Gonadotropin-releasing hormone agonist triggering with concomitant administration of low doses of human chorionic gonadotropin or a freeze-all strategy in high responders

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ABSTRACT

الأهداف: مقارنة معدلات المواليد الأحياء ومعدلات متلازمة فرط تحفيز المبيض المتوسطة و الشديدة (OHSS) لنهجين مختلفين باستخدام الناهض للهرمون المحرر لهرمونات الغدة التناسلية (GnRH) في النساء ذوات الاستجابه العالية.

الطريقة: اجريت دراسة التعرض استعادية لتقييم نتائج الحقن المنوي داخل الرحم (ICSI) ونقل الأجنة (ET) في النساء ذوات الاستجابة العالية اللاتي خضعن لتحريض الإباضة باستخدام بروتوكول GnRH في الفترة الممتدة بين ابريل عام 2011 حتى مارس 2015. في المجموعة الأولى (74 مريض) تم استخدام ناهض GnRH لتحفيز الاباضه باستخدام 1500 وحدة دولية من هرمون موجهة الغدد التناسلية المشيمية البوليه (hCG) مباشرة بعد استرجاع البويضه يليها نقل الأجنة ودعم اصفري قياسي. في المجموعة الثانية (48 مريض) تم استخدام ناهض GnRH المحفز بعد تجميد جميع الأجنة ومايليه من نقل الأجنة المجمدة والمذابة (FET) ويسمى هذا النهج بتجميد الكل.

النتائج: كانت الخصائص الأساسية متماثلة بين المجموعات. وكانت معدلات الحمل السريري للمجموعة الأولى 45.9% و في المجموعة الثانية 43.8 (اختبار مربع كاي p=0.812) وكانت معدلات المواليد الأحياء للمجموعة الاولى 40.5% والمجموعة الثانية 41.7% (اختبار مربع كاي p=0.902) قابلة للمقارنة بين المجموعات. لوحظ في المجموعة الاولى حالتين متأخرة من OHSS (حالة واحدة شديدة وحالة معتدلة واحدة) في اثنين من المرضى (2.7%). في المجموعة الثانية لم يعاني أي من المرضى من OHSS سواء بشكل معتدل أو شديد.

الخاتمة: معدل المواليد الأحياء مع ناهض GnRH المحفز ومايصاحبه من استخدام 1500 وحدة دولية من hCG مباشره بعد استرجاع البويضات كانت مماثله لتلك التي تم الحصول عليها باتباع نهج تجميد الكل. و FET في جرعة لاحقة وتسبب اعطاء جرعة منخفضه من hCG في مجموعات ناهض GnRH المحفز في حالات متوسطة أو شديدة في OHSS في 2.7% من المرضى.

Objectives: To compare the live birth rates and moderate/severe ovarian hyperstimulation syndrome (OHSS) rates of 2 different approaches using gonadotropin-releasing hormone (GnRH) agonist triggering in high responder women.

Methods: A retrospective cohort study was performed to evaluate intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) outcomes in high responder women who underwent ovulation induction with a GnRH antagonist protocol between April 2011 and March 2015. In group 1 (n=74), GnRH agonist was used for ovulation triggering with the concomitant use of 1500 IU of urinary human chorionic gonadotropin (hCG) immediately after oocyte retrieval followed by fresh ET and standard luteal support. In group 2 (n=48), GnRH agonist was used for triggering after freezing all embryos and subsequent frozen/thawed embryo transfer (FET); this approach is considered the "freeze-all" approach.

Results: Baseline characteristics were similar between the groups. The clinical pregnancy rates for group 1 was 45.9% and group 2 was 43.8% (p=0.812, chi-squared test) and live birth rates for group 1 was 40.5% and for group 2 41.7% (p=0.902, chi-squared test) were comparable between groups. In group 1, late-onset OHSS was observed (one severe case and one moderate case) in 2 patients (2.7%). In group 2, none of the patients experienced moderate/severe OHSS.

