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ARTICLE

The value of HCG serum concentrations after trigger in predicting pregnancy and live birth rates in IVF–ICSI

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Abstract The aim of this study was to determine if an association existed between serum human chorionic gonadotrophin (HCG) level at 12 h after trigger and IVF and intracytoplasmic sperm (ICSI) treatment outcomes. Women undergoing initial IVF–ICSI and embryo transfer treatment using the long luteal phase gonadotrophin-releasing hormone agonist protocol between April 2012 and March 2013 for tubal factor were included ($n = 699$). In the clinical pregnancy group, HCG after trigger was significantly elevated (276.0 ± 5.1 versus 198.5 ± 6.1 mIU/mL; $P < 0.001$). The optimal cut-off value proposed by the receiver operating characteristic analysis (area under curve = 0.730) for HCG was 201.2 mIU/ml. Compared with the lower HCG group, the clinical pregnancy rate in the higher HCG group was increased in obese and non-obese patients (77.8% versus 57.3%, $P < 0.05$; 85.6% versus 53.0%, $P < 0.01$, respectively). Adjusted for age and body mass index, an increase of HCG was associated with a better IVF–ICSI treatment outcome (OR 4.39, 95% CI 2.99 to 6.45). Clinical pregnancy rate was significantly higher across increasing quartiles of HCG. An elevated level of serum HCG at 12 h after trigger was associated with a better IVF–ICSI outcome.

KEYWORDS: HCG, ICSI, IVF, pregnancy

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Introduction

Successful implantation after IVF and embryo transfer depends on various factors related to the embryo, as well as the endometrial receptivity (von Grothusen et al., 2014). It is important that the embryo reaches the endometrial cavity during the period in which the endometrium is receptive, the 'implantation window'. It is estimated that about 50–70% of lost pregnancies are caused by implantation failure (Achache and Revel, 2006).

Implantation is a complex process that is regulated by many factors, the most important of which is human chorionic gonadotrophin (HCG) (Tsampalas et al., 2010). In primates, HCG is one of the early embryonic signals that is secreted by the embryo before its implantation. It is a glycoprotein from the same family as the pituitary gonadotrophins (FSH and LH), and shares marked structural similarity with LH. Because of its similarity to LH, HCG can act as a surrogate for LH surge to induce final oocyte maturation and ovulation in ovarian stimulation cycles. It stimulates similar periovulatory events, including softening of the connective tissue of follicle, which allows easy detachment of oocyte cumulus complex from the follicle wall, enabling aspiration during oocyte retrieval (Bjercke et al., 2000).

Studies have shown that early ovarian hyperstimulation syndrome (OHSS) was apparently caused by the administration of exogenous HCG, the more doses of HCG used, the earlier OHSS occurred (Nargund et al., 2007). One study reduced the dose of HCG to 4000 IU in triggering oocyte final maturation in IVF–ICSI. They found that the number or proportion of mature oocytes retrieved and the OHSS rate in the lower and higher doses groups were similar, but the reduced dose of HCG could obviously affect clinical pregnancy rates (Lin et al., 2011). It can be speculated that HCG might help embryo implantation.

Gonadotrophin-releasing hormone agonist (GnRHa) is also used as a trigger of final oocyte maturation to prevent OHSS. Compared with HCG triggering, GnRHa triggering reduced moderate to severe OHSS incidence, but also decreased the live birth rate and ongoing pregnancy rate, and increased ectopic pregnancy rate (Sahin et al., 2015; Youssef et al., 2014). Many involved genes known to play a role in implantation and the receptivity of the endometrium were highly up-regulated in HCG triggering compared with GnRHa triggering (Humaidan et al., 2012). Therefore, the reason for higher pregnancy rate and lower ectopic pregnancy rate in HCG-triggered cycles relative to GnRHa triggered cycles may be the increased receptivity of the endometrium (Sahin et al., 2015). Evans and Salamonsen (2013), however, also showed that, although acute high-dose HCG enhanced endometrial epithelial cell adhesion and elicited the maxim ERK1/2 phosphorylation response, chronic low-dose HCG exposure may detrimentally affect endometrial receptivity.

On the basis of these studies, we postulated that trigger with HCG contributes to final oocyte maturation and also plays an important role in modulating endometrial receptivity. Furthermore, to the best of our knowledge, no study has evaluated the association of the HCG levels after trigger with pregnancy rate. The aim of this study was to investigate the value of HCG serum levels after trigger in predicting pregnancy and live birth rates in IVF and intracytoplasmic sperm injection (ICSI) treatments.

