Continuous use of gonadotropin-releasing hormone (GnRH)-agonist maintains high luteinizing hormone concentrations at the time of oocyte retrieval in women undergoing GnRH-agonist long protocol assisted reproductive technology treatment and stimulation with recombinant follicle-stimulating hormone

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Abstract

Introduction: The objective of this study was to evaluate the changes in serum luteinizing hormone (LH) concentrations in infertile women undergoing continuous gonadotropin-releasing hormone (GnRH)-agonist long protocol and rec-follicle-stimulating hormone (rec-FSH) assisted reproductive technology (ART) treatment from the time of human chorionic gonadotrophin (hCG) administration until oocyte retrieval.

Material and methods: Between October 2006 and October 2007, 100 GnRH-agonist long protocol and rec-FSH ART treatment cycles from 86 patients who underwent ART were divided into two groups according to the duration of GnRH-agonist administration. Assisted reproductive technology treatment cycles during which administration of GnRH-agonist was stopped 36-40 h before oocyte retrieval comprised the standard GnRH-a group (n = 37). The remaining cycles during which GnRH-agonist was administered until oocyte retrieval constituted the continuous GnRH-a group (n = 63). Hormonal profiles and ART outcomes were compared between treatments in a retrospective study.

Results: On the day of hCG administration, LH concentrations in the standard (1.1 ±0.1 IU/l) and continuous (1.3 ±0.4 IU/l) GnRH-a groups were similar. However, on oocyte retrieval, LH concentration of the standard group was dramatically reduced (0.1 ±0.003 IU/l), while that of the continuous GnRH-a group remained unchanged and was significantly greater than the standard group (0.6 ±0.1 IU/l, p < 0.0001). The standard and continuous GnRH-a treatment groups exhibited comparable pregnancy rates (18.8% in the standard group and 24.1% in the continuous group).

Conclusions: Continuous administration of GnRH-agonist until oocyte retrieval maintains high LH concentrations and does not negatively affect ART outcome.

Key words: assisted reproductive technology, gonadotropin-releasing hormone agonist, luteinizing hormone, long protocol, rec-follicle-stimulating hormone, rec-luteinizing hormone.

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Introduction

Gonadotrophin-releasing hormone (GnRH)-agonists and gonadotrophins were first used in assisted reproductive technology (ART) treatment for ovarian stimulation in 1984 [1]. Since that time, GnRH-agonists have become a key component of successful ART treatment and numerous studies have demonstrated the efficacy of this treatment protocol. Consequently, GnRH-agonists are widely used for ovarian stimulation during ART treatment, and the vast majority of ART programmes use this approach as the first choice for controlled ovarian hyperstimulation. During the 1990s, the definition of the optimal GnRH-agonist protocol was a topic of considerable debate. One review article stated that the GnRH-agonist long protocol was superior to the short and ultra-short protocols [2]. Currently, GnRH-agonist induced ovarian stimulation during ART treatment is indispensable for the following reasons: reduction in the number of cancelled cycles; prevention of asynchronous follicular development; and augmentation of the number of oocytes collected.

Typically, administration of GnRH-agonist is stopped on the day of human chorionic gonadotrophin (hCG) administration, regardless of protocol duration, i.e., long, short or ultra-short protocols [3]. The primary function of using GnRH-agonist is to prevent a premature luteinizing hormone (LH) surge. Hence, GnRH-agonist is stopped on the day of hCG administration [4]. Oocyte retrieval is performed approximately 36 h after cessation of the GnRH-agonist. During this time, oestradiol (E2) and follicle-stimulating hormone (FSH) concentrations decrease rapidly because ovarian stimulation and GnRH-agonist are stopped simultaneously. However, changes in LH concentrations at the time of oocyte retrieval have not been evaluated because the gonadotrophins typically used for ovarian stimulation contain high levels of FSH and low levels of LH. Moreover, FSH is thought to play a predominant role in follicular growth.

The object of this study was to evaluate changes in serum LH concentrations in infertile women undergoing continuous GnRH-a long protocol and rec-FSH ART treatment from the time of hCG administration until oocyte retrieval.

Material and methods

Patients and ovarian hyperstimulation

Eighty-six normogonadotropic patients who underwent ART treatment at the division of Reproductive Medicine, Department of Perinatal Medicine and Maternal Care, National Centre for Child Health and Development, were recruited into this study between October 2006 and October 2007. Informed consent was obtained from all patients and the study was approved by the Institutional Review Board of the National Centre for Child Health and Development.

