J Assist Reprod Genet (2008) 25:359–364
DOI 10.1007/s10815-008-9243-0

ASSISTED REPRODUCTION

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

Koji Nakagawa · Shirei Ohgi · Akira Nakashima · Takashi Horikawa · Rikikazu Sugiyama · Hidekazu Saito

Received: 5 March 2008 / Accepted: 5 August 2008 / Published online: 28 August 2008

Abstract

Purpose To establish an index to predict ART outcomes and to identify infertile patients who need LH supplementation during ovarian stimulation.

Methods Serum LH concentrations were measured during the mid- and late-follicular phase in 86 normogonadotropic infertile patients who underwent ART treatment using GnRH-agonist long protocol with recombinant-FSH. The relationships between serum LH concentrations at both time points and ART outcomes were retrospectively analyzed, and the relationships between the ratio of late-follicular to mid-follicular LH concentrations and ART outcomes were also evaluated.

Results There were no significant correlations between the mid- or late-follicular LH concentrations and ART outcomes. The ratio of late-follicular to mid-follicular LH concentrations <1.0 was considered the relatively LH decreased group (RD group) and ratio≥1.0 was considered the relatively LH increased group (RI group). The number of usable embryos in RD group was similar to that in RI group, but the pregnancy and implantation rates in the RD group (9.7% and 5.8%) were significantly lower than those in the RI group (31.1% and 17.2%; $p<0.05$).

Conclusions Relatively decreased LH concentrations during ovarian stimulation using GnRH-agonist long protocol with rec-FSH had a negative effect on ART outcomes. Therefore, the ratio of mid- to late-follicular phase LH concentrations is suggested to be an efficient index to identify patients who might benefit from LH supplementation.

Keywords ART outcome · GnRH-agonist · LH concentration · Long protocol · Recombinant-FSH · Pregnancy rate

Capsule A relative decrease in LH concentration during ovarian stimulation in a GnRH-a long protocol negatively affected the ART outcomes. This ratio can be used to identify patients who need LH supplementation.

K. Nakagawa (✉) · S. Ohgi · A. Nakashima · T. Horikawa · H. Saito
Division of Reproductive Medicine,
Department of Perinatal Medicine and Maternal Care,
National Center for Child Health and Development,
2-10-1 Okura, Setagaya,
Tokyo 157-8535, Japan
e-mail: nakagawa-jiko@spice.ocn.ne.jp

R. Sugiyama
Division of Reproductive Medicine, Sugiyama Ladies Clinic,
Tokyo, Japan

Introduction

In the mid-1980s, gonadotrophin-releasing hormone (GnRH) agonists were introduced to assisted reproductive technology (ART) to allow for ovarian stimulation while successfully circumventing the problems associated with premature LH surge [1]. This down-regulation protocol was termed "GnRH agonist long protocol." The GnRH agonist long protocol is used for ovarian stimulation following treatment with human menopausal gonadotropin (HMG), which contains equal amounts of FSH and LH activity. Use of this GnRH agonist long protocol subsequent to HMG treatment is employed worldwide. Since its approval in 1994, the recombinant FSH (rec-FSH) has been used for ovarian stimulation [2]. Currently, rec-FSH preparations,

which have no LH activity, are the primary gonadotropin used for ovarian stimulation in GnRH agonist long protocols. Consequently, the role of LH in ovarian stimulation induced by rec-FSH during treatment with the GnRH agonist long protocol is of interest.

Circulating concentrations of endogenous LH are reduced in women treated with GnRH agonist long protocols that use rec-FSH for ovarian stimulation. Recently, the effect of LH on follicular maturation and pregnancy outcome during the course of ovarian stimulation has drawn increasing attention in the field of ART [3, 4]. LH concentrations during ovarian stimulation with rec-FSH as part of a GnRH agonist long protocol were substantially lower than those observed during natural cycles and during ovarian stimulation without GnRH agonists. Several investigators have examined the relationship between serum LH concentrations and ovarian/IVF outcome resulting from ovarian stimulation with rec-FSH in a GnRH agonist long protocol with rec-FSH [5–9]. A recent systematic review of studies investigating an association between endogenous LH concentrations during ovarian stimulation with GnRH agonists failed to detect a significant negative effect of low endogenous LH concentrations on viable pregnancies after 12 weeks of gestation [4].