Materials and methods

Study population

A cohort of couples who underwent initial IVF–ICSI and embryo transfer treatment using the long luteal phase GnRH agonist protocol between April 2012 and March 2013 at the IVF centre of Nanjing Drum Tower Hospital for tubal factor was included. Inclusion criteria were age 35 years or younger, regular cycles (24–35 days), basal FSH less than 10 mIU/ml, and antral follicle count 10 or over. Women with polycystic ovarian syndrome and poor ovarian reserve, immunological disease, endometriosis, uterine abnormality, endometrial thickness less than 8 mm before embryo transfer, fewer than two good-quality embryos available for transfer or patients with inadequate data for analysis were excluded. Poor responders were defined when one or more of the following criteria was present in the first or in at least one previous failed assisted reproduction technique cycle: fewer than four mature oocyte retrieved, level of oestradiol less than 500 pg/ml on the day of HCG administration, or a prior cancelled stimulation cycle owing to poor ovarian response. Independent ethical approval was obtained from the Nanjing Drum Tower Hospital Research Ethics Committee on 22 March 2014 (no. SZ200-802). Informed consent was provided by all couples at recruitment.

Ovarian stimulation protocol

All patients received standard ovarian stimulation with recombinent FSH under pituitary suppression with GnRH agonist according to a routinely used protocol (Ding et al., 2013). In all women, pituitary desensitization was achieved by subcutaneous administration of triptorelin (0.1 mg daily, which was reduced to 0.05 mg after ovarian arrest was confirmed) started in the mid-luteal phase of the previous cycle. Gonadotrophin stimulation of the ovaries was started when serum oestradiol concentrations declined to less than 40 pg/ml and a vaginal ultrasound scan showed an absence of follicles over 10 mm in diameter. Ovarian stimulation was started with 150–250 IU/day of recombinant FSH (Gonal F; Serono, Switzerland); the initial dose was determined by the treating clinician, based on the patient's age, body mass index (BMI), basal FSH and oestradiol. Transvaginal ultrasound and oestradiol measurement were used to monitor follicular growth, and gondotrophin doses were adjusted accordingly. Ovulation was triggered by intramuscular administration of 10,000 IU of HCG (Ferring Pharmaceuticals) when at least two follicles reached a diameter of 18 mm. Serum HCG concentrations were measured at 12 h after HCG trigger using the immunoassay (ARCHITECT i2000SR; Abbott, USA). Oocytes were retrieved 36 h after the injection of HCG.

Ovum pick-up, insemination and embryo incubation

After retrieval, oocytes were fertilized using IVF–ICSI. After IVF insemination for 6 h in droplets G-IVF™ (Vitrolife, Sweden) fertilization medium under mineral oil (Ovoil ®, Vitrolife) in a conventional incubator (37°C, 5% O_2, 6% CO_2), the cumulus and corona cells were removed by mechanical pipetting. Then,

embryos were individually placed in microdrops (50 μl) containing G-1™ (Vitrolife, Sweden) under mineral oil for further culture. For ICSI insemination, the cumulus–oocyte complexes were denuded using hyaluronidase (Cook). The ICSI procedure was carried out 1 h after oocyte denudation and the in-vitro culture was carried out in 50 μl of G-1™ under mineral oil until day 3 in conventional incubators with 5% O_2, 6% CO_2 at 37°C.

Embryo evaluation and transfer

All assessments were made using an inverted microscope with Hoffman modulation contrast. Fertilization was checked at 19 ± 1 h after insemination. Individually cultured embryos were checked at 44 ± 1 h and 68 ± 1 h after insemination. The consensus scoring system was applied for embryo assessment (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). Embryos were evaluated on the basis of the number of blastomeres, rate of fragmentation and symmetry. Good-quality embryo were considered to be two pronuclei embryo 18–20 h after insemination, four cells at day 2 and seven to eight cells at day 3, even size, and less than 10% fragmentation.

On day 3 after oocyte retrieval, two good-quality cleavage-stage embryos were selected and transferred into the uterus. Progesterone supplementation (intramuscular progesterone 60–80 mg daily) for luteal support was carried out 10 weeks after embryo transfer. Clinical pregnancy was diagnosed by increasing serum concentration of beta-HCG 14 days after embryo transfer, and the subsequent demonstration of an intrauterine gestational sac by ultrasonography on 30 days after embryo transfer. Number of oocytes retrieved, number of good-quality embryos and live birth rates were secondary outcome measures.