All treatment cycles ($n = 100$) for the 86 patients were hyperstimulated using a GnRH-agonist long protocol with rec-FSH as previously described [5]. Briefly, ovarian hyperstimulation using our GnRH agonist protocol was performed as follows: 600 μg per day of buserelin acetate (Buserecur, Fuji Pharma, Tokyo) was administered intranasally starting in the midluteal phase of the pre-treatment menstrual cycle and was continued until the day of human chorionic gonadotropin (hCG) injection. On days three and four of the menstrual cycle, 225 International Units (IU) of rec-FSH (Follistim, Organon, Osaka, Japan) were administered, 150 IU rec-FSH were administered on subsequent days until a dominant follicle reached 16 mm in diameter. In our stimulation protocol, the daily dosage of rec-FSH does not alter follicle growth or individual characteristics. Oocyte retrieval was performed 35 h after administration of 10,000 IU of hCG.

IVF/intracytoplasmic sperm injection procedure and embryo transfer

The IVF procedure used in this study has been previously described [6]. Oocytes were retrieved transvaginally using a needle-guided technique, aided by ultrasonography. All follicles with a mean diameter of > 15 mm were aspirated individually, using an 18-gauge needle connected to a tube and a 20-ml syringe for suction. The needle was removed after aspiration of each follicle. Aspiration was interrupted and a new syringe was used if blood appeared in the tube connected to the syringe, thus avoiding contamination with blood. Culture medium was not used to wash the follicle. Semen was produced by masturbation and, after washing, motile sperm were separated using a 30-60 min swim-up period. In vitro insemination was performed by the incubation of each oocyte with 50-100 × 10³ motile sperm within 5-6 h of collection. In vitro insemination was not performed when there was evidence of male factor infertility; instead, intracytoplasmic sperm injection (ICSI) was performed, as previously described [7]. Oocytes were examined using a dissecting microscope 16-18 h after insemination or ICSI. The presence of two pronuclei with extrusion of the second polar body was taken as evidence of successful fertilization.

Embryos were replaced into the patient's uterus transcervically 72 h after insemination or ICSI. The number of transferred embryos was no more than two to prevent multiple pregnancies. On days 1, 4 and 7 following embryo transfer (ET), 3,000 IU hCG were injected for luteal support. A combination

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of oestrogen and progesterone was administered orally for 10 days after ET. Hydroxyprogesterone caproate, rather than hCG, was given every 4 days to patients at elevated risk for ovarian hyperstimulation syndrome.

Hormone assays and comparison of assisted reproductive technology parameters

Serum FSH, LH, E2, and progesterone (P) concentrations were measured on the day of hCG administration and oocyte retrieval. Follicle-stimulating hormone was assayed using a commercially available ELISA kit (DPC IMMULIZE FSH (2000), Diagnostic Products Corporation, Los Angeles, CA). The lower limit of detection for FSH was 0.1 IU/l, and the inter- and intra-assay coefficients of variation were 7.2 and 4.9%, respectively. The concentration of LH in serum was measured using a chemiluminescent microparticle immunoassay (ARCHITECT LH®; Abbott Japan, Tokyo). The lower limit of detection for LH was 0.07 IU/l, and the inter- and intra-assay coefficients of variation were 2.1 and 3.3%, respectively. Serum E2 and P were assayed using a commercially available RIA kit (IMMULIZE 2000; Diagnostic Products Corporation, Los Angeles, CA).

Embryos that had developed to at least the 8-cell stage with less than 10% fragmentation on day 3 following oocyte retrieval were considered usable. Pregnancy was defined as the presence of an intrauterine gestational sac by transvaginal ultrasonography 21 days after oocyte retrieval. Miscarriage was defined as a pregnancy that was lost before 22 weeks of gestation.

According to the standard GnRH-agonist long protocol, GnRH-agonist administration is stopped 36-40 h before oocyte retrieval. Thus, the 100 cycles examined in this retrospective study were divided into two groups based on the duration of GnRH-agonist administration. The standard group included cycles during which administration

of GnRH-agonist was stopped 36-40 h before oocyte retrieval. The remaining cycles, during which GnRH-agonist was used until oocyte retrieval, comprised the continuous GnRH-a group. Hormonal profiles and ART parameters were compared between treatments.

Statistical analysis

All data are presented as the mean ± SEM (standard error of the mean). Significance of the differences between groups was determined using the unpaired t-test and the χ^2 test.

