Circulating luteinizing hormone level after triggering oocyte maturation with GnRH agonist may predict oocyte yield in flexible GnRH antagonist protocol

Shi-Ling Chen*,†, De-Sheng Ye†, Xin Chen, Xin-Hong Yang, Hai-Yan Zheng, Yan Tang, Yu-Xia He, and Wei Guo

Center for Reproductive Medicine, Department of Gynecology and Obstetrics, Nanfang Hospital, Southern Medical University, Guangzhou 510515, People’s Republic of China

*Correspondence address. Tel: +86-20-62787604; Fax: +86-20-87280183; E-mail: chensl_92@163.com

Submitted on November 22, 2011; resubmitted on January 19, 2012; accepted on February 1, 2012

BACKGROUND: The use of gonadotrophin-releasing hormone (GnRH) agonist for triggering final oocyte maturation and ovulation can reduce ovarian hyperstimulation syndrome (OHSS) in high-risk patients. LH levels post-trigger with GnRH agonist might be correlated with oocyte yield and maturity. Our aim was to evaluate the relationship between serum LH level at 12-h post-trigger and oocyte yield, maturity, and fertilization rate in patients at high risk of OHSS and therefore who were treated with a flexible GnRH antagonist protocol in which final oocyte maturation was triggered with GnRH agonist.

METHODS: In a prospective cohort study, 91 patients at high risk of OHSS were treated with a flexible GnRH antagonist protocol and divided into six groups according to their serum LH levels at 12-h after GnRH agonist administration: ≤ 15.0, 15.1–30.0, 30.1–45.0, 45.1–60.0, 60.1–75.0 and >75.0 IU/l. The oocyte yield, maturity, fertilization rate and clinical outcomes for each LH interval were analyzed.

RESULTS: There was a statistically significant reduction in oocyte yield with a concentration of serum LH ≤ 15.0 IU/l (P < 0.05), whereas no statistically significant differences in the oocyte maturity and fertilization rate among the six groups (P > 0.05) were seen. Only 5 out of 91 patients (5.5%) had a serum LH ≤ 15.0 IU/l at 12-h post-trigger with GnRH agonist. In addition, no statistically significant difference was seen regarding high-quality embryos, implantation rate, clinical pregnancy rate and early miscarriage between patients with LH ≤ 15.0 IU/l and >15.0 IU/l (P > 0.05).

CONCLUSIONS: Serum LH level at 12-h post-trigger with GnRHa ≤ 15.0 IU/l is associated with a dramatically lower oocyte yield but not with the oocyte maturity and fertilization rate. Serum LH levels post-trigger with GnRH agonist do not affect clinical outcomes.

Key words: GnRH agonist / GnRH antagonist / LH level / oocyte maturation

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a rare iatrogenic and potentially life-threatening complication during controlled ovarian stimulation. As an alternative to hCG, the use of gonadotrophin-releasing hormone (GnRH) agonist for triggering final oocyte maturation and ovulation can reduce OHSS in patients at high risk of OHSS (Babayof et al., 2006; Engmann et al., 2008; Humaidan et al., 2010). This is due to the short half-life of the elicited endogenous LH and FSH surge triggered by GnRH agonist are more physiological and the simultaneous FSH surge is known to promote LH receptor formation in luteinizing granulosa cells, nuclear maturation (Zelinski-Wooten et al., 1995; Yding, 2002) and cumulus expansion (Eppig, 1979; Strickland and Beers, 1976).

Triggering final oocyte maturation and ovulation with GnRH agonist has been known to be dependent solely on LH activity. Studies on the relationship between an LH surge and oocyte maturation in humans (Seibel et al., 1982) and monkeys (Zelinski-Wooten et al., 1991) after triggering with GnRH agonist compared with hCG (Imoedemhe et al., 1991; Humaidan et al., 2005). This increase may be due to the possibility that the endogenous LH and FSH surge triggered by GnRH agonist are more physiological and the simultaneous FSH surge is known to promote LH receptor formation in luteinizing granulosa cells, nuclear maturation (Zelinski-Wooten et al., 1995; Yding, 2002) and cumulus expansion (Eppig, 1979; Strickland and Beers, 1976).

These authors contributed equally to this work.
have suggested that 14–18 h of an LH surge were required to reinitiate meiosis. Furthermore, only 5% of the normal LH surge amplitude is necessary for oocyte maturation, whereas 85% of the surge is required for ovulation in rats (Peluso, 1990).