However, the need for LH supplementation in women being treated with a GnRH agonist long protocol using rec-FSH is a controversial issue. Several studies have indicated that the use of recombinant LH in women undergoing GnRH agonist/rec-FSH therapy has variable benefits in certain patient populations [10, 11]. Unequivocal LH cut-off values that identify patients who require supplemental LH were not determined in previously published studies. Thus, an efficient index to identify these patients is needed.

Thus, the object of the present study was to establish an index, based on ART outcomes, which identifies the patients who need LH supplementation during ovarian stimulation. To accomplish this goal, serum LH concentrations were measured in the mid- and late-follicular phases in patients treated for infertility with a GnRH agonist long protocol using rec-FSH.

Materials and methods

Patients and ovarian hyperstimulation

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

All treatment cycles (n=100) in the 86 patients were hyperstimulated with a GnRH agonist long protocol using rec-FSH according to our previous reports [12]. Briefly, ovarian hyperstimulation with the 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 pretreatment cycle and was continued until the day of human chorionic gonadtropin (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 administrated on subsequent days until a dominant follicle reached 16 mm in diameter. In our stimulation protocol, the daily dosage of rec-FSH did not alter follicle growth or previous poor ovarian response. Oocyte retrieval was performed 35 hours after administration of 10,000 IU of hCG.

IVF/ICSI procedure and embryo transfer

The IVF procedure used in this study has been previously described [13]. 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 the aspiration of each follicle. The aspiration was interrupted and a new syringe was used if blood appeared in the tube connected to the syringe, thus avoiding contamination by blood. Culture media 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 incubating each oocyte with $50–100\times10^3$ motile sperm within 5–6 h of collection. In vitro insemination was not performed when there was evidence of male factor infertility, rather intracytoplasmic sperm injection (ICSI) was performed according to a previous report [14]. 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 transcervically into the patient's uterus 72 hours after insemination or ICSI. The number of transferred embryos was no more than two to prevent multiple pregnancies. On day 1, 4 and 7 following embryo transfer (ET), 3,000 IU hCG were injected for luteal support. A combination of estrogen and progesterone was administered orally for 10 days after ET. Hydoxyprogesterone caproate instead of hCG was given every 4 days to patients at elevated risk of developing ovarian hyperstimulation syndrome.

Hormone assays and comparison of ART parameters

Serum FSH, LH and estradiol (E2) concentrations were measured on the fifth day after the start of ovarian stimulation (mid-follicular phase) and on the day of hCG administration (late follicular phase). Measurement of serum LH was done using a chemiluminescent microparticle immunoassay (ARCHITECT LH®; Abbott Japan, Tokyo) and the results were expressed as IU/l. 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.

The embryos that met the following criterion were defined as usable embryos: developed to at least the 8-cell stage with less than 15% fragmentation on day 3 following oocyte retrieval. 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.

Indices evaluated based on ART outcome

The following indices were evaluated by examining their relationship between ART outcomes and mid-follicular LH concentrations, LH concentrations on the day of hCG administration and the ratio of late-follicular to mid-follicular LH concentrations. The LH ratio was calculated as the late-follicular phase LH concentration divided by the mid-follicular phase LH concentration.

Statistics

All data are presented as the mean±SEM (standard error of the mean). Significant differences were determined using unpaired *t*-tests and the chi-square test.

