the mid-luteal phase had no adverse effect on outcome in 763 assisted reproduction cycles. Hum Reprod 15:1903–1908

30. **Gidley-Baird AA, O'Neill C, Sinosich MJ, Porter RN, Pike IL, Saunders DM** 1986 Failure of implantation in human in vitro fertilization and embryo transfer patients: the effects of altered progesterone/estrogen ratios in humans and mice. Fertil Steril 45:69–74 Abstract

31. **Hutchinson-Williams KA, Lunenfeld B, Diamond MP, Lavy G, Boyers SP, DeCherney AH** 1989 Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and nonconeption cycles in an in vitro fertilization program. Fertil Steril 52:441–445 Abstract

32. **Kamat BR, Brown LF, Manseau EJ, Senger DR, Dvorak HF** 1995 Expression of vascular permeability

factor/vascular endothelial growth factor by human granulosa and theca lutein cells. Role in corpus luteum development. Am J Pathol 146:157–165 Abstract

33. **Gordon JD, Mesiano S, Zaloudek CJ, Jaffe RB** 1996 Vascular endothelial growth factor localization in human ovary and fallopian tubes: possible role in reproductive function and ovarian cyst formation. J Clin Endocrinol Metab 81:353–359 Full Text

34. **Agrawal R, Conway G, Sladkevicius P, Tan SL, Engmann L, Payne N, Bekir J, Campbell S, Jacobs H** 1998 Serum Vascular endothelial growth factor and Doppler blood flow velocities in in vitro fertilization: relevance to ovarian hyperstimulation syndrome and polycystic ovaries. Fertil Steril 70:651–658 Abstract

35. **Molskness TA, Stouffer RL, Burry KA, Gorrill MJ, Lee DM, Patton PE** 2004 Circulating levels of free and total vascular endothelial growth factor (VEGF)-A, soluble VEGF receptors-1 and -2, and angiogenin during ovarian stimulation in non-human primates and women. Hum Reprod 19:822–830 Abstract

36. **Lee A, Christenson LK, Stouffer RL, Burry KA, Patton PE** 1997 Vascular endothelial growth factor levels in serum and follicular fluid of patients undergoing in vitro fertilization. Fertil Steril 68:305–311 Abstract

37. **Krasnow JS, Berga SL, Guzick DS, Zeleznik AJ, Yeo KT** 1996 Vascular permeability factor and vascular endothelial growth factor in ovarian hyperstimulation syndrome: a preliminary report. Fertil Steril 65:552–555 Abstract

38. **Abramov Y, Barak V, Nisman B, Schenker JG** 1997 Vascular endothelial growth factor plasma levels correlate to the clinical picture in severe ovarian hyperstimulation syndrome. Fertil Steril 67:261–265 Abstract

39. **Pellicer A, Albert C, Mercader A, Bonilla-Musoles F, Remohi J, Simon C** 1999 The pathogenesis of ovarian hyperstimulation syndrome: in vivo studies investigating the role of interleukin-1β, interleukin-6, and vascular endothelial growth factor. Fertil Steril 71:482–489 Abstract

40. **Artini PG, Monti M, Fasciani A, Battaglia C, D'Ambrogio G, Genazzani AR** 2002 Vascular endothelial growth factor, interleukin-6 and interleukin-2 in serum and follicular fluid of patients with ovarian hyperstimulation syndrome. Eur J Obstet Gynecol Reprod Biol 101:169–174 Abstract

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