Conclusion: The live birth rate with GnRH agonist triggering and concomitant use of 1500 IU of hCG immediately after oocyte retrieval was similar to that obtained with the freeze-all approach and FET in a subsequent cycle. The administration of a low dose of hCG in GnRH agonist trigger cycles caused moderate/severe OHSS in 2.7% of the patients.

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Ovarian hyperstimulation syndrome (OHSS) is a potentially life threatening, iatrogenic complication of ovulation induction. It is estimated that the incidence is 3-6% for moderate, and 0.1-2% for severe OHSS.[1] It is clinically presented with cystic enlargement of the ovaries with or without ascites following a number of follicles (≥20) and high E2 concentration. Human chorionic gonadotropin (hCG) triggering for the final oocytes maturation seems to be the main factor for the development of OHSS.[2] The final maturation of the oocytes is necessary to resume the arrested meiosis and to separate the oocyte from the underlying follicle, which allows its recovery and subsequent fertilization through in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).[3] The use of hCG has been the most common method to induce final maturation of the oocyte in IVF cycles. However, the longer biological half-life due to the increased glycosylation of hCG may generate a predisposition to OHSS. The expanding use of gonadotropin-releasing hormone (GnRH) antagonist protocols in controlled ovarian hyperstimulation has allowed the use of a GnRH agonist as the trigger for final oocyte maturation.[4] GnRHa trigger may provide a more physiologic environment with the release of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), and reduce the risk of severe OHSS because of the shorter half-life of LH. However, the outcome of IVF cycles were reported to be suboptimal with the GnRH agonist trigger in some studies.[5,6,7] This may be related to the luteal phase deficiency rather than the effect on oocyte/embryo since frozen-thawed embryos and donor oocytes obtained in GnRH agonist trigger cycles yielded satisfying pregnancy rates.[8,9] Recent studies reported different luteal support methods to overcome the negative effects of GnRH agonist trigger, such as intensive steroid support,[10] low dose hCG together with GnRH agonist (dual trigger)[11] or the concomitant use of low dose hCG on the day of oocyte retrieval.[12-15] and satisfying pregnancy rates were obtained with the different risk of OHSS.[10-12,14]

On the other hand, as we know that extremely elevated estradiol levels as a response to ovarian stimulation may be detrimental on endometrium. Oocyte/embryo freezing followed by the transfer of thawed embryos in a subsequent cycle has been reported as a strategy to avoid detrimental effects of supraphysiologic levels of steroids on the endometrium.[16-18] Taken together, optimum approach in high responder patients undergoing an IVF cycle with the high risk of OHSS is still a subject of debate. In this study, we aimed to compare the live birth and moderate/severe OHSS rates of women having GnRH agonist triggering with the concomitant use of low dose of hCG immediately after oocyte retrieval and the "freeze-all" approach and frozen/thawed embryo transfer (FET).

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

Methods. *Study Design.* A retrospective cohort study was performed in the IVF Unit of Ota-Jinemed Hospital to evaluate ICSI/ET outcome in high responder women who underwent ovulation induction with GnRH antagonist protocol and GnRH agonist trigger for final oocytes maturation in a private IVF center between April 2011 and March 2015. The study was approved by the Institutional Review Board of Ota-Jinemed Hospital, and the protocols of the study were in accordance with the Helsinki Committee requirements. Patients were defined as high responder if they had ≥15 follicles ≥ 12 mm and/or serum estradiol levels ≥3500 pg/ml on the day of GnRH agonist trigger.

A total of 122 women was included in the study population. All participants were divided into 2 groups according to their subsequent treatment after GnRH agonist trigger. Group 1 (n=74), GnRH agonist was used for ovulation triggering with the concomitant use of 1500 IU of urinary hCG within an hour after oocyte retrieval followed by fresh embryo transfer with standard luteal support. Group 2 (n=48), GnRH agonist was used for triggering with freeze of all embryos and subsequently had a FET; this approach is considered the "freeze-all" approach.

