



Comparison of Ovulation Triggering Outcomes by Standard Dose of hCG versus Declined hCG Dose With GnRH Agonist in Antagonist Cycles: A Randomized Clinical Trial

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Abstract

Objectives: Today, infertility is a global complication affecting 8%-12% of couples. The use of assisted reproductive techniques (ARTs) gives couples to have taken the home baby chance. In this study, we compare the outcomes of the ARTs after using the standard dose of human chorionic gonadotropin (hCG) hormone and gonadotropin-releasing hormone (GnRh) agonist plus declined dose of hCG for final oocyte maturation in antagonist cycles.

Materials and Methods: This randomized clinical trial was conducted in Al-Zahra hospital, Tabriz, Iran from June to December 2019 on 100 infertile women who were candidate for in vitro fertilization in two groups: group A received standard dose of hCG and group B received GnRH agonist 0.2 mg and hCG 2500 international unit as dual trigger. The number of oocytes retrieved, embryo obtained, implantation rate, pregnancy rate, and ovarian hyperstimulation syndrome (OHSS) were compared between two groups.

Results: The number of retrieved oocytes in the group B was more than group A ($P=0.024$), and the embryo obtained in both groups was similar. The implantation and pregnancy rate in the group B increased compared to group A ($P= 0.001$). There was no OHSS case in the group B, while in the group A two cases suffered from OHSS.

Conclusions: Dual trigger with GnRH agonist plus declined hCG dose increased the number of retrieved oocytes, implantation, and pregnancy rates and decreased the risk of OHSS compared to standard hCG trigger. The gynecologists should select the best strategy based on the patient's condition.

Keywords: GnRH agonist, Human chorionic gonadotropin hormone, Ovarian hyperstimulation syndrome, Reproductive techniques, Assisted

Introduction

Infertility is defined as failure to achieve clinical pregnancy in women under 35 years old after 12 months of unprotected intercourse and after 6-month regular intercourse in women more than 35 years old according to the World Health Organization (1,2). Approximately 8%-12% or 60-80 million couples suffer from infertility worldwide (3-5). It mostly occurs because of anovulation, fallopian tube disease, pelvic adhesions, endometriosis, and unexplained infertility (6). Assisted reproductive techniques (ARTs) give infertile couples a chance to have healthy offspring (7). The first step to achieve ART cycles is ovarian stimulation (8,9).

Ovarian hyperstimulation syndrome (OHSS) occurs in 20-30% of ovarian stimulation cycles (10-11). Due to the structural and biological similarity of human chorionic gonadotropin (hCG) and luteinizing hormone (LH) specialists administrate the hCG hormone (10) instead of endogen LH. hCG has some side effects like releasing vasoactive substances and vascular endothelial growth factor (VEGF), therefore it may increase the risk of OHSS (12). Another strategy to induce LH surge is gonadotropin-releasing hormone (GnRH) agonists.

GnRH agonists can increase the LH and follicle-stimulating hormone (FSH) surge simultaneously, so it may help to better oocyte maturation and granulosa cells expansion. Moreover, GnRH agonists administration does not effect on OHSS risk in luteal phase (13). To have the benefits of both triggers, specialist administrate another strategy as dual trigger, in this regards the hCG in lower dose and GnRH agonist are prescribed with together (14,15).

The main outstanding points to conduct this study were the importance of ovulation stimulation cycles, the risk of OHSS, and inconsistencies in the reported results. Given the promising results of this trigger strategy, in the present study, we administrated hCG in declined dose and GnRH agonist in trigger time in antagonist cycles. We then evaluated the outcome of IVF in both strategies.

Material and Methods

Study Design and Participants

This randomized clinical trial was conducted in Al-Zahra hospital, Tabriz, Iran from June to December 2019 on 100 infertile women who were candidate for in vitro fertilization in two groups ($n = 50$ /each). Group A received



Key Messages

- ▶ Dual trigger with GnRH agonist plus declined hCG dose reduced OHSS risk.

standard dose of hCG (10000 IU, Organon, Norway) and group B received 0.2 mg GnRH agonist (Decapeptyl, Ferring, Germany) and hCG 2500 IU as a dual trigger. Our inclusion criteria were infertile women candidates for IVF/ICSI aged between 20 to 40 years old who were at risk of OHSS.