Results

The characteristics and ART outcomes of the patients who underwent ovarian hyperstimulation using the GnRH-agonist long protocol with rec FSH are summarized as follows. The indications for ART treatment included tubal factor infertility (19.8%), unexplained infertility (45.3%), endometriosis (15.1%) and the male factor infertility (19.8%). Most of male factor infertility cases were treated by using ICSI. The mean age and previous ART attempts were 38.5±0.4 years old and 1.9±0.1, respectively. The duration of stimulation and the average gonadotrophin dose administered until oocyte retrieval were 10.0±0.2 days and 1,710±35 IU, respectively. The numbers of retrieved oocytes, fertilized oocytes and transferred embryos were 8.1±0.5, 4.8±0.4 and 1.7±0.1, respectively. In 92 of 100 cycles embryo transfer (ET) was performed between the third and fifth days after oocyte retrieval. Twenty-two pregnancies were produced in 92 ET cycles, cycles, but 7 pregnancies resulted in miscarriage. The pregnancy rate per ET and implantation rate were 23.7% and 13.7%, respectively.

The treatment cycles were classified into three groups based on LH concentration in the mid-follicular phase as follows: LH≥1.2 comprised the high LH group (H group); 1.2>LH≥0.6 constituted the intermediate LH group (IM group); and, cycles 0.6<LH made up the low LH group (L group). The cut-off values were selected based on a previously published study that defined a low LH concentration to be <1.2 IU/l [15]. The ART outcomes for each group are summarized in Table 1. The number of stimulated cycles in the H, IM and L groups were 15, 53 and 32, respectively. The duration of stimulation and the dosage of rec-FSH were comparable among the 3 groups. In addition, the other parameters were similar among groups, except for age. The subjects in the group H were significantly younger than those in other two groups ($p<0.05$). ART outcomes

Table 1 ART outcomes according to mid-follicular phase LH concentrations

	H group (LH≥1.2)	IM group (1.2>LH≥0.6)	L group (0.6<LH)
Number of stimulated cycles	15	53	32
Number of ET cycles	14	47	31
Age (years)[a]	36.7±1.0[b]	38.1±0.6[c]	39.9±0.5[c]
Duration of stimulation (days)[a]	9.5±0.6	10.3±0.3	9.8±0.3
Dosage of rec-FSH (ampoules)[a]	22.9±1.6	22.9±0.6	22.9±0.9
No. of retrieved oocytes[a]	8.7±1.6	7.5±0.6	8.7±0.9
No. of fertilized oocytes[a]	4.3±0.7	4.8±0.5	5.0±0.6
No. of usable embryo[a]	2.3±0.7	2.0±0.3	2.5±0.4
Pregnancy rate per ET (%)	21.4	25.5	22.6
Implantation rate (%)	12.5	13.5	14.3

[a] Values are mean±SE
[b] vs. the L group ($p<0.05$)
[c] vs. the L group ($p<0.05$)

did not differ among patients with low, intermediate and high mid-follicular LH concentrations. Hence, mid-follicular LH concentrations appeared unrelated to ART outcome.

Alternatively, the treatment cycles were classified into the three groups described above, but using late-follicular phase, rather than mid-folicular phase, LH concentrations. The ART outcomes for the three groups are summarized in Table 2. The pregnancy rates in the H, IM and L groups were 30.8, 21.7 and 20.0, respectively, with no significant differences among three groups. Likewise, there were no differences in ART outcomes among the groups. Therefore, LH concentrations in the late-follicular phase do not appear to predict ART outcomes.

The treatment cycles were divided into two groups based on the LH ratio: cycles with an LH ratio<1.0 were considered the relatively decreased LH group (RD group); and cycles with an LH ratio≥1.0 comprised the relatively increased LH group (RI group). The duration of stimulation and dose of rec-FSH were similar in both groups, as were the numbers of retrieved and fertilized oocytes (Table 3). The number of usable embryos in the RD group was also similar to that in the RI group. However, in the RD group only three pregnancies were produced in 31 ET cycles and the pregnancy rate per ET was 9.7%, and this was significantly lower than that in the RI group (31.1%, $p<0.05$). Consequently, the implantation rate in the RD group (5.8%) was also significantly lower than that in the RI group (17.2%, $p<0.05$). In the RD group 1 pregnancy was miscarried and the miscarriage rate resulted in high percentage, but there was no significant difference in miscarriage rate between the two groups according to the small number.