All of the patients had normal uterine cavity confirmed with hysteroscopy or hysterosalphingography without any other medical problems. Eighty-five percent of patients underwent the first treatment cycle. ICSI was performed on all oocytes with the best spermatozoa available in the ejaculate. Only cycles with the presence of ejaculated spermatozoa were enrolled.

Controlled ovarian stimulation. Transvaginal ultrasound was carried out on cycle day 3 in all women and ovarian stimulation was initiated with recombinant FSH (Gonal F, Merck Serono). Initial dose was ranging from 150 to 225 IU, adjusted according to the woman's age, BMI and ovarian response in the previous attempt, if any. Gonadotropin-releasing hormon antagonist (Cetrotide 0.25 mg, SC, Merck Serono) was started on cycle day 6 and continued until the day of hCG. Patients were followed with serial estradiol assessments and transvaginal sonogram. Trigger with GnRH agonist (1 mg leuprolide acetate, Lucrin, Abbott) was

performed subcutaneously when at least two leading follicles reached 17 mm in diameter and oocyte retrieval was carried out 35-36 hours later. After oocyte retrieval, 1500 IU of urinary hCG (Pregnyl 1500IU, Merck Sharp & Dohme Limited, Hertfordshire, UK) was administered within an hour (Group 1). Oocytes were cultured in a medium (G-IVF-Plus,Vitrolife AB, Sweden) supplemented with 10% Human Serum Albumin at 37°C under 6% CO_2. Following cumulus-corona removal, ICSI was performed 2 hours after incubation on all metaphase II oocytes as described by Van Steirteghem et al.[19] Embryos were initially cultured in a culture medium G-1 PLUS (G5 series, Vitrolife AB, Sweden) until day 3, and then transferred to G-2 PLUS (G5 series, Vitrolife AB, Sweden). Transferring fresh embryos or freezing all of them for a subsequent cycle was determined after counseling with the patient.

Embryological freeze/thaw technique and luteal support. Embryos were frozen on day 3 or at the blastocyst stage. Vitrification method was used to freeze for both stages of embryos. The embryos were vitrified and warmed by the method developed by Kuwayama et al.[20] In short, they were equilibrated in the equilibration solution 7-10 minutes before exposure to the vitrification solution then loaded onto the tip of the cryotop within 45 seconds. The cryotop was immediately plunged into liquid nitrogen. For the warming procedure, the cryotop tip with embryos was plunged directly into pre-warmed sucrose solution for 1 minute. All steps were completed on the stage warmer of a dissecting microscope at 38°C and zona-intact embryos were then cultured in medium until transfer. Women underwent FET in a subsequent artificially prepared cycle within 3 months. Cryopreserved embryos were transferred on the day they were frozen. In group 1 (fresh embryo transfer (ET) cycles), luteal phase support was achieved with the injection of 50 mg progesterone IM, starting on the day of oocyte pick-up until the fetal heart beat was detected in group 1. In group 2 (FET cycles), endometrium was prepared with oral estradiol tablets (2 mg estradiol valerate) once a day starting on cycle day 3, and the dose was increased by 2 mg every 4 days until endometrial thickness reached ≥7 mm. Luteal phase P (50 mg IM) together with 4 mg of estradiol valerate were used until the detection of fetal heart beat in group 2. Clinical pregnancy was defined with the presence of fetal heart beat on transvaginal sonogram at 7 weeks of gestation. Miscarriage was defined as the loss of the pregnancy until 20 weeks of gestation. In addition, all clinical and laboratory procedures and follow-up of patients were performed in the same IVF center

Outcome variables. The primary outcome variables were the live birth rate and the incidence of moderate/severe OHSS defined by Golan et al.[21] All patients were examined one week after embryo transfer for the symptoms and signs of OHSS.

