Human chorionic gonadotropin (hCG) plasma levels at oocyte retrieval and IVF outcomes

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Abstract

Purpose The aim of our study was to ascertain the influence of hCG levels at oocyte pick-up on IVF outcomes, and their relationship with clinical parameters.

Methods A prospective study was performed including 473 women undergoing IVF, aged under 40 years. Blood samples to analyze hCG levels were obtained at the time of follicular aspiration, 36 h after the administration of 250 µg of recombinant hCG.

Capsule After the administration of 250 mcg of recombinant hCG, plasma hCG levels are not responsible for failure to recover oocytes or for recovering a reduced number of oocytes.

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Results Neither the numbers of oocytes obtained or fertilized, nor the pregnancy rate, were correlated with hCG levels. Moreover, hCG values were very similar in women who did and did not become pregnant (123.3 ± 48.7 and 117.5 ± 44.7 mUI/mL). Cases in which no oocytes were recovered after follicular aspiration had similar hCG levels to those in which more than 1 oocyte was obtained. On the other hand, hCG levels were negatively related to body mass index, weight, and age.

Conclusions These data indicate that after the administration of 250 µg of recombinant hCG, hCG levels are not responsible for failure to recover oocytes. Specifically, there was no correlation between plasma hCG levels and the number of oocytes obtained or other markers of IVF outcome. There was, however, an inverse relationship with BMI, body weight and age.

Keywords Human chorionic gonadotropin · Oocytes · IVF · Body mass index · Pregnancy rate

Introduction

Human chorionic gonadotropin (hCG) administration plays a crucial role in assisted reproduction. During ovarian stimulation in in vitro fertilization (IVF) cycles, hCG administration is usually used as a surrogate luteinizing hormone (LH) surge to induce final oocyte maturation. The administration of urinary hCG (u-hCG) has been used for decades to achieve final oocyte maturation and ovulation in controlled ovarian hyperstimulation protocols at a dose of 5,000–10,000 IU. Compared with 5,000 IU of u-hCG, a 250 µg dose of recombinant hCG (r-hCG) achieves similar clinical results [1–3] and has a similar pharmacokinetic profile [4]. Moreover, when doses of 500 µg and 250 µg of r-hCG were compared, although the mean number of oocytes retrieved was the same [5], as was

the number of metaphase II oocytes [6], the numbers of fertilized oocytes and cleaved embryos were found to be significantly higher with 500 µg r-hCG [5].

Failure of oocyte retrieval after follicular aspiration occurs in 1–2 % of IVF cycles [7, 8]. This has been attributed to the following factors: empty follicles, technical difficulties with oocyte collection, ovulation occurring earlier than expected, and inaccurate hCG administration. Since hCG plasma levels depend on the hCG dose [5, 6] and on body mass index (BMI) [9, 10], it could be speculated that lower hCG levels could be associated with i) a higher rate of aspiration failure; and ii) a smaller number of oocytes being retrieved. Moreover, in IVF cycles after oocyte aspiration the number of oocytes obtained is sometimes much lower than expected and it could be that this is due to insufficiently high levels of hCG.

Previous studies focusing on hCG dose have used the total number of oocytes [1, 2, 5] or number of metaphase II oocytes retrieved as primary end points. We have also considered pregnancy rates, in order to assess any effect on implantation. The aim of our study was to ascertain whether hCG plasma levels on the day of oocyte retrieval are related to the number of oocytes retrieved and/or pregnancy rates.

Material and methods

Over an 8-month period, plasma hCG levels were measured at the time of oocyte retrieval for all the women undergoing IVF or ICSI with their own oocytes at our assisted reproduction unit. A total of 473 consecutive women were studied. All of them were aged <40 years old, as this is the general inclusion criterion for patients in our center.

The mean age of the women was 34.66 ± 2.87 years, while their mean duration of infertility was 4.36 ± 1.96 years. The main indications for IVF/ICSI were: male factor (67.6 %), failure of intrauterine insemination (10.1 %), endometriosis (9.7 %) and tubal factor (8.0 %). Exclusion criteria were: i) age ≥ 40 years, ii) three or more failed IVF cycles, iii) two previous cycles with poor response, iv) poor ovarian reserve (antral count <6 follicles), v) infection risk, vi) prenatal genetic diagnosis, and vii) contraindication for pregnancy. In 48.6 % of cases it was the first IVF cycle, in 37.1 % the second and in 14.3 % the third.