All women with diminished ovarian reserve (AFC antral follicle count (AFC) < 4 or anti-Mullerian hormone (AMH) < 1) were excluded.

Sample Size

The sample size was estimated to be a minimum of 100 (50 in each group) by considering the significance level of 95%, the power of 80%, based on the study by Schachter and others (18) and used the following formula:

$$n = \frac{(Z_1 - \frac{\alpha}{2} + Z - \beta)^2 (S_1^2 + S_2^2)}{\Delta^2}$$

Randomization and Blinding

A computer-generated randomization list with a block size of 4, with 1:1 allocation was used. In such a case, one control will be checked for each intervention and with each of the 4 participants enrolled in the study; the two groups are balanced in terms of control and intervention. The analyzer was not aware of grouping and type of treatment in each group.

IVF Protocol and Assessments

All participants had specific and confidential codes, and then randomly divided into two groups using a computer system with a block size of 4, with 1:1 allocation. To ovarian stimulation, an antagonist protocol was administered to all participants. Thus, on days 3-7 of the menstrual cycle, Letrozole (5 mg/daily, orally) and then recombinant FSH Gonal-f® (EMD Serono, USA) 150 unit subcutaneous per day were administered from days 8 to 10 of the menstrual cycle. Follow-up with sonography to check the size of the follicles started on the 7th day and was performed every other day. When the follicles in 13-14-mm diameter were detected in sonography, 0.25 mg of Cetrotide (Cetrorelix acetate; EMD Serono, USA) as a GnRH antagonist was administered. When the follicle growth was ≥18 mm, they were triggered with hCG and GnRH agonist or only hCG according to their study group. The embryos were transferred three days after oocyte retrieval, and two weeks after embryo transfer, blood β-hCG was tested.

Outcomes and Data Collection

Finally, the number of the retrieved oocyte, obtained embryo, OHSS cases, also implantation and pregnancy

rates were recorded and compared between two groups. Two weeks after embryo transfer, serum β-hCG was assessed in all participants. If it were positive, it was considered as the chemical pregnancy, and in these women, 14 days later, transvaginal sonography was performed to determine the fetal heartbeat. The existence of a heartbeat in the fetus was considered a clinical pregnancy.

Statistical Analysis

Data analysis was performed using STATA software (Software Statistics and Data Science, StataCorp., version 16) and graphs were drawn by GraphPad Prism version 7. Mean and standard deviation were used to describe quantitatively symmetric data. Kolmogorov-Smirnov test was used to assess the data normality. In addition, Student's *t* test was utilized for continuous variables and compared the means of the two groups. *P* value < 0.05 was considered statistically significant.

Results

Out of 231 patients referred to Al-Zahra hospital in Tabriz on the date, 124 patients were excluded from the study based on lack of inclusion criteria. Four people were excluded from the study due to non-acceptance of the study conditions and 3 people were excluded from the study due to personal reasons. Finally, we had 100 patients after completing the consent form entered the study. Then, dividing the subjects into two groups, 50 patients control and 50 patient intervention group. None of the participants was lost to follow-up (Figure 1).

Demographics and clinical characteristics of all participants were demonstrated in Table 1. There were no significant differences in age, body mass index (BMI), number of smokers (men or women), previous pregnancy, previous live birth, infertility type, endometrial thickness, the number of previous embryo -transferring and the number of embryos in previous transfers between two groups (Table 1).

In addition, the results of the studies in the two groups are shown in Table 2 and Figure 2.

The number of retrieved oocytes in the group A and B was consequently 8.7 and 10.4; there was a significant difference between the two groups (*P*=0.024) (Figure 2A). The difference between the two groups in the number of embryos obtained was insignificant (*P*=0.26) (Figure 2B). Based on Figure 2C, the implantation rate in the group A was 36%, and in the group B was 46% (*P*=0.001), so this difference was significant. The pregnancy rate in the group A was 32% and significantly lower than the group B (42%) (*P*=0.001) (Figure 2D). Based on Table 2, two cases of OHSS were reported in the hCG group, while in the dual trigger group, no cases suffered from OHSS.