Discussion

There is no consensus regarding the lower limit of LH, below which a reduced outcome is observed. Although the criteria for severe LH deficiency has been previously set at 1.2 IU/l [10, 16], LH deficiency during ovarian hyperstimulation remains to be defined. Moreover, the majority of LH assays used in previous studies had low sensitivity; therefore, LH concentrations≤1.0 IU/l were not detectable [3, 10]. However, the high-sensitivity LH assay used in this study indicated that the lower limit of LH detection was 0.07 IU/l. Thus, the high-sensitivity LH assays a more detailed analysis of the relationship between low serum LH concentrations and ART outcomes.

In this study, the treatment cycles were classified into three groups based on mid- and late-follicular phase LH concentrations. A low LH concentration has been defined previously as <1.2 IU/l [15]. There was no relationship among late-follicular LH concentrations and ART outcomes (Table 2), similar to previous reports [5–9]. In addition, the results of this study also indicated that there were no differences among groups with low, intermediate or high mid-follicular phase LH concentrations and ART outcomes (Table 1). Based on these results, ART outcomes could not be predicted by LH concentrations measured at one time point. The inability of single time point LH values to predict ART outcomes is likely due to sensitivity of the pituitary gland to GnRH-agonist and to the large inter-individual variation in the magnitude of the GnRH agonist induced down-regulation of the pituitary gland.

In contrast to the single time point measurements of LH concentrations, the ratio of late-follicular to mid-follicular LH concentrations was used 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. An LH ratio≥1.0 indicated that the LH concentration was greater than the LH concentration during the mid-follicular phase, reflecting the change in LH concentration during ovarian stimulation. In this study, approximately 30% of stimulated cycles showed a relative decrease in LH concentration (LH ratio<1.0). ART

Table 2 ART outcomes according to late-follicular phase LH concentrations

	H group (LH≥1.2)	IM group (1.2>LH≥0.6)	L group (0.6<LH)
Number of stimulated cycles	31	48	21
Number of ET cycles	26	46	20
Age (years)[a]	37.0±0.7[b, c]	39.2±0.5	39.0±0.7
Duration of stimulation (days)[a]	10.0±0.4	10.0±0.2	10.1±0.4
Dosage of rec-FSH (ampoules)[a]	23.1±1.0	22.5±0.8	23.3±0.9
No of retrieved oocytes[a]	8.4±0.9	8.1±0.8	7.5±1.1
No of fertilized oocytes[a]	5.3±0.7	4.8±0.5	4.1±0.7
No. of usable embryo[a]	2.6±0.6	2.1±0.3	1.7±0.3
Pregnancy rate per ET (%)	30.8	21.7	20.0
Implantation rate (%)	16.0	13.1	11.4

[a] Values are mean±SE
[b] vs. the IM group ($p<0.05$)
[c] vs. the L group($p<0.05$)

Table 3 ART outcomes according to the LH ratio (late-follicular LH levels /mid-follicular phase LH concentrations)

	RD group (LH ratio<1.0)	RI group (LH ratio≥1.0)
Number of stimulated cycles	33	67
Number of ET cycles	31	61
Age (years)[a]	37.8±0.7	38.8±0.4
Duration of stimulation (days)[a]	10.0±0.4	10.0±0.2
Dosage of rec-FSH (ampoules)[a]	1,673±53	1,723±45
No of retrieved oocytes[a]	8.3±1.1	8.0±0.6
No of fertilized oocytes[a]	4.9±0.8	4.8±0.4
No of usable embryos[a]	2.1±0.4	2.3±0.3
Pregnancy rate per ET (%)	9.7	31.1[b]
Implantation rate (%)	5.8	17.2[b]
Miscarriage rate (%)	66.7	26.3

[a] Values are mean±SE
[b] vs RD group, $p<0.05$

parameters, such as duration of stimulation, required gonadotrophin doses, numbers of retrieved, fertilized oocytes and usable embryos in this group, were similar between the two groups. However, the pregnancy and implantation rates were significantly lower in the RD group compared with the RI group. Thus, based on these two ART outcomes, the ratio of late-follicular to mid-follicular phase LH concentrations predicted ART outcomes. Thus, it appeared that a relative decrease in LH concentrations during the late-follicular phase might impair follicular development.