Recently, two patients with polycystic ovarian syndrome (PCOS) were reported to fail in oocyte retrieval post-trigger with GnRH agonist, but a successful recovery was attained using hCG 36 h later (Honnma et al., 2011). In addition, a study demonstrated that an LH level at 12-h post-trigger with GnRH agonist is necessary for oocyte retrieval. The authors hypothesized that failure to trigger oocyte maturation may be due to a shorter duration and/or a lower LH level post-trigger with GnRH agonist.

The aim of the present study was to investigate the relationship between serum LH level at 12-h post-trigger with GnRH agonist and oocyte yield, maturity and fertilization rate in patients at high risk of OHSS and treated with a flexible GnRH antagonist protocol for undergoing in vitro fertilization and embryo transfer (IVF-embryo transfer).

Materials and Methods

Subjects
This was a prospective cohort study of all women attending the Center for Reproductive Medicine, Department of Gynecology and Obstetrics, Nanfang Hospital, affiliated with Southern Medical University for IVF and/or ICSI from September 2009 to July 2011. Women at high risk of OHSS who received IVF and/or ICSI treatment with a flexible GnRH antagonist protocol were recruited to participate in this study. This study was approved by the ethics committee of Nanfang Hospital and written informed consent was obtained from each participant. The inclusion criteria for patients at high risk of OHSS were as follows: (i) patients with PCOS (The RotterdamESHRE/ASRM-sponsoredPCOS Consensus Workshop Group, 2004) or polycystic ovarian morphology on ultrasound (Balen et al., 2003); (ii) patients who previously experienced an ovarian stimulation cycle, with a high response to gonadotrophins. Patients were excluded due to coasting and past ovarian surgery.

Ovarian stimulation protocols
All patients underwent pretreatment with oral contraceptive pills (OCPs) starting on Day 2 of spontaneous menses of the cycle or induced menses prior to the treatment cycle, after blood tests confirmed the presence of a baseline hormone profile. Ovarian stimulation was initiated on Day 2 of withdrawal bleeding with recombinant FSH (rFSH; Gonal F, Merck Serono, Modugno, Italy) or highly purified FSH (HP-FSH; Lishenbao, Livzon, Guangdong, China). The gonadotrophin started at 112.5–225.0 IU per day for 4 days according to age, body mass index (BMI), antral follicle count (AFC) and previous ovarian response. Thereafter, the dose was adjusted on the basis of ovarian response and serum E2, Cetrotrelax (Cetrotide, Merck Serono, Halle, Germany) of 0.25 mg per day was initiated when at least one of the following criteria was reached: (i) the presence of at least one follicle measuring ≥14 mm, (ii) serum E2 ≥600 pg/ml or (iii) serum LH level ≥10 IU/l (Lainas et al., 2010) and was continued until and including the day of trigger. When at least three follicles reached 17 mm or two follicles reached 18 mm in diameter, and at least one of the following criteria was reached: (i) serum E2 ≥3500 pg/ml, (ii) ≥25 follicles measuring ≥10 mm or (iii) 18–24 follicles measuring ≥10 mm and serum E2 ≥3000 pg/ml and <3500 pg/ml, a single bolus of 0.2 mg triptorelin (Diphereline, IPSEN, Signes, France) was administered for triggering final oocyte maturation. Oocytes were retrieved transvaginally 34–38 h after triptorelin administration. Conventional IVF and/or ICSI were performed as indicated. Two or three embryos were transferred on Days 2–3 after oocyte pick-up (OPU). The luteal phase was supported with 60 mg of progesterone in oil and 6 mg of estradiol (Prognova, Schering, Lys llez Lannoy, French), starting on the day of OPU and continuing until 8 weeks of gestation in the presence of a positive hCG test. A single dose of 2000 IU hCG was administered on the day of OPU to supplement corpus luteum function.

Hormone measurement
Basal values of FSH, LH and E2 were routinely determined before the initiation of OCPs, ovulation induction and GnRH antagonist. Serum E2, LH and P were measured on the day of, and approximately at 12 h after, GnRH agonist administration. The serum FSH, LH, E2 and P concentrations were measured using electrochemiluminescence immunoassay (cobas e601, Roche Diagnostics GmbH, Germany). The inter-assay coefficients of variation (CV) for FSH, LH, E2 and P were 4.5, 2.2, 4.9 and 4.8%, respectively. The intra-assay CV for FSH, LH, E2 and P were 2.8, 1.2, 3.3 and 2.9%, respectively.