Results

Of the 100 ART treatment cycles examined, 37 were classified as standard GnRH-agonist treatment cycles and the remaining cycles were classified as continuous (Table I). The two groups were similar in age and there were no differences in ART-treatment indications between groups. In both groups, most cases of male factor infertility were treated using ICSI. In the continuous GnRH-a group, the average gonadotrophin dose administered until oocyte retrieval was 1,774 ±47 IU and the duration of stimulation was 10.3 ±0.3 days. The gonadotrophin dose and duration were higher in the continuous group compared with the standard group (1,616 ±49 IU, $p = 0.029$ and 9.5 ±0.2 days, $p = 0.042$, respectively). The numbers of retrieved and fertilized oocytes in the continuous GnRH-a group were 7.3 ±0.6 and 4.3 ±0.5, respectively; these values were significantly lower than those in the standard group (8.1 ±0.5, $p = 0.049$ and 5.6 ±0.5, $p = 0.071$, respectively). However, the number of usable embryos in the continuous GnRH-a group (1.9 ±0.3) and standard group (2.6 ±0.4) were similar. The pregnancy rate per embryo transfer cycle for the continuous GnRH-a group (24.1%) was also comparable to that observed for the standard group (18.8%) (Table II).

Changes of hormone concentrations from the time of hCG administration to oocyte retrieval are summarized in Table III. Mean LH concentrations did not differ between standard (1.1 ±0.1 IU/l) and continuous GnRH-a (1.3 ±0.4 IU/l) groups on the day of hCG administration. However, on the day of oocyte retrieval, the LH concentration in the continuous GnRH-a group (0.6 ±0.1 IU/l) was significantly higher than that in the standard group (0.1 ±0.003 IU/l, $p < 0.0001$). Changes in FSH concentration during the same period revealed a pattern similar to that observed for changes in LH. Mean FSH concentrations were similar in the standard (12.1 ±0.7 IU/l) and continuous GnRH-a (13.4 ±0.5 IU/l) groups on the day of hCG administration. However, on the day of oocyte

Table I. Backgrounds of groups with or without continuous GnRH-agonist administration

	Standard group	Continuous GnRH-a group	Difference
Number of stimulated cycles	37	63	
Average age [years]*	38.4 ±0.6	38.5 ±0.5	n.s.
Indications of ART			
tubal factor	8	10	n.s.
male factor	5	12	n.s.
endometriosis	6	11	n.s.
Unexplained infertility	18	30	n.s.
IVF/ICSI	31/6	53/10	n.s.

*Values are mean ± SE, n.s. – no statistical difference

Table II. Several ART parameters of groups with or without continuous GnRH-agonist administration

	Standard group	Continuous GnRH-a group	P value
Total doses of gonadotropins [IU]	1616 ±49	1774 ±47	0.029
Stimulation period [days]	9.5 ±0.2	10.3 ±0.3	0.042
Endometrial thickness on hCG [mm]	10.9 ±0.4	11.1 ±0.3	0.767
Number of retrieved oocytes	9.4 ±0.8	7.3 ±0.6	0.049
Number of fertilized oocytes	5.6 ±0.5	4.3 ±0.5	0.071
Number of usable oocytes	2.6 ±0.4	1.9 ±0.3	0.185
Clinical pregnancy rates [%; /ET]	18.8	24.1	0.5687

*All values are mean ± SE except clinical pregnancy rates

Table III. Hormone profiles of groups with or without continuous GnRH-agonist administration

	Standard group	Continuous GnRH-a group	P value
LH levels on hCG [IU/l]	1.1 ±0.1	1.3 ±0.1	0.280
LH levels on OPU [IU/l]	0.1 ±0.003	0.6 ±0.1	< 0.0001
FSH levels on hCG [IU/l]	12.1 ±0.7	13.4 ±0.5	0.127
FSH levels on OPU [IU/l]	5.6 ±0.3	7.2 ±0.3	0.0008
E2 levels on hCG [ng/ml]	2045 ±222	1830 ±182	0.464
E2 levels on OPU [ng/ml]	813 ±79	787 ±87	0.8456
P levels on hCG [pg/ml]	0.77 ±0.07	0.65 ±0.06	0.251
P levels on OPU [pg/ml]	7.2 ±0.9	5.2 ±0.5	0.0347

* All values are mean ± S.E except clinical pregnancy rates, OPU – oocyte pick up

retrieval, the FSH concentration in the continuous GnRH-a group (7.2 ±0.3 IU/l) was significantly greater than the standard group (5.6 ±0.3, IU/l, $p = 0.0008$). E2 concentrations were similar in the standard and continuous groups on both days. However, the P concentration of the standard group was significantly greater than the continuous GnRH-a group on the day of oocyte retrieval ($p = 0.0347$).