Similar to the results of the present study, a recent investigation found that a relative reduction in mid-follicular LH concentrations during treatment with the GnRH agonist long protocol lead to reduced live birth rates [17]. That study, compared LH concentrations measured on the day before ovarian stimulation and during the mid-follicular phase. Because the effects of LH are more important during the late follicular phase than during mid-follicular phase, changes in LH concentrations from mid- to late-follicular phase are more meaningful than those between the early and mid-follicular phases.

Why did this difference occur? It has been suggested that the residual endogenous LH remaining during GnRH-agonist induced down-regulation of the pituitary may be sufficient to achieve adequate follicular maturation during ovarian stimulation with recombinant FSH preparations in most clinical cases. Nevertheless, it is also possible that in some cases GnRH agonist induced down-regulation may result in profound suppression of LH concentrations with an adverse effect on steroidogenesis and oocyte quality, thereby negatively impacting IVF outcome.

All patients in this present study were treated under the same GnRH agonist long protocol and, therefore, received the same dose of GnRH-agonist nasal spray and were similarly stimulated with rec-FSH. There is no theoretical explanation for our findings which show various LH concentrations during the ovarian stimulation using GnRH-a long protocol with rec-FSH. We compared the FSH and estradiol concentrations between the RD and RI group, but these concentrations of each group showed similar (data not shown), because the dosage of rec-FSH during ovarian stimulation and the number of retrieved oocytes were similar in both group. We hypothesize the following explanation for our results. During follicle development, serum estradiol concentrations are elevated by ovarian stimulation, inducing increased GnRH secretion from the hypothalamus. GnRH-receptors on the pituitary gland that were not suppressed by the GnRH-agonists might be stimulated, as a result, LH concentrations on late-follicular phase might be elevated. That is to say, inadequate estradiol elevation caused by inadequate follicular growth during the ovarian hyperstimulation, especially from the mid-follicular to late-follicular phases would result in a decline in LH concentrations. If this hypothesis is correct, these patients require LH supplementation during later ovarian stimulation.

Recently, several reports demonstrating beneficial effects of exogenous rec-LH supplementation during ovarian stimulation on ART outcomes have been published. [10, 11, 18]. The authors of these studies noted that the calibrated LH administration improved ovarian outcome in specific patient populations: patients >35 years of age, patients with an abnormal initial ovarian response to rec-FSH; and, in women with a poor prognosis treated with GnRH antagonists. Many researchers have attempted to identify an index to easily detect patients who need LH supplementation, but, to date, no such index has been established. Based on the results of the present study, we propose that the LH ratio can be used to easily identify patients who need LH supplementation. Additional studies are needed to determine whether LH supplementation in patients with a relative decrease in LH during ovarian stimulation with rec-FSH as part of a GnRH-agonist long protocol improves ART outcome. Should a beneficial effect of LH supplementation be demonstrated, the optimal number of doses also should be investigated. These questions are currently under investigation by our research group.

In conclusion, a relative decrease in the LH concentration (LH ratio<1.0) during ovarian stimulation with rec-FSH in a GnRH-agonist long protocol negatively affected the ART outcomes. This ratio could easily be used to identify patients who need LH supplementation during ovarian stimulation. Our next study aims to develop an effective LH supplementation protocol for patients who exhibited a relative decrease in LH concentrations from the mid-to late-follicular phase.

