Failure of GnRH agonist-triggered oocyte maturation: its cause and management

Yoshimasa Asada • Fumiaki Itoi • Hiroyuki Honnma •
Shuji Takiguchi • Noritaka Fukunaga •
Yoshiki Hashiba • Tsuyoshi Baba • Toshiaki Endo

Received: 1 January 2013 / Accepted: 26 February 2013 / Published online: 15 March 2013
© Springer Science+Business Media New York 2013

Abstract

Objective In the present study we investigated the cause of GnRHa's failure to trigger oocyte maturation and present an effective rescue protocol for use when failure occurs.

Design Case reports.

Setting One in vitro fertilization (IVF) center.

Patient(s) Eight cases of failure of GnRH agonist (GnRHa)-triggered oocyte maturation and one case of GnRHa successfully triggered oocyte maturation, despite a weak response in the LH-RH test.

Capsule In cases where GnRHa fails to trigger oocyte maturation, a rescue protocol entailing re-triggering with HCG can produce successful outcomes.

Y. Asada • F. Itoi • H. Honnma (✉) • S. Takiguchi • N. Fukunaga •
Y. Hashiba
Asada Ladies Clinic, RENAK 5F, Matsushincho 1-4,
Kasugai, Aichi 486-0931, Japan
e-mail: hihonma@sapmed.ac.jp

Y. Asada
e-mail: y_asada@ivf-asada.jp

F. Itoi
e-mail: f_itoi@ivf-asada.jp

S. Takiguchi
e-mail: s_takiguchi@ivf-asada.jp

N. Fukunaga
e-mail: N_fukunaga@ivf-asada.jp

Y. Hashiba
e-mail: y_hashiba@ivf-asada.jp

T. Baba • T. Endo
Department of OB & GY, Sapporo Medical University
School of Medicine, South 1 West 16,
Chuo-ku, Sapporo 060-8543, Japan

T. Baba
e-mail: tbaba@sapmed.ac.jp

T. Endo
e-mail: endot@sapmed.ac.jp

Main Outcome Measure(s) All cases were successfully rescued by re-triggering maturation using HCG, with seven of the eight patients achieving ongoing pregnancy and successful delivery.

Result(s) In three patients the cause of the oocyte maturation failure was likely temporal or continuous severe down-regulation of the hypothalamus-pituitary-axis, the cause was unknown in the other five patients.

Conclusion(s) In cases where GnRHa fails to trigger oocyte maturation, a rescue protocol entailing re-triggering with HCG can produce successful outcomes.

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a potentially life-threatening condition and is the most serious complication of in-vitro fertilization-embryo transfer (IVF-ET). The most common form occurs a few days after triggering oocyte maturation by administration of HCG [1]. It is therefore noteworthy that using a GnRH agonist (GnRHa) to trigger the final oocyte maturation in a GnRH antagonist protocol may prevent OHSS in high responders, such as those with polycystic ovary syndrome (PCOS) [2, 3]. It has also been reported that greater numbers of metaphase 2 (M2) oocytes are retrieved when maturation is triggered by GnRHa than when triggered by HCG [4]. These results likely reflect endogenous surges of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) triggered by GnRHa. Moreover, it was recently reported that a suboptimal LH response to GnRHa clearly reduces oocyte yields and maturity [5]. Furthermore we previously reported two cases in which GnRHa failed to trigger oocyte maturation in a PCOS patient, though we were subsequently able to re-trigger maturation using HCG [6]. Similar cases were reported as the empty follicle syndrome (EFS) [7].

We have encountered eight cases in which GnRHa failed to triggering oocyte maturation in a flexible GnRH

antagonist protocol, and all of these cases were successfully rescued by re-triggering maturation using HCG. In the present study we investigated the cause of GnRHa's failure to trigger oocyte maturation and present an effective rescue protocol for use when failure occurs.

Material and methods

Ovarian stimulation protocol

Ovarian stimulation was conducted from cycle day 3 using 150–300 IU of either human menopausal gonadotropin (Menopur; Ferring Pharmaceuticals) or recombinant FSH (Gonal-f; Merck Serono). Cycles were monitored using both ultrasound scanning and serum estradiol (E2) assays. A GnRH antagonist, Ganrelix (Ganirest; MSD) or Cetrorelix (Cetrotide; Nippon Kayaku), was administered when the leading follicle reached a maximum diameter of 14–16 mm or a premature LH surge was suspected based on serum LH monitoring [8]. When three or more follicles reached a maximum diameter of 18–20 mm, final oocyte maturation was triggered by administering 300 µg of nasal buserelin 35 h and then 34 h (total dose: 600 µg) prior to oocyte retrieval, as described previously [6]. Oocyte retrieval was carried out using ultrasound-guided transvaginal needle aspiration after the patient had received a sedative and an analgesic.