Data analysis

All data were presented as mean ± standard error of mean (SEM). Variables among groups were compared using the independent-samples Student's t-test, Mann–Whitney and chi-squared tests, respectively. $P < 0.05$ was considered to be statistically significant. Three factors (HCG value 12 h after trigger, BMI and age) were selected for inclusion in multivariable logistic regression model. A receiver operating characteristic (ROC) curve was constructed to evaluate the performance of each factor. Odds ratios (OR) and 95% confidence interval (CI) for clinical pregnancy associated with variables was estimated by multivariate logistic regression analysis. The Statistical Package for Social Sciences (SPSS version 14.0 for Windows, Chicago, IL, USA) was used for statistical analysis.

Results

A total of 699 cycles met the inclusion criteria during the study period. Baseline characteristics and outcomes for the whole group are presented in Table 1. In this group of young women, the clinical pregnancy rate was 74.2%, whereas the live birth rate was 68.4%, which were consistent with our previous studies (Ding et al., 2013; Zhou et al., 2012, 2013).

Table 1 Baseline characteristics and clinical outcomes of patients undergoing long luteal phase gonadotrophin-releasing hormone agonist cycles for IVF and intracytoplasmic sperm injection.

Characteristics	*Study group (n = 699)*
Age (years)	29.6 (20–35)
Body mass index (kg/m²)	22.2 (15.9–35.5)
Oestradiol on day of HCG (pg/ml)	4314 (972.39–10140)
P on day of HCG (ng/ml)	0.77 (0.01–3.51)
HCG 12 h after trigger (mIU/mL)	260.3 (61.17–963.9)
Oestradiol 12 h after trigger (pg/ml)	4759.7 (910.44–11163)
P 12-h after trigger (ng/ml)	9.6 (1.62–25.84)
Endometrial thickness (mm)	11.4 (8–18)
Number of retrieved oocytes per cycle	10.74 (4–23)
Number of fertilized oocytes per cycle	8.96 (2–22)
Number of cleaved embryos per cycle	8.8 (2–22)
Number of good-quality embryos per cycle	5.3 (2–19)
Number of good quality embryos transferred per cycle	2
Implantation rate, % (*n*)	51.6 (722/1398)
Clinical pregnancy rate per cycle, % (*n*)	74.2 (519/699)
Live birth rate per cycle, % (*n*)	68.4 (478/699)

No significant difference was observed between clinical pregnancy and non-clinical pregnancy groups in patients' age, oestradiol value on day of HCG, *P*-value on day of HCG, *P*-value–oestradiol ratio on day of HCG, endometrial thickness, number of retrieved oocytes, fertilized oocytes, cleaved embryos and good-quality embryos, which were thought to be associated with IVF outcomes. At 12 h after trigger, HCG serum value was significantly higher in the clinical pregnancy group (276.0 ± 5.1 versus 198.5 ± 6.1 mIU/ml, $P < 0.001$), whereas BMI was significantly lower (21.9 ± 0.1 versus 22.9 ± 0.2, $P < 0.01$) (Table 2). Age and BMI were negatively correlated with HCG value ($r = -0.11$, $P < 0.01$; $r = -0.30$, $P < 0.01$, respectively).

A ROC analysis comparing HCG value or BMI between clinical pregnancy and non-clinical pregnancy groups was conducted. The area under ROC curve for HCG value was 0.730, whereas, for BMI, it was only 0.600 (Figure 1). The optimal cut-off value proposed by the ROC analysis for HCG value was 201.2 mIU/mL for this group of young women. The World Health Organization (WHO) recommended cut-off value for obesity was used: that Asians should be considered obese when the BMI is greater than 25 kg/m² (WHO Expert Consultation, 2004). So the study population was then divided into four groups based on BMI and HCG value. No significant difference was observed among four groups in *P*-value on day of HCG, endometrial thickness, number of retrieved oocytes, fertilized oocytes, cleaved embryos and good-quality embryos. When compared with the lower HCG value group, the implantation rate and the clinical pregnancy rate in the higher HCG value group were higher in obese patients and non-obese patients. The HCG value was significantly lower in obese patients than that in non-obese patients (189.8 ± 7.6 versus

Table 2 Predicators for clinical pregnancy in patients undergoing long luteal phase gonadotrophin-releasing hormone agonist cycles for IVF and intracytoplasmic sperm injection.