Our IVF protocol has been described previously [11–13]. Briefly, it consists of down-regulation with gonadotrophin-releasing hormone analogue, triptorelin acetate (Decapeptyl, Ipsen Farma, Madrid, Spain) on a long protocol; ovarian stimulation with recombinant FSH (Gonal F, Merck Serono, Madrid, Spain) and highly purified urinary menopausal gonadotropins (Menopur, Ferring, Madrid Spain) or

recombinant LH (Luveris, Merck Serono), follicular growth being monitored by measuring plasma estradiol levels and vaginal ultrasound; and triggering of ovulation with 250 µg r-hCG (Ovitrelle, Merck Serono) when at least 3 follicles >18.5 mm are observed. Transvaginal ultrasound-guided oocyte retrieval was scheduled 36 h after self-administered subcutaneous injections of 250 µg of r-hCG. Oocyte aspiration was performed under sedation with local anesthesia, with a single-lumen 18-gauge needle. All the follicles observed, even if less than 10 mm, were aspirated. Oocyte aspiration was always performed by a staff gynecologist with more than 20 years of experience in the procedure.

Our IVF policy has also been described in recent publications [12, 14]. Normal sperm following WHO criteria [15], with no previous fertilization failures or with no previous artificial insemination failures were subjected to conventional IVF, while ICSI was used in the remaining cases.

Ultrasound-guided embryo transfer was performed on day 2–3. In cases with a good prognosis, two embryos were transferred, while three were used in the remaining cases.

For this study, institutional board approval and informed consent were obtained. A fasting blood sample was taken immediately before oocyte retrieval. Retrieval rate was defined as the percentage of punctured follicles that contained an oocyte.

Serum concentrations of E2 (during ovarian stimulation and at oocyte retrieval) and of hCG (at oocyte retrieval) were measured using commercially available kits (ACS-180 Automated Chemiluminescence System, Bayer, Tarrytown, NY). The sensitivity of the E2 assay was 10.0 pg/ml, and the intra- and inter-assay coefficients of variation were 8.1 % and 8.7 %, respectively; while the sensitivity of the hCG assay was 2 IU/L, and the intra- and inter-assay coefficients of variation for hCG were 1.8 % and 4.9 %, respectively. All healthcare personnel involved were blind to the hCG results and this data were not analyzed until the end of the study period. Thus, the embryo transfer policy and luteal phase management were not influenced by any findings of this study.

Among women who had transfers carried out, the mean number of embryos transferred was 2.39 ± 0.75 . A priori sample-size calculations were as follows: assuming a common standard deviation in hCG levels of 45 mUI/mL (on the basis of our previous unpublished data), a ratio of 0.61 between study cases and controls (on the basis of our 39 % per transfer pregnancy rate), and an alpha of 0.05 and beta of 0.20 for two-sided comparisons, 243 cases of embryo transfer resulting in pregnancy and 156 cases with no pregnancy would be needed to detect a 13 mUI/mL difference between the means in the two groups. Assuming that 85 % of patients reached embryo transfer, a total of 459 cases of oocyte retrieval would be needed.

Statistical analysis was performed using Anova, Pearson tests, Chi square tests, Chi square tests for linear trend, Pearson r correlation and multivariate regression analysis following the standard applicability criteria. Pregnancy was defined as the visualization of the gestational sac 3–4 weeks after the transfer. In the study population, 396 women reached embryo transfer and the pregnancy rate per transfer was 40.4 % (160/396).

Results

The levels of hCG were very similar across the subgroups of patients from whom 1 or more oocytes were obtained: 121.9 ± 51.9 mIU/mL in the 1–5 oocytes group; 119.5 ± 48.0 in the 6–10 oocytes group; 116.0 ± 39.1 mIU/mL in the 11–15 oocytes group; and 125.7 ± 43.6 mIU/mL in the group of women from whom 16 or more oocytes were obtained. The group of patients from whom no oocytes were obtained had a mean value somewhat lower (77 ± 58.4 mIU/mL) (Table 1). There were, however, only 4 cases in this group: the first was a patient who forgot to administer the hCG (hCG level=0); the second involved follicular aspiration after prolonged coasting (hCG=138); the third had had a previous IVF cycle in which oocyte retrieval had also failed (hCG=102); and, lastly, the fourth was a woman with severe recurrent endometriosis and follicular aspiration had been very difficult (hCG=67). If the patient who forgot their dose of hCG is excluded, the mean hCG value is 103.3 ± 35.5 mIU/mL, and the difference compared to the groups in which oocytes were obtained is not then statistically significant. The lowest hCG value in our series (excluding the patient who did not take the hCG dose) was 23 mIU/mL.