Oocytes were fertilized conventionally or via intracytoplasmic sperm injection (ICSI). Fertilized oocytes were all cryopreserved at the pronuclear stage to prevent OHSS, and frozen-thawed embryo transfer was performed during artificial hormone replacement cycles using identical endometrial preparation protocols [8].

Hormone assays

In our clinic, an LH-RH (GnRH) test was administered to each patient at her first medical examination. Basal FSH and LH levels were measured on cycle day 3, after which 100 µg of LH-RH were administered intramuscularly, and 30 min later FSH and LH were re-measured [8]. Each patient's age and BMI were determined on days 2 to 5 of the prestimulation cycle. At the same time, peripheral blood was collected, and serum AMH, basal FSH and basal LH were determined as described previously [8]. Briefly, the AMH concentration was measured in duplicate using an enzyme-linked immunosorbent assay (EIA AMH/MIS A16507; Immunotech, Beckman-Coulter, Marseille, France). The conversion factor for the AMH concentration was 1.0 ng/mL. 7.143 pmol/L. Serum FSH, LH, E2 and P4 were measured using an automated electro-chemiluminescence immunoassay on a Cobas e 411 Analyzer (Roche Diagnostics K.K., Tokyo, Japan).

This study was approved by the institutional review board and conducted in accordance with the principles of

the Declaration of Helsinki. Written informed consent was obtained from all patients prior to AMH measurement and description of their treatment outcomes.

Results

Between October 2007 and December 2011, we encountered eight cases in which GnRHa failed to trigger oocyte maturation in a flexible GnRH antagonist protocol. However, all of these cases were successfully rescued by re-triggering maturation through HCG administration. We summarize these eight cases in Table 1, which also includes one case where GnRHa successfully triggered oocyte maturation, despite a weak response in the LH-RH test (case 9). At first oocyte retrieval, 6 of the 8 patients (cases 1–4, 6 and 7) presented no oocytes from unilateral ovaries, even after extensive flushing. A few granulosa cells were identified upon analysis of the aspirates and flushes, but no cumulus masses or immature oocytes were seen. In the remaining two patients (cases 5 and 8) only a few oocytes were collected, even after extensive flushing, despite high serum E2 and the presence of numerous follicles on transvaginal ultrasound before the attempted retrieval. Subsequently, urinary HCG was administered, and a second oocyte retrieval was scheduled for 34 h after the HCG administration. With this second oocyte retrieval, numerous oocytes were obtained from bilateral ovaries (total numbers of oocytes, 8–35).

The oocytes were then fertilized conventionally or via intracytoplasmic sperm injection (ICSI). Once fertilized, they were cryopreserved at the pronuclear stage until frozen-thawed embryo transfer was performed during artificial hormone replacement cycles using identical endometrial preparation protocols [8]. In seven of the patients, ongoing pregnancy (after 12 weeks of pregnancy) and live birth in their first embryo transfer cycles was confirmed. Only one patient (case 4), who was 40 years of age, did not become pregnant in that IVF treatment cycle.

We suspect the reasons the usual doses of GnRHa failed to trigger oocyte maturation in our eight patients were (a) temporally severe down-regulation of the hypothalamus-pituitary-axis (cases 1 and 2); (b) continuous severe down-regulation of the hypothalamus-pituitary-axis (case 3); or (c) an unknown cause, though not down-regulation of the hypothalamus-pituitary-axis (cases 4–8). The causes of the infertility were male factors in three cases, PCOS in three cases, and were unexplained in two cases. The patients' ages ranged from 29 to 40 years. Seven patients showed a normal or strong response to 100 µg of LH-RH; however, patients 3 and 9 responded poorly to LH-RH due to severe down-regulation of the hypothalamus-pituitary-axis. The total dose of gonadotropin varied widely (1650–7150 IU), and the GnRH antagonist was administered 2 to 4 times. Serum AMH levels ranged from 17.9 to 91.9 pmol/l (not measured