Characteristics	*Clinical pregnancy group (n = 519)*	*Non-clinical pregnancy group (n = 180)*	*P-value*
Age (years)	29.6 ± 0.1	29.7 ± 0.3	NS
Body mass index (kg/m^2)	21.9 ± 0.1	22.9 ± 0.2	<0.001
Oestradiol on day of HCG (pg/ml)	4318.9 ± 66.9	4303.3 ± 112.2	NS
P on day of HCG (ng/ml)	0.77 ± 0.02	0.79 ± 0.04	NS
P/ oestradiol on day of HCG	0.20 ± 0.15	0.21 ± 0.15	NS
HCG value at 12 h after trigger (mIU/mL)	276.0 ± 5.1	198.5 ± 6.1	<0.001
Endometrial thickness (mm)	11.4 ± 0.1	11.2 ± 0.2	NS
Number of oocytes retrieved per cycle	10.63 ± 3.65	11.05 ± 4.19	NS
Number of fertilized oocytes per cycle	8.93 ± 3.57	9.06 ± 3.98	NS
Number of cleaved embryos per cycle	8.8 ± 3.85	8.81 ± 4.03	NS
Number of good-quality embryos per cycle	5.31 ± 3.00	5.19 ± 3.08	NS

NS = not significant.

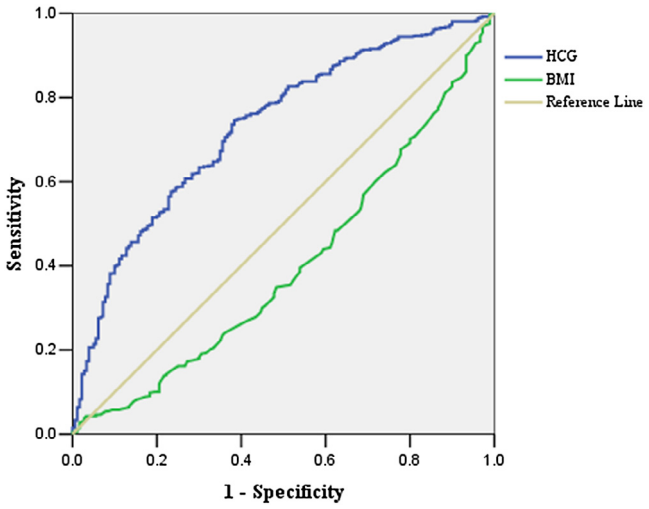

Figure 1 Receiver operating characteristic curve (ROC) of HCG and body mass index (BMI) in predicting clinical pregnancy in patients undergoing IVF–ICSI treatment. Area under ROC curve for HCG was 0.730; 95% CI 0.688 to 0.771; BMI was 0.600; 95% CI 0.552 to 0.648).

274.8 ± 7.0 mIU/ml, $P < 0.01$), as well as the implantation rate and the clinical pregnancy rate (43.3% versus 53.4%, $P < 0.01$; 65.0% versus 76.2%. $P < 0.05$, respectively). When the HCG value was 201.2 mIU/mL or over, the implantation rate and the clinical pregnancy rate had no significant difference between obese and non-obese patients (52.2% versus 60.2% and 77.8% versus 85.6%, respectively) (**Table 3**).

Statistics from the multivariable model are presented in **Table 4**. The modified Hosmer–Lemshow goodness-of-fit chi-squared test statistics was 1.834 (not statistically significant), which suggested that the multivariable model was a good fit. The HCG value showed a high OR (OR 4.391, 95% CI 2.990 to 6.450), which is a good predictor for better IVF outcomes with a sensitivity of 74.6%, specificity of 61.7%, positive predictive value of 66.1% and negative predictive value of 70.8%. Neither BMI (OR 0.98, 95% CI 0.92 to 1.04) nor age (OR 1.02, 95% CI 0.97 to 1.08) had an effect on IVF outcome.

Furthermore, to evaluate the prognostic effect of HCG value at 12 h after trigger on clinical pregnancy, a model including HCG value quartiles was fitted as a categorical dummy variable using the first quartile as the reference. Clinical pregnancy rate and implantation rate were significantly higher across increasing quartiles of HCG value ($P < 0.01$). Compared with that in the first quartile of HCG value, the clinical pregnancy rate in the other quartiles was OR 2.36, 3.90, and 11.70 for the second, third and fourth quartile, respectively. Additional adjustment for age and BMI did not change our findings (**Table 5**).