Discussion

The lower detection limits for the conventional methods used to determine LH concentrations – ELIZA and RIA – were 0.7 and 1.0 IU/l, respectively. However, the LH concentrations in the sera of patients undergoing ART treatment with the GnRH-agonist long protocol and rec-FSH were often below the detection limit during the late follicular phase. However, because the chemiluminescent assay had a lower limit of detection (0.1 IU/l), changes in LH concentration during the late follicular phase could be measured using this method. Consequently, we were able to investigate the role of LH in ovarian stimulation induced by rec-FSH during ART treatment using the GnRH-agonist long protocol. Follicle-stimulating hormone SH concentrations measured in patient sera reflected both endogenous FSH and exogenous FSH administered during ovarian stimulation with rec-FSH. However, detected LH reflected only endogenous LH because the rec-FSH showed no LH activity.

Many clinicians have assumed that during adequate down-regulation of the pituitary gland resulting from GnRH-agonist administration, secretion of endogenous LH is inhibited. Indeed, this assumption has been a presumed weakness of the GnRH-agonist long protocol with rec-FSH. Several recent reports demonstrated improved ART outcome with exogenous rec-LH supplementation during ovarian stimulation [8-10].

In the present study, we found that on the day of hCG administration, LH concentrations were detectable in both the standard and continuous GnRH-a groups. These data suggest that secretion of endogenous LH from the pituitary gland is maintained during GnRH-agonist administration. It has been suggested that GnRH-agonist stimulates the pituitary gland via a flare-up mechanism and, consequently, LH concentrations are not low during GnRH-agonist treatment. In patients undergoing an ultra-short GnRH-agonist ART treatment protocol, a spontaneous LH surge was observed approximately 10 days after cessation of nasal GnRH-agonist spray, indicating recovery of hypothalamic and pituitary function by that time

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[11]. In addition, we have also observed a second flare-up response after re-initiation of GnRH-agonist therapy after cessation for 48 h. This observation implies that the low level of gonadotropin release after cessation of the GnRH-agonist is due to low endogenous GnRH activity, and not a lack of pituitary responsiveness [12].

Surprisingly, on the day of oocyte retrieval, the LH concentration in the standard group was extremely low. This result may be explained by the rapid and significant decline in endogenous LH secretion from the pituitary gland after cessation of GnRH-agonist stimulation. Sungurtekin *et al.* also observed a decrease in LH concentrations after cessation of GnRH-agonist in patients treated with an ultra-short GnRH-agonist protocol and ovarian stimulation with urinary FSH derivatives [12]. Thus, these results suggest that GnRH-agonists do not completely suppress LH secretion, i.e., secretion of endogenous LH from the pituitary gland may occur even after down-regulation of the pituitary gland has been achieved. We suspected that this mechanism might prevent the decline in LH that is associated with use of standard protocols, such as the GnRH-agonist long protocol. Therefore, we decided to administer the GnRH-agonist continuously until the day of oocyte retrieval (continuous GnRH-a group). In patients administered continuous GnRH-agonist, LH concentrations on the days of oocyte retrieval and hCG administration were similar; therefore, continuous use of GnRH-agonist prevented the decline in LH. The success of this protocol is significant for patients whose GnRH-agonist long protocol ART treatment failed due to low LH concentrations during the late-follicular phase.

However, in the present study, the dose of gonadotrophin used and duration of stimulation in the continuous GnRH-a group were significantly greater than the standard group. Consequently, the numbers of retrieved and fertilized oocytes in the continuous GnRH-a group were also lower than those in the standard group. There is no theoretical explanation for these negative findings in the continuous GnRH-a group. All patients were stimulated under the same conditions, using the same protocol. The only difference between groups was the administration of GnRH-agonist after the hCG administration until oocyte retrieval. Despite the deduction in retrieved and fertilized oocytes in the continuous group, the number of usable embryos and pregnancy rates in the continuous and standard groups were comparable. From this point of view, continuous use of GnRH agonist until oocyte retrieval did not appear to negatively affect ART outcomes. The results of the present study contradict the commonly held assumptions that GnRH-agonist

administration lowers LH concentrations and that cessation of GnRH-agonist administration improves the hormonal profile. Rather, we found that cessation of GnRH-agonist administration after hCG injection had an adverse effect on LH concentrations.