Table 1 Cases of failure of triggering oocytes maturation by GnRHa and a case of successively triggered by GnRHa

Case No.	1	2	3	4	
Age	31	29	40	33	
0–30 min FSH (ng/ml)	6.04–12.05	3.2–9.9	0.1–1.6	4.4–6.4	
0–30 min LH (ng/ml)	9.25–71.37	0.2–17.0	0.1–4.5	3.5–15.9	
Basl FSH/LH (ng/ml)	0.1/0.1	0.2/0.1	0.2/0.1	6.8/3.6	
Suspected cause	a	a	b	c	
AMH (pmol/l)	–	–	17.9	32.4	
Indication of IVF-ET	PCOS	PCOS	Unexplained	Unexplained	
Amplges of GnRHantagonist	3	4	4	2	
Total dose of gonadotropin (IU)	3405	1725	7150	3600	
Serum E2 at the day of GnRHa (pg/ml)	5772	11492	7197	9169	
Serum P4 at the day of GnRHa (ng/ml)	2.04	2.13	2.94	1.22	
Number of oocytes at 1st	Rt 0	Rt 0	Rt 0	Rt 0	
Serum E2 at the day of HCG (pg/ml)	7848	–	–	–	
Serum P4 at the day of HCG (ng/ml)	2.5	–	–	–	
The dose of HCG (IU)	5000	5000	5000	10000	
Number of oocytes at 2nd	Rt 9: Lt 26	Rt 3: Lt 25	Rt 4: Lt 4	Rt 6: Lt 8	
Number of M2 oocytes (Fertilization rate)	26 (76.5 %)	11 (84.6 %)	3 (100 %)	10 (71.4 %)	
IVF outcome	Live birth	Live birth	Not pregnant	Ongoing	
Case No.	5	6	7	8	9
Age	34	34	33	31	32
0–30 min FSH (ng/ml)	5.2–8.4	6.7–11.2	5.22–8.82	3.6–5.6	0.3–1.0
0–30 min LH (ng/ml)	3.0–14.1	5.2–18.1	2.53–22.36	3.5–12.5	0.1–3.7
Basl FSH/LH (ng/ml)	6.9/5.3	10.0/7.1	4.4/3.2	6.3/16.8	0.5/0.1
Suspected cause	c	c	c	c	
AMH (pmol/l)	43.8	23.6	91.9	–	66.6
Indication of IVF-ET	Male factor	Male factor	PCOS	Male factor	PCOS
Amplges of GnRHantagonist	3	2	3	3	6
Total dose of gonadotropin (IU)	3600	6000	1650	3600	5150
Serum E2 at the day of GnRHa (pg/ml)	7312	15428	7023	26134	7056
Serum P4 at the day of GnRHa (ng/ml)	1.56	3.06	4.53	7.27	1.85
Number of oocytes at 1st	Rt 0	Rt 0	Rt 2	Rt 3	13
Serum E2 at the day of HCG (pg/ml)	6407	8732	–	21189	–
Serum P4 at the day of HCG (ng/ml)	1.53	2.43	–	11.28	–
The dose of HCG (IU)	5000	5000	5000	5000	–
Number of oocytes at 2nd	Rt 3: Lt 11	Rt 3: Lt 9	Rt 3: Lt 11	Rt 9: Lt 16	–
Number of M2 oocytes (Fertilization rate)	6 (50.0 %)	7 (87.5 %)	8 (72.7 %)	14 (66.7 %)	–
IVF outcome	Live birth	Ongoing	Live birth	Live birth	Live birth

Eight cases of failure of triggering oocytes maturation (case1–8) and one case of successively triggered by GnRHa with severe down-regulation of the hypothalamus-pituitary-axis (case 9) were presented.

0–30 min FSH, before and 30 min after FSH levels of the LH-RH administration: 0–30 min LH, before and 30minuites after LH levels of the LH-RH administration: Suspected cause, suspected cause of failure of triggering oocytes maturation; (a) temporallysevere down-regulation of the hypothalamus-pituitary-axis, (b) continuoussevere down-regulation of the hypothalamus-pituitary-axis and (c) unknown cause; however not down-regulation of the hypothalamus-pituitary-axis: E2, estradiol: P4, progesterone: Number of oocytes at 1st, number of oocytes at first oocytes retrieval: Number of oocytes at 2nd, number of oocytes at second oocytes retrieval: Ongoing, ongoing pregnancy

in 3 cases). On the day GnRHa was administered, serum E2 levels were high (5772–26134 pg/ml) and serum P4 levels were 1.22–7.27 ng/ml. On the day HCG was administered (for the second oocyte maturation), serum P4 was measured

in 4 patients (cases 1, 5, 6 and 8). Among them, three patients showed serum P4 levels that were not higher than on the day GnRHa was administered (cases 1, 5 and 6). The fertilization rates were 50–100 %.