Discussion

Today, infertility has affected 8%-12% of couples worldwide. Specialists in this field have always tried to

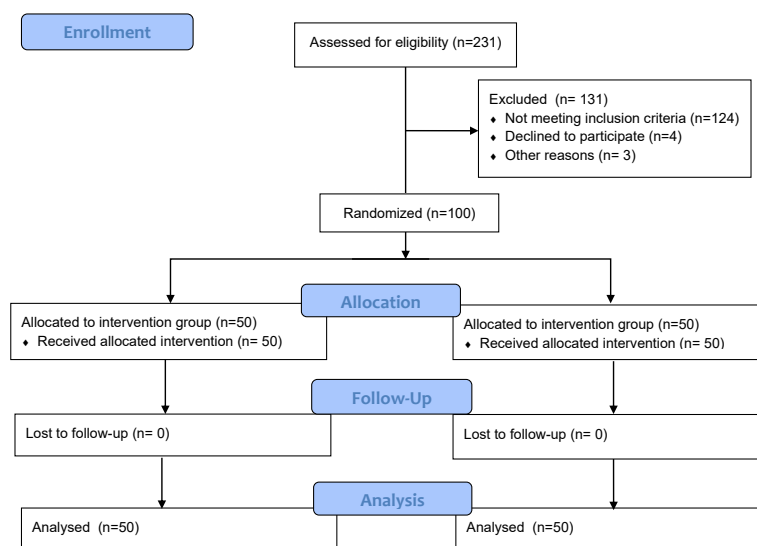


Figure 1. The Study CONSORT Flowchart.

improve the results of infertility treatment and increase the couple's chance to take home a baby. Here we evaluated the effect of the dual trigger by a declined dose of hCG and GnRH agonist and traditional trigger with hCG on ART outcomes.

Based on our results, dual triggers increased the number of retrieved oocytes. Other studies have confirmed our data. According to them, using dual trigger with a standard

dose of hCG at trigger time increased the number of the oocyte (19). Even in poor responders, dual trigger with a declined dose of hCG increased the number of mature retrieved oocytes (20). A study about the different doses of hCG and GnRH agonists indicated that the 1000 IU hCG and GnRH agonist increased the number of mature oocytes (21). More retrieved oocyte in the dual trigger cycle maybe cause of LH and FSH surge together like natural

Table 1. Demographics and Clinical Characteristics of the Study Participants (n=50/each)

| Variables | Group A (hCG) | Group B (Dual Trigger) | P Value |
|--|------------------------|------------------------|-------------------|
| Age (y), mean \pm SD (age gap) | 34.7 \pm 3.5 (21-40) | 35.1 \pm 2.9 (22-40) | 0.29 ^a |
| Body mass index (kg/m ²), mean \pm SD | 28.8 \pm 4.3 | 29.6 \pm 3.7 | 0.17 ^a |
| History of smoking, n (%) | 0 (0) | 0 (0) | ^c |
| History of smoking in the spouse, n (%) | 9 (18) | 8 (16) | 0.13 ^b |
| Previous pregnancy, n (%) | 14 (27.9) | 15 (30.3) | 0.05 ^b |
| Previous live birth, n (%) | 8 (16.27) | 9 (18.18) | 0.16 ^b |
| Primary infertility, n (%) | 34 (67.44) | 33 (66.66) | 0.24 ^b |
| Secondary infertility, (%) | 32.59% | 33.33% | 0.03 ^b |
| Endometrial thickness, mean \pm SD | 10.6 \pm 1.6 | 10.5 \pm 1.4 | 0.41 ^a |
| Number of previous embryo-transferring, mean \pm SD | 4.32 \pm 2.2 | 4.45 \pm 2.64 | 0.46 ^a |
| Number of embryos in previous transfers, mean \pm SD | 8.3 \pm 4.2 | 9.2 \pm 6.8 | 0.15 ^a |

^a Student's t test.

^b Fisher exact test.

^c Since there was no smoker patient in our study, we did not do any statistical work on this parameter.

Table 2. Comparison of the Study Outcomes in two Groups (n=50/each)

| Variables | Group A (hCG) | Group B (Dual Trigger) | P Value |
|----------------------------------|---------------|------------------------|--------------------|
| Retrieved oocytes, mean \pm SD | 8.7 \pm 3.3 | 10.4 \pm 4.1 | 0.024 ^a |
| Obtained embryos, mean \pm SD | 4.6 \pm 2.2 | 5.2 \pm 3.1 | 0.26 ^a |
| Implantation rate, n (%) | 18 (36) | 23 (46) | 0.001 ^a |
| Pregnancy rate, n (%) | 16 (32) | 21 (42) | 0.001 ^a |
| OHSS, n (%) | 2 (4) | 0 (0) | |

^a Student's t test.