Definition of oocyte yield, maturity and fertilization rate
Oocyte yield was defined as the ratio of the total number of collected oocytes to the number of follicles measuring ≥10 mm on the day of oocyte retrieval. Oocyte maturity was defined as the ratio of MII oocytes to the number of collected oocytes in the patients undergoing with ICSI. Fertilization rate was defined as the ratio of normal fertilized oocytes (2PNs) to the number of oocytes used for fertilization (i.e. the denominator in IVF in calculating fertilization rate is all oocytes recovered, but in ICSI it is calculated using only the number of MII oocytes).

Statistical analysis
Patients were divided into six groups according to their serum LH levels at 12 h after GnRH agonist administration: ≤15.0, 15.1–30.0, 30.1–45.0, 45.1–60.0, 60.1–75.0 and >75.0 IU/L. Oocyte yield, maturity and fertilization rate were calculated for each LH interval and assessed for trend analysis. Data were analyzed using Student’s t-test and χ², as appropriate. A P < 0.05 was considered statistically significant. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS, version 16.0 for Windows).

Results
There were 220 patients who gave informed consent to participate in the study during their IVF and/or ICSI cycle at our center between September 2009 and July 2011. Of those, 109 patients at high risk of OHSS met the inclusion criteria of GnRH agonist trigger. A total of 18 patients were excluded due to coasting (n = 9) and past ovarian surgery (n = 9; Fig. 1). All patients were treated with a flexible GnRH antagonist protocol and received routine luteal phase support. However, two patients had to postpone the administration of 2000 IU hCG on the day of embryo transfer due to extremely high E2 levels, and one of them was coasted for 2 days. Both of them developed
severe OHSS. Thereafter we discontinued supplementing hCG as luteal phase support in later patients.

Baseline characteristics, hormone profiles and laboratory parameters of the 91 patients are shown in Table I. The average age and BMI were 29.3 years (range: 20–38 years) and 20.8 kg/m² (range: 17.1–27.0 kg/m²), respectively. The mean level of serum LH at 12-h post-trigger was 46.6 ± 23.2 IU/l (range: 9.7–151.2 IU/l). Oocyte maturity was calculated only for patients undergoing ICSI. Oocyte yield, maturity and fertilization rate were 61.1, 78.3 and 71.5%, respectively.

The relationships between LH level at 12-h post-trigger and oocyte yield, maturity and fertilization rate are shown in Fig. 2. As shown, there was a statistically significant reduction in oocyte yield with concentrations of serum LH ≤ 15.0 IU/l (Fig. 2A). Furthermore, the difference was statistically significant only between the ≤ 15.0 and 15.1–30.0 IU/l intervals (38.3 versus 68.8%, P < 0.05; Fig. 2A). These data suggest that a serum LH concentration of 15.0 IU/l at

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n = 91</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>29.3 ± 3.6</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.8 ± 2.2</td>
</tr>
<tr>
<td>Duration of infertility, years</td>
<td>4.4 ± 2.5</td>
</tr>
<tr>
<td>Procedure, % (n)</td>
<td></td>
</tr>
<tr>
<td>IVF</td>
<td>56.0 (51)</td>
</tr>
<tr>
<td>IVF/ICSI</td>
<td>20.9 (19)</td>
</tr>
<tr>
<td>ICSI</td>
<td>23.1 (21)</td>
</tr>
<tr>
<td>Basal FSH, IU/l</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td>Basal LH, IU/l</td>
<td>7.0 ± 4.0</td>
</tr>
<tr>
<td>Basal E₂, pg/ml</td>
<td>43 ± 30</td>
</tr>
<tr>
<td>E₂ at 12-h post-trigger, pg/ml</td>
<td>5201 ± 1943</td>
</tr>
<tr>
<td>LH at 12-h post-trigger, IU/l</td>
<td>46.6 ± 23.2</td>
</tr>
<tr>
<td>P at 12-h post-trigger, ng/ml</td>
<td>8.8 ± 4.2</td>
</tr>
<tr>
<td>Follicles ≥ 10 mm on the day of OPU</td>
<td>27.3 ± 7.8</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>16.7 ± 6.4</td>
</tr>
<tr>
<td>Oocyte yield, % (n)</td>
<td>61.1 (1519/2486)</td>
</tr>
<tr>
<td>Oocyte maturity, ICSI, % (n)</td>
<td>78.3 (318/406)</td>
</tr>
<tr>
<td>Fertilization rate, % (n)</td>
<td>71.5 (1011/1414)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or percentage (number), unless otherwise noted.