Statistical Analysis. Medcalc Statistical Software Program Version 16.8.4 was used for statistical analysis. Categorical variables were compared with chi-square or Fisher's exact test and continuous variables with independent samples t-test. Values are expressed as mean±SD or n (%). Statistical significance was defined as *p*-value <0.05.

Results. Women's age, FSH and anti-müllerian hormone levels, antral follicle count, duration and the etiology of infertility were similar between the 2 groups. Potential confounders are shown in **Table 1.** There were also no significant differences between the 2 groups in terms of number of follicles, E2 level on the day of trigger, the number of oocytes and metaphase II oocytes collected, fertilization rate, the number of embryos transferred and the endometrial thickness (**Table 2**). In fresh ET group, 55 out of 74 (74.3%), and in FET, 35 out of 48 (72.9%) women underwent blastocyst transfer. All women included had supernumerary embryos to be frozen in group 1.

The pregnancy (48.6% versus (vs) 50.0%, *p*=0.884), clinical pregnancy (45.9% vs. 43.8%, *p*=0.812) and live birth rates (40.5% vs. 41.7%, *p*=0.902) were comparable between groups. Miscarriage (13.3% vs. 15.0%, *p*=0.862) and twin pregnancy rates (23.3% vs. 25.0%, *p*=0.845) did not differ between the groups. (**Table 2**).

Table 1 - Patients' demographics of the 2 groups underwent ovulation induction with GnRH antagonist protocol and GnRH agonist trigger for final oocytes maturation in a private IVF center between April 2011 and March 2015.

Variables	Group 1 Concomitant hCG+fresh ET (n=74)	Group 2 Frozen-Thawed ET (n=48)	*p*-value
	n(%)		
Women's age (years)	30.5±4.6	29.8±4.4	0.448**
Basal FSH level (mIU/ml)	6.3±2.4	6.2±2.1	0.647**
No. of antral follicles	11.7±4.3	12.9±4.6	0.154**
AMH level (ng/ml)	3.5±1.8	3.7±1.7	0.405**
Duration of infertility (years)	3.9±2.1	3.8±1.7	0.726**
Etiology of infertility			
Male factor	13 (17.6)	14 (29.1)	0.135*
PCOS	38 (51.4)	23 (47.9)	
Tubal factor	10 (13.5)	4 (8.3)	
Unexplained	13 (17.6)	7 (14.6)	

Values are expressed as mean±SD. *P*-values are based on *chi-squared test, **independent samples t-test, PCOS - polycystic ovary syndrome, FSH - Follicle Stimulating Hormone, AMH - anti-müllerian hormone

Table 2 - Characteristics and the outcome of ovarian stimulation-ICSI cycles in the 2 groups.

Variable	Group 1 Concomitant hCG + fresh ET (n=74)	Group 2 Frozen-Thawed ET (n=48)	*p*-value
No. of follicles	18.3±3.1	19.2±3.8	0.463**
E2, day of trigger (ng/ml)	4050±768	4159±601	0.502**
No. of oocytes	16.5±5.8	15.1±4.4	0.147**
No. of MII oocytes	11.7±4.8	10.8±4.3	0.255**
Fertilization rate (%)	(79.9)	(78.2)	0.261**
No. of embryos transferred	1.6±0.7	1.4±0.7	0.226**
Endometrial thickness (mm)	9.4±3.4	8.6±4.1	0.353**
Transfer date n (%)			
Day-3 transfer	19 (25.7)	13 (27.1)	0.863*
Day-5 transfer	55 (74.3)	35 (72.9)	
Pregnancies	36 (48.6)	24 (50.0)	0.884*
Clinical pregnancies	34 (45.9)	21 (43.8)	0.812*
Live birth rate	30 (40.5)	20 (41.7)	0.902*
No. of twins	7/30 (23.3)	5/20 (25.0)	0.862*
No. of miscarriages	4/30 (13.3)	3/20 (15.0)	0.845*
Severe/moderate OHSS	2/74 (2.7)	0	0.518†