Discussion

Investigators continue to seek for more sensitive and specific tests to predict cycle outcome and optimize treatment of couples undergoing IVF–ICSI and embryo transfer. In relation to this, the associations of elevated *P*-values and *P*- values–oestradiol ratio on day of, or day after, HCG administration with cycle outcomes have been extensively investigated (Keltz et al., 2012; Liu et al., 2015; Santos-Ribeiro et al., 2014; Sonntag et al., 2013), whereas the relationship of HCG value after trigger and cycle outcomes was seldomly studied.

An important role is played by HCG in modulating endometrial receptivity. The direct effect of HCG on the human endometrium was studied as early as 1998. Licht et al. (1998) found that intrauterine 500 IU of HCG provoked a significant stimulation of leukaemia inhibitory factor, vascular endothelial growth factor and matrix metalloproteinase 9. It also directly increased the expression of HOXA10 and other markers of endometrial receptivity, supporting the role of HCG as a candidate protein for blastocyst–endometrial communication (Fogle et al., 2010; Racicot et al., 2014). Schumacher et al. (2013) found that HCG produced mainly by the trophoblast was one of the factors attracting human regulatory T cells efficiently to the fetal–maternal interface contributing to their local accumulation, so HCG was deemed as a central regulator of pregnancy immune tolerance. This local accumulation of human regulatory T cells might also be favoured by other immune cells secreting HCG at the fetal–maternal interface (Schumacher et al., 2009). Our

Table 3 Baseline characteristics and clinical outcomes of non-obese and obese patients undergoing long luteal phase gonadotrophin releasing hormone agonist cycles for IVF and intracytoplasmic sperm injection.

Characteristics	*Non-obese*		*Obese*	
HCG value 12 h after trigger (mIU/ml)	*<201.2 (n = 168)*	*≥201.2 (n = 411)*	*<201.2 (n = 75)*	*≥201.2(n = 45)*
Age (years)	30.2 ± 0.2	29.3 ± 0.2[a]	29.9 ± 0.4	29.5 ± 0.4
P on day of HCG (ng/ml)	0.78 ± 0.03	0.82 ± 0.02	0.66 ± 0.05	0.58 ± 0.06
P/ oestradiol on day of HCG	0.20 ± 0.01	0.21 ± 0.01	0.20 ± 0.02	0.16 ± 0.02
Endometrial thickness (mm)	11.4 ± 0.2	11.4 ± 0.1	11.4 ± 0.2	11.1 ± 0.3
Number of oocytes retrieved per cycle	10.61 ± 3.79	10.73 ± 3.75	10.91 ± 4.14	10.98 ± 3.82
Number of fertilized oocytes per cycle	9.08 ± 3.84	8.91 ± 3.56	8.64 ± 3.90	9.58 ± 3.77
Number of cleavaged embryos per cycle	8.89 ± 3.89	8.70 ± 3.54	9.07 ± 5.60	9.27 ± 3.56
Number of good-quality embryos per cycle	5.29 ± 3.25	5.32 ± 2.93	4.76 ± 2.80	5.73 ± 3.26
Ovarian hyperstimulation syndrome (moderate to severe), % (*n*)	1.2 (2/168)	2.2 (9/411)	1.3 (1/75)	2.2 (1/45)
Implantation rate, % (*n*)	36.6 (123/336)	60.2[b] (495/822)	38.0 (57/150)	52.2[c] (47/90)
Clinical pregnancy rate per cycle, % (*n*)	53.0 (89/168)	85.6[b] (352/411)	57.3 (43/75)	77.8[c] (35/45)
Live birth rate per cycle, % (*n*)	50.0 (84/168)	78.8[b] (324/411)	49.3 (37/75)	73.3[c] (33/45)

[a]Compared with lower HCG value non-obese patients, $P < 0.05$.
[b]Compared with lower HCG value non-obese patients, $P < 0.01$.
[c]Compared with lower HCG value obese patients, $P < 0.05$.

Table 4 Multivariate logistic regression analysis of factors related to clinical pregnancy.