The combination of GnRH-a down-regulation with FSH results in lower LH concentrations than in the normal menstrual cycle. However, recent trials have shown that pure FSH is effective both in ovulation induction and COH in these suppressed cycles, suggesting that in most women the endogenous LH levels present are sufficient for folliculogenesis [13, 14]. But, recently, we measured the LH concentration during ovarian stimulation with rec-FSH in a GnRH-agonist long protocol, and used the ratio of late-follicular to mid-follicular LH concentrations as an index to evaluate the change of LH concentration during ovarian stimulation. The ratio was calculated as the late-follicular concentration divided by the mid-follicular LH concentration. As a result of this analysis we demonstrated that approximately 30% of stimulated cycles showed a relative decrease in LH concentration (LH ratio < 1.0) and the pregnancy and implantation rates were significantly lower in this relative decrease in LH concentration group compared with the relative increase in LH concentration group [15]. Therefore, approximately 30% of stimulated cycles with rec-FSH in a GnRH-agonist long protocol brought inadequate LH increase and to improve the results of these cycles a relative increase in LH concentration is needed. According to the results of this study, increased frequency of GnRH-agonist use per day might increase the LH concentration, at this point of view, this study is meaningful.

In conclusion, continuous use of GnRH agonist until oocyte retrieval maintained high LH concentrations. Therefore, this modified long protocol might improve the extremely low LH concentrations during oocyte retrieval in women undergoing GnRH-agonist long protocol and rec-FSH ART treatment. In particular, continuous administration of GnRH-agonist might benefit patients who require LH supplementation.

References

1. Poter RN, Smith W, Craft IL, Abdulwahid NA, Jacobs HS. Induction of ovulation for in vitro fertilization using buserelin and gonadotrophins. Lancet 1984; 2: 1284-5.
2. Tan SL. Luteinizing hormone-releasing hormone agonists for ovarian stimulation in assisted reproduction. Curr Opin Obstet Gynecol 1994; 6: 166-72.
3. Nakagawa K, Yamano S, Aono T. The ovarian stimulation in assisted reproductive technology. Obstet Gynecol (Tokyo) 1997; 64: 205-10.
4. Bayer SR, Apler MM, Penzias AS. The Boston IVF Handbook of Infertility. New York, The Parthenon Publishing Group 2002; 78-83.

5. Nakagawa K, Ohgi S, Kojima R, et al. Recombinant-FSH has more effective potent than urinary human menopausal gonadotropin in ovarian hyperstimulation for ART treatment. Reprod Med Biol 2007; 6: 27-32.

6. Nakagawa K, Ohgi S, Kojima R, et al. Reduction of perifollicular arterial blood flow resistance after hCG administration is a good indicator of the recovery of mature oocytes in ART treatment. J Assist Reprod Genet 2006; 23: 433-8.

7. Nakagawa K, Yamano S, Moride N, Yamashita M, Yoshizawa M, Aono T. Effect of activation with Ca ionophore A23187 and puromycin on the development of human oocytes that failed to fertilize after intracytoplasmic sperm injection. Fertil Steril 2001; 76: 148-52.

8. Humaidan P, Bungum M, Bungum L, Yding Andersen C. Effects of recombinant LH supplementation in women undergoing assisted reproduction with GnRH agonist down-regulation and stimulation with recombinant FSH: on opening study. Reprod BioMed Online 2004; 8: 635-43.

9. Humaidan P. To add or not to add LH: comments on a recent commentary. Reprod BioMed Online 2006; 12: 284-5.

10. Kol S. To add or not to add LH: consideration of LH concentration changes in individual patients. Reprod Biomed Online 2005; 11: 664-6.

11. Gardner D, Weissman A, Howles CM, Shoham Z (eds.) Textbook of Assisted Reproductive Techniques; Laboratory and Clinical Perspectives. UK: Martin Dunitz 2001; 483-91.

12. Sungurtekin U, Jansen RP. Profound luteinzing hormone suppression after stopping the gonadotropin-releasing hormone – agonist leuprolide acetate. Fertil Steril 1995; 63: 663-5.

13. Schats R, Sutter PD, Bassil S, Kremer JA, Tournaye H, Donnez J. Ovarian stimulation during assisted reproduction treatment: a comparison of recombinant and highly purified urinary human FSH. On behalf of The Feronia and Apis study group. Hum Reprod 2000; 15: 1691-7.

14. Frydman R, Howles CM, Truong F. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. The French Multicentre Trialists. Hum Reprod 2000; 15: 520-5.

15. Nakagawa K, Ohgi S, Nakashima A, Horikawa T, Sugiyama R, Saito H. The ratio of late-follicular to mid-follicular phase LH concentrations efficiently predicts ART outcomes in women undergoing ART treatment with GnRH-agonist long protocol and stimulation with recombinant FSH. J Assist Reprod Genet 2009; 25: 359-64.