Figure 2 Relationship between serum LH level at 12-h post-trigger with GnRH agonist and oocyte yield (n = 91, A), maturity (ICSI patients, n = 21, B) and fertilization rate (n = 91, C). *P < 0.05 for comparison with previous LH level interval; ^trend analysis using χ². LH, luteinizing hormone.
12-h post-trigger may represent the critical threshold level below which there might be a negative impact on oocyte yield. No significant differences were observed in oocyte maturity or fertilization rate among the groups (Fig. 2B and C).

In the 91 patients, only 5 (5.5%) had a serum LH ≤ 15.0 IU/l at 12-h post-trigger with GnRH antagonist. The LH levels on Day 2 of menses, on the day administered with gonadotrophin, on the day administered with GnRH antagonist and on the trigger day in patients with LH > 15.0 IU/l were higher than those with LH ≤ 15.0 IU/l; however, only LH levels on the days administered with gonadotrophin and GnRH antagonist were significantly different (Table II). Patients with LH > 15.0 IU/l demonstrated significantly more oocytes retrieved and a higher oocyte yield compared with those with LH ≤ 15.0 IU/l (Table II). There were no statistically significant differences regarding high-quality embryos and embryos transferred (Table II). Patients with LH level > 15.0 IU/l at 12-h post-trigger were further grouped according to whether hCG was supplemented on the day of oocyte retrieval or not. The implantation rate, clinical pregnancy rate and early miscarriage were comparable among the three groups (Table III).

### Discussion

This study shows that GnRH agonist (triptorelin of 0.2 mg) is sufficient for triggering final oocyte maturation in GnRH antagonist protocol cycles. Only 5.5% of 91 patients had a lower serum LH at 12-h post-trigger with GnRH agonist. A serum LH level of 15.0 IU/l at 12-h post-trigger with GnRH agonist was identified as an appropriate threshold to define an adequate level for oocyte yield. The findings of this study also demonstrate that oocyte maturity, fertilization rate and clinical outcomes were comparable despite the serum LH levels at 12-h post-trigger with GnRH agonist.

The correlation between serum LH level at 12-h post-trigger with GnRH agonist and oocyte yield and maturity was assessed recently (Shapiro et al., 2011b). The study showed that LH level at 12-h post-trigger < 52.0 IU/l was suboptimal with the risk of submaximal oocyte yield and maturity, while < 12.0 IU/l was clearly inadequate and associated with dramatically lower oocyte yield and maturity (Shapiro et al., 2011b). Our study showed that LH levels < 15.0 IU/l were inadequate for oocyte yield, which was similar to 12.0 IU/l in the study by Shapiro et al. However, our results do not support that lower LH levels may have a dramatic effect on oocyte maturity. In contrast to the present study, Shapiro et al. used a defined low oocyte yield and maturity combined with a receiver operating characteristic curve analysis to estimate the impact of LH levels on the outcome of oocyte retrieval. We believe that the defined low oocyte and maturity might not be suitable for the assessment, as there is a scarcity of references. Moreover, the definition of oocyte maturity was different between the two studies (the ratio of MII oocytes to the number of oocytes retrieved in the present study versus the ratio of MII oocytes to the number of follicles > 10 mm on the trigger day in the study by Shapiro et al.).

Oocyte yield but not oocyte maturation or fertilization rate was significantly different between LH > 15.0 IU/l and ≤ 15.0 IU/l in the present study, which could be explained by different requirements of level and/or duration of gonadotrophin for oocyte maturation and ovulation. As the resumption of meiosis required 14–18 h of an LH surge (Seibel et al., 1982; Zelinski-Wootten et al., 1991), and the threshold level for follicular rupture was higher than that for induction of oocyte maturation (Peluso, 1990), we hypothesize that higher LH levels (> 15.0 IU/l in the present study) continuing for > 14–18 h
might be required for expanding cumulus cells that lead to ovolation in natural cycles or oocyte retrieval in IVF cycles. However, compared with natural cycles (Zelinski-Wooten et al., 1991) and cycles triggered with hCG, a shorter duration and a lower amount of gonadotrophin were triggered by GnRH agonist (Hoff et al., 1983), in which an effective duration of action might be shortened. Further investigation is required to analyze whether a threshold level and duration of LH level post-trigger are necessary for oocyte yield.