Factors	*B*	*Standard error (b)*	*Wald Chi-square*	*P-value*	*Odds raio*	*95% Confidence interval*
HCG	1.48	0.196	56.935	0.00	4.39	2.99 to 6.45
Body mass index	−0.02	0.031	0.511	NS	0.98	0.92 to 1.04
Age	0.02	0.029	0.517	NS	1.02	0.97 to 1.08

The modified Hosmer–Lemshow goodness-of-fit Chi-square test statistics was 1.834 (not signficiant). NS = not significant.

Table 5 Odds ratios and 95% confidence intervals for clinical pregnancy in HCG quartiles.

Quartile	n	*Odds ratios (95% CI)*	*Adjusted odds ratios[a] (95% CI)*	*Clinical pregnancy rate (%)*	*Implantation rate (%)*	P
Quartile 1 (<179 mIU/ml)	174	1	1	51.7	33.6	1
Quartile 2 (179–233 mIU/ml)	173	2.36 (1.51–3.69)	2.55 (1.60–4.06)	71.7	51.7	<0.01
Quartile 3 (234–314 mIU/ml)	176	3.90 (2.42–6.29)	3.67 (2.18–6.18)	80.7	56.0	<0.01
Quartile 4 (>314 mIU/ml)	176	11.70 (6.18–22.16)	13.16 (6.42–26.98)	93.1	65.6	<0.01

[a]Adjusted for age and body mass index. CI = confidence interval.

previous studies had proved that pregnancy immune tolerance plays a critical role in successful implantation (Zhou et al., 2012, 2013). Recently, Mansour et al. (2011) and Zarei et al. (2014) both found that intrauterine injection of HCG before embryo transfer significantly improved the implantation and pregnancy rates in IVF–ICSI cycles. Evans and Salamonsen (2013) also found that prolonged HCG exposure may detrimentally affect endometrial receptivity (Evans and Salamonsen, 2013). These studies support that only the appropriate application of HCG can improve endometrial receptivity.

In the present study, the relationship between HCG value at 12 h after trigger and IVF treatment outcome was explored. Results revealed that women who were likely to achieve a pregnancy after IVF treatment had the higher HCG value than that in non-pregnant group, whereas patients' age, oestradiol value on day of HCG, progesterone value on day of HCG, progesterone-to-oestradiol ratio on day of HCG, endometrial thickness, number of oocytes retrieved, number of good-quality embryos had no significant difference. These effects were also dependent on HCG concentration, as the clinical pregnancy rate and implantation rate increased with

increasing HCG quartiles. The findings were compatible with other studies. Arce and Smitz (2011) showed that increased serum concentrations of exogenous HCG at the mid-follicular or at the end of stimulation were associated with higher live birth rates in infertile women treated with highly purified human menopausal gonad (Arce and Smitz, 2011, 2013). The mechanism of implantation and the precise role of HCG in implantation are still not fully understood, but it can be speculated that an excess HCG after trigger might play a positive role in successful implantation.

Obese women have a larger volume of distribution than non-obese women, and this may lead to a lower serum concentration after drug administration. A negative correlation between BMI and serum HCG levels at 12 h after injection was observed in the present study. Shah et al. (2014) also showed higher HCG levels in non-obese patients compared with obese and slimmer patients, and have a higher pregnancy rate. In the present study, however, when HCG value at 12 h after injection was 201.2 mIU/ml or greater, obese patients achieved the same good IVF outcomes as non-obese patients. Furthermore, only the HCG value at 12 h after injection was a good predictor of better IVF outcomes by using multivariate logistic regression analysis; neither BMI nor age had an effect on IVF outcome in this group of young women. Threfore, the HCG concentration at 12 h after injection was not only likely to be a reflection of HCG bioactivity associated with BMI and age, but the elevated level of HCG value *per se* was associated with a good IVF–ICSI treatment outcome.

Different doses of HCG have been used in various IVF treatment protocols to induce final maturation. No agreement has yet been reached on the minimum dose required, but evidence has suggested that different patients have different thresholds for their response to HCG. In this study, HCG value at 12 h after trigger greatly varied (from 61.17 to 963.9 mIU/ml), although all the patients received intramuscular HCG at a dose of 10,000 IU. Age and BMI were the two important factors associated with the HCG value, which were both negatively correlated with HCG value. An increasing trend of moderate to severe OHSS was observed when HCG was 201.2 mIU/ml or over in obese patients and non-obese patients. As HCG is a well known promoter of OHSS and seems to initiate the complex cascade that leads to the development of OHSS, preventive strategies attempt either to limit the dose or concentration of HCG without inducing a detrimental effect on endometrial and oocyte quality. In this study, optimal cut-off value of HCG at 12 h after trigger was 201.2 mIU/ml in our group of young women, whereas 64.7% of the patients, after intramuscular administration of 10,000 IU uHCG, had HCG values above this level, so these patients can reduce the HCG dose for trigger; in contrast, the other 35.3% patients should use a larger dose of HCG. The individual use of HCG for trigger according to patient's age and BMI might result in a higher pregnancy rate with lower OHSS occurrence.