Values are expressed as mean±SD or n (%). *P*-values are based on *chi-square test or **independent samples t-test, or †Fisher's exact test, Metaphase II, ICSI - intracytoplasmic sperm injection

In Group 1, 2 patients (2.7%) developed OHSS (one severe and one moderate) around the time of first pregnancy test (12 days after ET). The severe OHSS case was a 31-year-old woman with 32 oocytes retrieved. One blastocyst was transferred which resulted in a singleton pregnancy. She required removal of ascitic fluid twice and hospitalization for two days. The moderate OHSS case was a 30-year-old woman with 24 oocytes at retrieval and was also pregnant, carrying a singleton. She was followed on an outpatient basis with mild analgesics. In Group 2, none of the patients experienced moderate/severe OHSS.

Discussion. The present study revealed that there was no significant difference in the live birth rate and OHSS rate between the two approaches: the freeze-all strategy and subsequent FET and the administration of a low dose of hCG (1500 IU) immediately after oocyte retrieval. However, addition of low doses of hCG resulted in moderate/severe OHSS in 2.7% of the patients. According to our knowledge, this is the first study comparing two approaches that follow GnRH agonist triggering in women who underwent ovulation induction with GnRH antagonist protocol.

The use of hCG triggering for the final oocytes' maturation seems to be the main factor for the development of OHSS in high responder women.[2] In addition to the high risk of OHSS, high estradiol (E2) levels during embryo transfer may also be associated with abnormal implantation and abnormal placentation characterized by fetal growth restriction and pregnancy-induced hypertension.[22] Detrimental effects of extremely high E2 levels on the endometrium as well as the embryo can be avoided by freezing all embryos and subsequent FET. Grisinger et al[8] reported similar live birth rates in FET cycles with oocytes obtained through GnRh agonist triggering as compared to conventional hCG triggering for final oocyte maturation. Bodri et al[9] found an ongoing pregnancy rate of 32.1% with donor oocytes obtained through GnRH agonist triggering. In this study, a live birth rate of 41.7% in the FET cycles following the freeze-all strategy was higher than the 14.6% rate reported in the study by Griesinger et al.[17] Different patient populations or endometrial preparation protocols might have played a role in the inconsistency. In a recent meta-analysis, Roque et al[23] concluded that cryopreservation of all embryos and subsequent FET resulted in better outcomes compared to fresh embryo transfer, assumingly due to better physiologic synchronization of the embryo and endometrium. Apparently, FET seems to be the best strategy, but this approach requires experienced staff and a laboratory for performing FET cycles.

The main purpose of GnRH agonist triggering in high responder women is to minimize the risk of OHSS. However, previous studies have shown that GnRH agonist triggering is related to a lower ongoing pregnancy rate[22] compared to conventional hCG triggering. Furthermore, it was found that the impaired pregnancy rates associated with the GnRH agonist trigger may be attributed to insufficient luteal phases rather than oocyte immaturity.[5-7] Therefore, some strategies have been developed to support the luteal phase and ameliorate the outcome of GnRH agonist triggering within IVF cycles. In a recent report, Humaiden et al[12] administered a low dose of 1500 IU of hCG in the morning of oocyte retrieval and found a significant improvement in the pregnancy rate. Radesic et al[13] found an ongoing pregnancy rate of 52.1% with GnRH agonist triggering and with 1500 IU of hCG administered on the day of oocyte retrieval. Datta et al[14] compared the concomitant use of 1500 IU of hCG immediately after oocyte retrieval in GnRH agonist trigger cycles with 5000 IU of hCG administered in high responders. Although it was not statistically significant, the live birth rate was found to be slightly higher (35.5% vs. 24.1%) in the group with GnRH agonist triggering. In a recent international multicenter retrospective study, the clinical pregnancy rate was 41.8% per cycle started

with GnRh agonist triggering and 1500 IU of urinary hCG or 1500 IU of recombinant hCG administered within an hour of oocyte retrieval.[15] In our study, the live birth rate of 40.5%, obtained in cycles with GnRH agonist triggering and fresh ET, was similar to the rates reported in previous studies.[13,14]