References

1. Wildt L, Diedrich K, van der Ven H, al Hasani S, Hubner H, Klasen R. Ovarian hyperstimulation for in-vitro fertilization controlled by GnRH agonist administered in combination with human menopausal gonadotrophins. Hum Reprod. 1986;1:15–9.
2. Devroy P, Mannaerts B, Smitz J, Coelingh Bennink H, Van Steirteghem A. Clinical outcome of a pilot efficacy study on recombinant human follicle-stimulating hormone (Org 32489) combined with various gonadotropin-releasing hormone agonist regimens. Hum Reprod. 1994;9:1064–9.
3. Cabrera RA, Stadtmauer L, Mayer JF, Gibbons WE, Oehninger S. Follicular phase serum levels of luteinizing hormone do not influence delivery rates in in vitro fertilization cycles down-regulated with a gonadotropin-releasing hormone agonist and stimulated with recombinant follicle-stimulating hormone. Fertil Steril. 2005;83:42–8. doi:10.1016/j.fertnstert.2004.06.050.
4. Kolibianakis EM, Collins J, Tarlatzis B, Papanikolous E, Devroy P. Are endogenous LH levels during ovarian stimulation for IVF using GnRH analogues associated with the probability of ongoing pregnancy? A systematic review. Hum Reprod Update. 2006;12:3–12. doi:10.1093/humupd/dmi030.
5. Fleming R, Lloyd F, Herbert M, Fenwick J, Griffiths T, Murdoch A. Effects of profound suppression of luteinizing hormone during ovarian stimulation on follicular activity, oocyte and embryo function in cycles stimulated with purified follicle stimulating hormone. Hum Reprod. 1998;13:1788–92. doi:10.1093/humrep/13.7.1788.
6. Westergaard LG, Laursen SB, Andersen CY. Increased risk of early pregnancy loss by profound suppression of luteinizing hormone during ovarian stimulation in normogonadotrophic women undergoing assisted reproduction. Hum Reprod. 2000;15:1003–8. doi:10.1093/humrep/15.5.1003.
7. Balasch J, Vidal E, Peñarrubia J, Casamitjana R, Carmona F, Creus M, et al. Suppression of LH during ovarian stimulation: analyzing threshold values and effects on ovarian response and the outcome of assisted reproduction in down-regulated women stimulated with recombinant FSH. Hum Reprod. 2001;16:1636–43. doi:10.1093/humrep/16.8.1636.
8. Esposito MA, Barnhart KT, Coutifaris C, Patrizio P. Role of periovulatory luteinizing hormone concentrations during assisted reproductive technology cycles stimulated exclusively with recombinant follicle-stimulating hormone. Fertil Steril. 2001;75:519–24. doi:10.1016/S0015-0282(00)01745-3.
9. Humaidan P, Bungum L, Bungum M, Andersen CY. Ovarian response and pregnancy outcome related to mid-follicular LH levels in women undergoing assisted reproduction with GnRH agonist down-regulation and recombinant FSH stimulation. Hum Reprod. 2002;17:2016–21. doi:10.1093/humrep/17.8.2016.
10. 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.
11. Humaidan P. To add or not to add LH: comments on a recent commentary. Reprod Biomed Online. 2006;12:284–5.
12. Nakagawa K, Ohgi S, Kojima R, Sugawara K, Horikawa T, Ito M, I Irahara M, Saito H. Recombinant-FSH has more effective potent than urinary human menopausal gonadotropin in ovarian hyperstimulation for ART treatment. Reprod Med Biol. 2007;6:27–32. doi:10.1111/j.1447-0578.2007.00161.x.
13. Nakagawa K, Ohgi S, Kojima R, Ito M, Horikawa T, Irahara M, 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. doi:10.1007/s10815-006-9087-4.
14. 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. doi:10.1016/S0015-0282(01)01839-8.
15. The European recombinant human LH study group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced folliclar development in LH- and FSH-deficient anovulatory women: a dose-finding study. J Clin Endocrinol Metab 1998;83:1507–14. doi:10.1210/jc.83.5.1507.
16. Shoham Z, Balen A, Patel A, Jacobs HS. Results of ovulation induction using human menopausal gonadotropin or purified follicle-stimulating hormone in hypogonadotorpic hypogonadism patients. Fertil Steril. 1991;56:1048–53.
17. Lauoud R, Al-Jefout M, Tyler J, Ryan J, Driscoll G. A relative reduction in mid-follicular LH concentrations during GnRH agonist IVF/ICSI cycles leads to lower live birth rates. Hum Reprod. 2006;21:2645–9. doi:10.1093/humrep/del219.
18. Kol S. To add or not to add LH: consideration of LH concentration changes in individual patients. Reprod Biomed Online. 2005;11:664–6.