On the other hand, there was a significant negative correlation (Pearson test, $r = -0.40$, $p < 0.05$) between body weight and hCG levels (Fig. 1). Specifically, the levels of hCG were 136.2 ± 37.8 mIU/mL in women under 45 Kg, progressively decreasing to 68.25 ± 56.5 in those over 90 Kg. A similar pattern was observed with BMI.

Table 1 Number of obtained oocytes and hCG levels at oocyte pick-up

Number of oocytes obtained	hCG level (mIU/mL) mean $\pm$ SD	N
0	77.0 ± 58.4 (103.3 ± 35.5) ^a	4 (3) ^a
1–5	121.9 ± 51.9	109
6–10	119.5 ± 48.0	187
11–15	116.0 ± 39.1	99
>15	125.7 ± 43.6	74

^a excluding one case in which hCG administration was forgotten

No significant differences

Fig. 1 hCG values at oocyte pick up and weight (Kg) ($p < 0.01$)

Namely, the values of hCG were 133.3 ± 54.5 mIU/mL for those with a BMI < 20, 113.6 ± 39.9 with a BMI of 20–25, 96.5 ± 43.6 with a BMI of 25–30 and 78.8 ± 36.8 with a BMI > 30, the differences being statistically significant (Pearson test $r = -0.36$, $p < 0.05$) (Fig. 2). A significant negative correlation was also found between hCG levels and age ($r = -0.12$, $p < 0.05$). Multivariate analysis confirmed that body weight, BMI and age were significantly correlated with hCG levels, while age was not correlated with either BMI or body weight.

No correlation was found with estradiol values the day of hCG administration, or the number of oocytes obtained, inseminated or fertilized or the recovery rate (Table 2). However, the number of oocytes fertilized was significantly lower in the groups with extreme levels of hCG (5–49 and >200), compared with those with intermediate values (50–99 and 100–199) (Table 2). The hCG levels did not vary by the number of IVF attempts. Indeed, when the analysis was restricted to first IVF cycles only, the results were similar (data not shown).

The levels of hCG at oocyte pick-up were very similar in women who did and did not become pregnant (123.3 ± 48.7 vs. 117.5 ± 44.7 mIU/mL). There were no significant differences

Fig. 2 hCG values at oocyte pick up and Body Mass Index ($p < 0.01$)

Table 2 IVF outcome markers and hCG levels at oocyte pick-up

hCG level (mIU/mL)	N	Estradiol levels the day of oocyte pick-up (mean $\pm$ SD)	Number of oocytes obtained (mean $\pm$ SD)	Oocyte recovery rate (%)	Number of fertilized embryos (mean $\pm$ SD)	BMI (mean $\pm$ SD)	Body weight in Kg(mean $\pm$ SD)	Pregnancy rate per puncture (%)	Pregnancy rate per transfer (%)
5–49	16	2,045.5 $\pm$ 1021.2	8.4 $\pm$ 3.9	66.7 $\pm$ 18.4	6.2 $\pm$ 2.8	29.6 $\pm$ 4.9	80.1 $\pm$ 15.9	18.8 (3/16)	25.0 (3/12)
50–99	150	2,206.7 $\pm$ 1063.1	10.0 $\pm$ 5.2	73.4 $\pm$ 21.5	8.1 $\pm$ 4.9	24.4 $\pm$ 3.9	64.8 $\pm$ 10.8	37.3 (56/150)	42.4 (56/132)
100–199	281	2,007.6 $\pm$ 934.4	10.8 $\pm$ 5.9	71.8 $\pm$ 19.8	8.7 $\pm$ 5.1	22.7 $\pm$ 3.3	58.6 $\pm$ 8.4	31.7 (89/281)	38.4 (89/232)
200–300	22	1,881.3 $\pm$ 938.2	8.7 $\pm$ 7.9	69.0 $\pm$ 16.2	6.3 $\pm$ 7.3	21.0 $\pm$ 2.7	54.9 $\pm$ 5.0	54.5 (12/22)	60.0 (12/20)
P value		NS	NS	NS	< 0.05	<0.01	< 0.01	NS	NS

in pregnancy rates either per transfer or per puncture as a function of hCG levels. We observed a trend towards higher pregnancy rates in cases with hCG values over 200 mIU/mL and lower rates in cases with hCG values ranging from 5 to 49 mIU/mL. Nevertheless, the chi square test for trend showed no significant differences. Taking into account the small numbers of extreme hCG values, however, a beta-error could not be ruled out.