Discussion

We have encountered eight cases in which GnRHa failed to trigger oocyte maturation in a flexible GnRH antagonist protocol. In all of these cases, however, oocyte maturation was rescued by re-triggering with HCG. In our clinic, a LH-RH (100 µg of GnRH) test was administered to all patients at their first medical examination on cycle day 3. In that regard, we previously reported two cases in which the failure to trigger oocyte maturation was due to severe down-regulation of the pituitary-hypothalamus-axis (cases 1 and 2) [6]. Those cases prompted us to use HCG to trigger oocyte maturation in patients predicted to have a weak response to GnRHa based on the LH-RH test. In other cases, however, oocyte maturation was not triggered, despite a normal or strong response in the LH-RH test.

Chen et al. recently reported that a suboptimal LH surge could result in dramatically lower oocyte yields when maturation was triggered with GnRHa in a flexible GnRH antagonist protocol [5]. In that study, the threshold serum LH level 12 h after triggering with GnRHa was 15 IU/l. They also mentioned that post-trigger serum LH levels did not affect oocyte maturation or the fertilization rate. This suggests the oocyte maturation failures we observed were due to suboptimal endogenous LH surge induced by GnRHa. However, although the cause of maturation failure might have been temporal or continuous severe down-regulation of the hypothalamus-pituitary-axis in three cases, the cause was unknown in the remaining five cases.

In case 7, two earlier oocyte retrievals were successfully triggered by GnRHa in a flexible GnRH antagonist protocol. We think this result is the product of human error or a pharmaceutical aberration in the same vein as empty follicle syndrome [7]. In addition, we experienced a curious case of oocyte maturation being successfully triggered by GnRHa, despite a weak response in the LH-RH test (case 9); in other words, despite severe down-regulation of the hypothalamus-pituitary-axis. In that case, the weak LH reaction to GnRHa inferred from the LH-RH test might have still been sufficient to mature the oocytes. Consistent with that idea, only 5 % of the normal LH surge amplitude is reportedly necessary for oocyte maturation in rats [9], and the serum LH level 12 h after triggering with GnRHa may be greater than 15 IU/l in this case. The circumstances of these two cases would make it difficult to predict GnRHa's inability to trigger oocyte maturation.

In cases 1, 5, 6 and 8, serum P4 levels were measured on the day of HCG administration (the day of first oocyte retrieval). The absence of P4 elevation in serum was indicative of the failure of GnRHa to trigger oocyte maturation in cases 1, 5 and 6. In case 8, only three oocytes were collected from a unilateral ovary in the first oocyte retrieval, despite an extremely high serum E2 level, though serum P4 was clearly higher on the day HCG was administered than the day of GnRHa administration. In this case the LH surge induced by GnRHa was apparently

suboptimal, and only several oocytes matured and were then collected. We therefore considered most oocytes to be mature after maturation was re-triggered by HCG. We would suggest that when a case with suboptimal LH surge is encountered, a second oocyte retrieval following HCG administration should be scheduled. This might enable retrieval to more oocytes and lead to successful outcomes in IVF-ET.

EFS is a condition of uncertain etiology in which no oocytes are retrieved from many growing follicles with normal E2 levels after ovarian stimulation for IVF treatment [7]. The available literature describes two subtypes of EFS; genuine EFS, presumably related to intrinsic ovarian factors, and false EFS. With false empty follicle syndrome, beta HCG levels are suboptimal due to pharmaceutical reasons or human error [10]. Therefore our cases were considered one of the false EFS.

We considered the possibility that re-triggering oocyte maturation using HCG could cause severe OHSS in high responders; however, we experienced no occurrences of severe OHSS requiring hospitalization among these patients. It has also been reported that unilateral aspiration during the first oocyte retrieval, before the optimal LH surge is induced by HCG, may reduce the risk of OHSS [11].