Criteria for the selection of the best approach for cycles of GnRH agonist triggering vary among studies; the number of follicles and the peak E2 levels are the most important parameters. Griesinger et al[8] applied the freeze-all approach in women with ≥20 follicles and/or an E2 level ≥4000 pg/ml. In this study, women with ≥ 15 follicles (≥12 mm) and/or E2 levels ≥3500 pg/ml met selection criteria for GnRH agonist triggering. The cut-off values for the number of follicles and E2 level were empirically chosen and need to be confirmed by further studies. Radesic et al[13] used GnRH agonist triggering and 1500 IU of hCG on the day of oocyte retrieval in patients with ≥14 follicles (≥12 mm) and obtained a clinical pregnancy rate of 52.1%.

At present, there is no standard approach regarding the addition of hCG in GnRH agonist trigger cycles to improve live birth rates. Kummer et al[25] recommended the assessment of LH levels the day after GnRH agonist triggering for the adjustment of low dose hCG administration, but the routine use of this approach needs further investigation.

The use of GnRH agonist triggering has been reported as an effective method to prevent clinically significant OHSS in high responders.[5,26,27] In a recent study, the expression of vascular endothelial growth factor (VEGF) and inhibin β was found to be lower in the granulosa cells collected from patients with GnRH agonist triggering than those from patients with hCG triggering, which may be another factor for the lower OHSS rate.[28] Datta et al[14] reported no severe OHSS and 16.2% mild-to-moderate OHSS in 62 women after GnRH agonist triggering and administration of 1500 IU of hCG immediately after oocyte retrieval. Iliodromiti et al.[15] reported severe OHSS in 2 out of 275 (0.72%) women with the administration of 500 IU of hCG one hour after oocyte retrieval. In our study, we observed 2 cases of moderate/severe OHSS (2.7%) in women who had GnRH agonist triggering with administration of 1500 IU of hCG immediately after oocyte retrieval, and our OHSS rate was similar to the rates found in previous studies. However, Seyhan et al[29] reported severe OHSS in 6 out of 23 women (26%) with the use of 1500 IU of hCG as a "luteal rescue" following GnRH agonist triggering. Further studies assessing the efficacy of hCG dosing lower than 1500 IU in GnRH agonist trigger cycles may help decrease OHSS rates. Although late-onset OHSS, which is a result of hCG secreted by the conceptus, can be avoided by cancellation of embryo transfer, GnRH agonist triggering per se does not completely eliminate OHSS. Recently, 2 studies reported severe OHSS following GnRH agonist triggering in the freeze-all approach.[30,31] We have not experienced any severe early-onset OHSS with the freeze-all strategy, but Griesinger et al[17] reported a single case of severe early-onset OHSS out of 51 women who underwent the same protocol as we used. In our study, the absence of early-onset OHSS in the freeze-all group may be due to the clinical approach that involved careful selection of an initial FSH dose based on qualitative factors such as age, polycystic ovary syndrome (PCOS) status, or previous hyperstimulation and quantitative factors such as antral follicle count and serum anti-mullerian hormone (AMH) levels.

The present study has some limitations, such as its retrospective design and relatively small number of patients. The choice of one of the strategies could have been affected by counseling with the patient and the physician's previous experience regarding high-risk patients. However, to the best of our knowledge, this is the first study comparing the 2 approaches following GnRH agonist triggering in women who underwent ovulation induction with GnRH antagonist protocol. Results may provide clinicians valuable data for clinical practice. In addition, all clinical and laboratory procedures and follow-up of patients were performed in the same IVF center.

In conclusion, the outcome of ICSI cycles with GnRH agonist triggering and concomitant use of 1500 IU of hCG immediately after oocyte retrieval is similar to that obtained with the freeze-all approach and FET in subsequent cycles in high responders.

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