In conclusion, our data suggest that an elevated level of HCG value 12 h after trigger is associated with an increased rate of clinical pregnancy and live birth in IVF–ICSI treatment. The HCG value after trigger in serum might be also a new therapeutic target for better IVF–ICSI treatment outcome. A larger prospective study is needed to establish the optimal level of HCG value in IVF–ICSI cycles.

References

Achache, H., Revel, A., 2006. Endometrial receptivity markers,the journey to successful mbryo implantation. Hum. Reprod. Update 12, 731–746.

Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum. Reprod. 26, 1270–1283.

Arce, J.C., Smitz, J., 2011. Exogenous hCG activity, but not endogenous LH activity, is positively associated with live birth rates in anovulatory infertility. Hum. Fertil. (Camb.) 14, 192–199.

Arce, J.C., Smitz, J., 2013. Live-birth rates after HP-hMG stimulation in the long GnRH agonist protocol: association with mid-follicular hCG and progesterone concentrations, but not with LH concentrations. Gynecol. Endocrinol. 29, 46–50.

Bjercke, S., Tanbo, T., Dale, P.O., Abyholm, T., 2000. Comparison between two hCG-to-oocyte aspiration intervals on the outcome of in vitro fertilization. J. Assist. Reprod. Genet. 17, 319–322.

Ding, L.J., Wang, B., Shen, X.Y., Yan, G.J., Zhang, N.Y., Hu, Y.L., Sun, H.X., 2013. Withdrawal of GnRH agonist decreases oestradiol and VEGF concentrations in high responders. Reprod. Biomed. Online 27, 131–139.

Evans, J., Salamonsen, L.A., 2013. Too much of a good thing? Experimental evidence suggests prolonged exposure to hCG is detrimental to endometrial receptivity. Hum. Reprod. 28, 1610–1619.

Fogle, R.H., Li, A., Paulson, R.J., 2010. Modulation of HOXA10 and other markers of endometrial receptivity by age and human chorionic gonadotropin in an endometrial explant model. Fertil. Steril. 93, 1255–1259.

Humaidan, P., Van Vaerenbergh, I., Bourgain, C., Alsbjerg, B., Blockeel, C., Schuit, F., Van Lommel, L., Devroey, P., Fatemi, H., 2012. Endometrial gene expression in the early luteal phase is impacted by mode of triggering final oocyte maturation in recFSH stimulated and GnRH antagonist co-treated IVF cycles. Hum. Reprod. 27, 3259–3272.

Keltz, M.D., Stein, D.E., Berin, I., Skorupski, J., 2012. Elevated progesterone-to-estradiol ratio versus serm progesterone alone for predicting poor cycle outcome with in vitro fertilization. J. Reprod. Med. 57, 9–12.

Licht, P., Lösch, A., Dittrich, R., Neuwinger, J., Siebzehnrübl, E., Wildt, L., 1998. Novel insights into human endometrial paracrinology and embryo-maternal communication by intrauterine microdialysis. Hum. Reprod. Update 4, 532–538.

Lin, H., Wang, W., Li, Y., Chen, X., Yang, D., Zhang, Q., 2011. Triggering final oocyte maturation with reduced doses of hCG in IVF/ICSI: a prospective, randomized and controlled study. Eur. J. Obstet. Gynecol. Reprod. Biol. 159, 143–147.

Liu, L., Zhao, L., Li, T.C., Zhu, H., Lin, X., Jin, X., Tong, X., Zhang, S., 2015. Comparison of progesterone measurement on day of, and day after, HCG administration in IVF-embryo transfer cycles. Reprod. Biomed. Online 30, 157–165.

Mansour, R., Tawab, N., Kamal, O., El-Faissal, Y., Serour, A., Aboulghar, M., Serour, G., 2011. Intrauterine injection of human chorionic gonadotropin before embryo transfer significantly improves the implantation and pregnancy rates in in vitro fertilization/intracytoplasmic sperm injection: a prospective randomized study. Fertil. Steril. 96, 1370–1374, e1.