The relationship between LH level at 12-h post-trigger and oocyte yield could be used to predict and optimize oocyte yield. In the study by Shapiro et al. (2008), patients at high risk of OHSS received leuprolide and hCG concomitantly on the trigger day in a GnRH antagonist protocol. No OHSS was diagnosed in any patients. The serum LH and hCG on the day post-trigger were 69.1 ± 36.5 IU/l and 62.2 ± 35.4 IU/l. The average number of oocytes retrieved was 20.4 ± 6.0. Interestingly, a patient with LH of 11.9 IU/l and hCG of 40 IU/l 9.6 h post-trigger had 29 oocytes retrieved, 24 of which were MII. The study implies that a low LH level could be compensated by hCG to increase oocyte yield. However, according to the relationship between hCG and OHSS, whether hCG is safe and effective to rescue the low LH in patients at high risk of OHSS requires further investigation.

Regarding hCG as the evocation of OHSS, other methods such as exogenous gonadotrophin and repeated GnRH agonist might be substituted for hCG. Repeated injections of GnRH agonist have been reported to increase the duration of LH secretion (LH level was >100 IU/l) up to >14 h (Zelinski-Wooten et al., 1991). Although the prolonged duration is significantly shorter than that of a natural cycle, it might be enough for the ovulation process. Recently a study found that the intrafollicular FSH concentration was significantly higher when an oocyte was retrieved rather than not retrieved despite the use of a stimulation protocol (Rosen et al., 2009). Furthermore, a statistically significant improvement in oocyte recovery with a concomitant FSH/hCG trigger injection, compared with conventional hCG trigger alone in routine GnRH agonist long protocol (Lamb et al., 2011), illustrated that FSH might promote LH receptor formation in luteinizing granulosa cells, nuclear maturation (Zelinski-Wooten et al., 1995; Yding, 2002) and cumulus expansion (Eppig, 1979; Strickland and Beers, 1976). Nevertheless, whether repeated GnRH agonist or an additional FSH bolus administered at the time of trigger in GnRH antagonist protocol can improve oocyte yield requires further investigation.

Comparable clinical outcomes between patients with LH >15.0 IU/l and LH ≤15.0 IU/l suggests that LH levels post-trigger with GnRH agonist may not affect clinical outcomes. The clinical outcomes we obtained in patients with LH >15.0 IU/l post-trigger were consistent with prior studies. These studies showed that supplementing with a low dose of hCG on the day of trigger (Shapiro et al., 2011a) or on the day of oocyte retrieval (Humaidan, 2009) for luteal corpus rescue improved clinical outcomes in a GnRH antagonist protocol with a GnRH agonist trigger in high-risk patients (Humaidan, 2009; Shapiro et al., 2011a).

In conclusion, our study shows that a serum LH level at 12-h post-trigger with GnRH agonist <15 IU/l is associated with a dramatically lower oocyte yield but not with changes in oocyte maturity and fertilization rate in GnRH antagonist protocol. Furthermore, serum LH levels post-trigger might not affect the clinical outcomes. Whether additional hCG or FSH or repeated GnRH agonist administration is safe and effective to rescue low LH levels post-trigger with GnRH agonist in patients at high risk of OHSS requires further investigation.

Acknowledgements

The authors are grateful to Le-Le Wang, Ya-Qin Wu, Chen Luo, Wei-Qing Zhang, Zhuo-Lin Qiu and Jie Yang from the Center for Reproductive Medicine, Department of Gynecology and Obstetrics, Nanfang Hospital for their technical support and valuable suggestions.

Authors’ roles

All the authors contributed substantially to the paper. S.-L.C. and D.-S.Y. contributed to the whole conception and clinical design, acquisition of data, analysis and interpretation of data and drafted the article and revised it critically for important intellectual content. X.C., X.-H.Y. and H.-Y.Z. made an effort to control the quality of this clinical trial, and contributed to acquisition of data and analysis of data. Y.T., Y.-X.H. and W.G. contributed to acquisition of data. All the authors revised it critically and approved the final form of manuscript prior to submission.

Funding

This work was supported by National Key Basic Research Development Project of China (973 Program; 2007CB948104), Comprehensive Strategic Sciences Cooperation Projects of Guangdong Province and Chinese Academy (04020416) and Guangzhou Science and Technology Program Key Projects (11C22120737).

Conflict of interest

All authors declare that they have no conflict of interest.

References