Our eight cases indicate that the failure to trigger oocyte maturation is sometimes caused by down-regulation of the hypothalamus-pituitary-axis, which is predictable, but the cause is often unknown, making prediction impossible. It is therefore difficult to completely prevent or precisely predict the failure of GnRHa to trigger oocytes maturation. Nonetheless, we successfully managed all eight of these patients by re-triggering maturation using HCG, with seven of the patients achieving ongoing pregnancy and delivery. We therefore consider a rescue protocol like that depicted in Fig. 1 to be very important for successful IVF outcomes. In the event that no oocytes or only a few are

Fig. 1 Flow chart depicting management of the failure of GnRHa-triggered oocyte maturation

retrieved when serum E2 levels are high and many follicles are detected by transvaginal sonography at first oocyte retrieval, we suggest that oocyte aspiration from a unilateral ovary should be stopped, and a second oocyte retrieval following HCG administration should be scheduled. We also found that it is better to compare serum P4 levels measured on the day of GnRHa with that on the day of first oocyte retrieval, in which case the lack of P4 elevation clearly indicates a failure of oocyte maturation. Finally, our cases show that oocyte aspiration from bilateral ovaries is needed at the second oocyte retrieval because re-aspiration from a same ovary subjected to the first aspiration yields only several oocytes.

In conclusion, our findings suggest that in cases where the usual dose of GnRHa fails to trigger oocyte maturation, the cause is sometimes a suboptimal endogenous LH surge due to severe down-regulation of the hypothalamus-pituitary-axis. Often, however, the cause may be unknown. Under both circumstances, a rescue protocol entailing re-triggering oocyte maturation using HCG can produce a successful outcome.

Acknowledgment None of the authors have a conflict of interest. We thank the nursing and embryology staffs of the Asada Ladies Clinic for their expert technical help.

References

1. Aboulghar M. Symposium: update on prediction and management of OHSS. Prevention of OHSS. *Reprod Biomed Online*. 2009;19:33–42.
2. Engmann L, Siano L, Schmidt D, Nulsen J, Maier D, Benadiva C. GnRH agonist to induce oocyte maturation during IVF in patients at high risk of OHSS. *Reprod Biomed Online*. 2006;13:639–44.
3. Humaidan P, Quartarolo J, Papanikolaou EG. Preventing ovarian hyperstimulation syndrome: guidance for the clinician. *Fertil Steril*. 2010;94:389–400.
4. Humaidan P, Bredkjaer HE, Bungum L, Bungum M, Grondahl ML, Westergaard L, et al. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. *Hum Reprod*. 2005;20:1213–20.
5. Chen SL, Ye DS, Chen X, Yang XH, Zheng HY, Tang Y, et al. Circulating luteinizing hormone level after triggering oocyte maturation with GnRH agonist may predict oocyte yield in flexible GnRH antagonist protocol. *Hum Reprod*. 2012;27:1351–6.
6. Honnma H, Hashiba Y, Asada Y, Endo T. Failure of triggering oocyte maturation with a GnRH agonist in polycystic ovary syndrome: two case reports. *Eur J Obstet Gynecol Reprod Biol*. 2011;157:239–40.
7. Stevenson TL, Lashen H. Empty follicle syndrome: the reality of a controversial syndrome, a systematic review. *Fertil Steril*. 2008;90:691–8.
8. Honnma H, Baba T, Sasaki M, Hashiba Y, Ohno H, Fukunaga T, et al. Trophoblast morphology significantly affects the rates of ongoing pregnancy and miscarriage in frozen-thawed single-blastocyst transfer cycle in vitro fertilization. *Fertil Steril*. 2012;98:361–7.
9. Peluso JJ. Role of the amplitude of the gonadotropin surge in the rat. *Fertil Steril*. 1990;53:150–4.
10. Castillo JC, Garcia-Velasco J, Humaidan P. Empty follicle syndrome after GnRHa triggering versus hCG triggering in COS. *J Assist Reprod Genet*. 2012;29:249–53.
11. Tomazevic T, Meden-Vrtovc H. Early timed follicular aspiration prevents severe ovarian hyperstimulation syndrome. *J Assist Reprod Genet*. 1996;13:282–6.

Authors' contributions

YA participated in the study as project supervisor coordinator overseeing the critical revision of the manuscript. FI carried out the bibliographic research. HH conceived the study and the design and drafted the manuscript. ST and YH helped draft the manuscript. NF, TB and TE analyzed the results. All authors read and approved the final manuscript.