Discussion

Ovulation triggering represents an important step in IVF treatment. Nowadays, ovulation is usually triggered with a subcutaneous injection of r-hCG. In the past, u-hCG was employed at two different doses, mainly administered intramuscularly. Abdalla et al. [15] reported that 2,000 IU of u-hCG was not as effective as 5,000 IU of u-hCG at inducing the final stages of ovulation, whereas doses of 5,000 IU and 10,000 IU were equally effective. Regarding r-hCG, it has been shown that increasing the dose (500 μ g vs 250 μ g) may led to a higher rate of ovarian hyperstimulation syndrome, but with no significant improvement in pregnancy rate [5]. Considering both safety and efficacy, 250 μ g has been proposed as the dose of choice for triggering ovulation [16].

Failure to obtain oocytes after follicular aspiration in patients with an adequate ovarian response occurs in 1–2 % of cycles [7, 8]. A number of hypotheses have been put forward to explain this lack of oocyte recovery: premature ovulation, deficient or insufficient administration of hCG, and “empty follicle” syndrome, as well as technical difficulties in oocyte pick-up. We tested the hypothesis that it could be due to lower plasma levels of hCG. However (excluding one individual who forgot to administer the dose of hCG, and who accordingly had a zero level), the mean hCG levels of patients from whom no oocytes were recovered were similar to the levels in those from whom oocytes were obtained. Thus, our findings indicate that insufficient hCG plasma levels are not responsible for the failure to recover oocytes in IVF cycles. Experimental research in rats suggests that the lowest

concentration of serum hCG at which mature oocytes could be successfully retrieved would be 5–10 IU/L [17, 18], a values that is considerably lower than the hCG levels found in our study all our patients. Indeed, the lowest serum hCG level on the day of hCG administration was 23 mIU/mL in our study.

Furthermore, we did not observe a dose–response relationship between hCG and the number of obtained oocytes. That is, considering cases in which 1 or more oocytes were successfully harvested, the hCG levels were almost identical, irrespective of the number of oocytes obtained. Our results are consistent with a study of urinary hCG, which showed that a low serum hCG level before oocyte recovery (<100 IU/L) was as effective as higher levels at initiating maximal oocyte maturity and led to a similar percentage of MII oocytes being recovered [18]. In our opinion, the range of hCG plasma levels obtained with a 250 μ g dose is likely to be similarly effective for triggering ovulation. Thus, increasing the dose of hCG should not be expected to increase the number of oocytes obtained.

It is well known that obese women do not respond well to ovarian stimulation, either with clomiphene [19] or with gonadotropins [20]. In our series, we found a strong negative correlation between serum hCG values and BMI. This is consistent with findings in smaller series with u-hCG administered intramuscularly [9, 21], though other authors did not observe this association when u-hCG was administered subcutaneously [22]. We also found a negative correlation with body weight.

Women with lower hCG values had a significantly smaller number of fertilized oocytes and tended to have a lower PR. However, taking into account the inverse correlation with age, and especially with BMI/weight, as well as the relatively small size of the group with low hCG values ($n=16$), it was not possible to ascertain whether the less good outcome was due to the low hCG levels or to the other factors known to be associated with poor prognosis in infertility (namely, BMI and age).

Regarding the finding of an inverse correlation between hCG levels and age, the following comments should be made. A number of age-related changes in pharmacokinetics and pharmacodynamics have been reported in studies mainly focusing on elderly people [23]. The majority of such changes

lead to higher drug concentrations [23]. There is also, however, a progressive reduction in total body water with an increase in plasma volume, and a decrease in lean body mass, resulting in a relative increase in body fat, especially in women [24]. In our opinion, the inverse correlation with age we found could be due to some subtle age-related changes in body mass, milder but similar to those described in the elderly [24], especially regarding the increase in body fat and in plasma volume. Other explanations are also possible such as the presence of confounding factors or that this was a spurious correlation, since the correlation was relatively weak.

It should be remembered that no patients aged ≥ 40 years were included in our study, in accordance with the inclusion criteria applied by all Spanish public hospitals. Thus, the results we obtained may not necessarily apply for women over 40 years.

Although more studies are needed to ascertain whether in cases of high BMI an increase in hCG could be beneficial, in general hCG plasma levels are not associated either with the number of oocytes obtained, failure to recover any oocytes, or pregnancy rates.

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