Nargund, G., Hutchison, L., Scaramuzzi, R., Campbell, S., 2007. Low-dose HCG is useful in preventing OHSS in high-risk women without adversely affecting the outcome of IVF cycles. Reprod. Biomed. Online 14, 682–685.

Racicot, K.E., Wünsche, V., Auerbach, B., Aldo, P., Silasi, M., Mor, G., 2014. Human Chorionic Gonadotropin Enhances Trophoblast-Epithelial Interaction in an In Vitro Model of HumanImplantation. Reprod. Sci. 21, 1274–1280.

Sahin, S., Ozay, A., Ergin, E., Turkgeldi, L., Kürüm, E., Ozornek, H., 2015. The risk of ectopic pregnancy following GnRH agonist triggering compared with hCG triggering in GnRH antagonist ivf cycles. Arch. Gynecol. Obstet. 291, 185–191.

Santos-Ribeiro, S., Polyzos, N.P., Haentjens, P., Smitz, J., Camus, M., Tournaye, H., Blockeel, C., 2014. Live birth rates after IVF are reduced by both low and high progesterone levels on the day of human chorionic gonadotrophin administration. Hum. Reprod. 29, 1698–1705.

Schumacher, A., Brachwitz, N., Sohr, S., Engeland, K., Langwisch, S., Dolaptchieva, M., Alexander, T., Taran, A., Malfertheiner, S.F., Costa, S.D., Zimmermann, G., Nitschke, C., Volk, H.D., Alexander, H., Gunzer, M., Zenclussen, A.C., 2009. Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. J. Immunol. 182, 5488–5497.

Schumacher, A., Heinze, K., Witte, J., Poloski, E., Linzke, N., Woidacki, K., Zenclussen, A.C., 2013. Human chorionic gonadotropin as a central regulator of pregnancy immune tolerance. J. Immunol. 190, 2650–2658.

Shah, D.K., Missmer, S.A., Correia, K.F., Ginsburg, E.S., 2014. Pharmacokinetics of human chorionic gonadotropin injection in obese and normal-weight women. J. Clin. Endocrinol. Metab. 99, 1314–1321.

Sonntag, B., Loebbecke, K.C., Nofer, J.R., Kiesel, L., Greb, R.R., 2013. Serum estradiol and progesterone in the mid-luteal phase predict clinical pregnancy outcome inIVF/ICSI cycles. Gynecol. Endocrinol. 29, 700–703.

Tsampalas, M., Gridelet, V., Berndt, S., Foidart, J.M., Geenen, V., Perrier d'Hauterive, S., 2010. Human chorionic gonadotropin: a hormone with immunological and angiogenic properties. J. Reprod. Immunol. 85, 93–98.

von Grothusen, C., Lalitkumar, S., Rao Boggavarapu, N., Gemzell-Danielsson, K., Lalitkumar, P.G., 2014. Recent advances in understanding endometrial receptivity: molecular basis and clinical applications. Am. J. Reprod. Immunol. 72, 148–157.

WHO Expert Consultation, 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 363, 157–163.

Youssef, M.A., Van der Veen, F., Al-Inany, H.G., Mochtar, M.H., Griesinger, G., Nagi Mohesen, M., Aboulfoutouh, I., van Wely, M., 2014. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst. Rev. (10), CD008046.

Zarei, A., Parsanezhad, M.E., Younesi, M., Alborzi, S., Zolghadri, J., Samsami, A., Amooee, S., Aramesh, S., 2014. Intrauterine administration of recombinant human chorionic gonadotropin before embryo transfer on outcome of in vitro fertilization/intracytoplasmic sperm injection: a randomized clinical trial. Iran J. Reprod. Med. 12, 1–6.

Zhou, J., Wang, Z., Zhao, X., Wang, J., Sun, H., Hu, Y., 2012. An increase of treg cells in the peripheral blood is associated with a better in vitro fertilization treatment Outcome. Am. J. Reprod. Immunol. 68, 100–106.

Zhou, J., Zhao, X., Wang, Z., Wang, J., Sun, H., Hu, Y., 2013. High circulating CD3+CD56+CD16+ natural killer-like T cell levels predict a better IVF treatment outcome. J. Reprod. Immunol. 97, 197–203.

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