^b Fisher exact test.

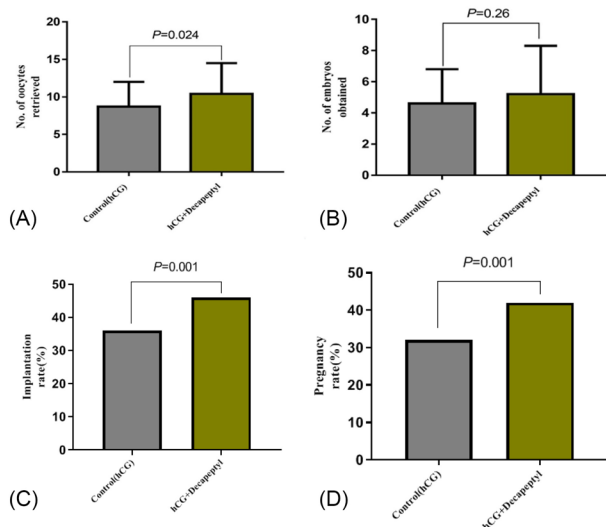


Figure 2. Comparison of the study outcomes in two study groups: (A) The number of retrieved oocyte ($P=0.024$), (B) The number of obtained embryo ($P=0.26$), (C) The implantation rate ($P=0.001$), and (D) The pregnancy rate ($P=0.001$).

cycle after GnRH agonist administration. FSH increases the LH receptors in granulosa cells, persuading them to expand and oocyte to continue meiosis and maturation (22). The number of embryos obtained in dual trigger and hCG were similar. Another study is corroborated our data that the fertilization rate in dual trigger and hCG had no difference (23).

On the other hand, another study indicated that dual trigger strategy increased the number of the blastocyst and improved the quality of embryos obtained (15). Based on our investigation, the implantation and pregnancy rate after dual trigger increased in patients. Another study in different condition patient indicated the dual trigger could improve the ART outcomes by increasing the implantation, pregnancy, and ongoing pregnancy rate (19). In addition, this strategy enhanced the chance of taking home a baby (15,23).

OHSS is one of the major problems in the ART cycle, and the excessive activity of LH causes it. In the ART cycle after the hCG trigger, LH activity remains for a long time and consequently increases the risk of OHSS (24). Since the hCG hormone have a long half-time, it increases the level of steroid hormones in the luteal phase, so it enhances the risk of OHSS and declines the endometrial receptivity (25). The risk of OHSS depends on hCG dose; in the IUI cycle that the hCG administration is in declined doses and follicle number is less, the OHSS occurrence is lower too (26). OHSS in a patient who received 2500 IU hCG and GnRH agonies as dual-trigger did not occur, while two cases in hCG trigger suffered from OHSS. Shapiro and co-workers confirmed our data that dual trigger declined the risk of OHSS in IVF candidate patient (27).

Moreover some studies revealed that the only GnRH agonist administration as a trigger decreased the OHSS

prevalence compared to dual trigger even with a lower dose of hCG (16,21). Generally, in hyper respond patients, GnRH agonies trigger a better choice (22). Soares and colleagues indicated that the level of VEGF in plasma and follicular fluid in patient who suffer from OHSS is more than in other patients (28). Still, another study revealed that the anti-angiogenic factor pigment epithelium-derived factor (PEDF) and the PEDF/VEGF balance could effect on OHSS (29). An in vitro study illustrated that GnRH agonist-induced this balance while the hCG had a negative effect on PEDF/VEGF balance (30). Therefore, this event can approve the effect of the hCG trigger on OHSS.

Limitations of the Study

The limit of our study was its relatively small sample size because of achieving the desired power of 80% and an alpha value of 0.05, 386 people are needed to study in each group (assuming a 1: 1 allocation) to show the needed power to indicate differences in live birth rates. Another limitation that could affect the sample size was the limited duration of the study interval. On the other hand, failure to investigate the molecular mechanisms involved in effect of GnRH agonist on increasing ovarian stimulation, increasing embryo implantation, and increasing endometrial acceptance was another limitation of the present study.

Suggestions

- It is suggested that a study with a higher sample size should be conducted if sufficient financial resources are allocated.
- For better results, the follow-up period of patients after IVF / ICSI can be extended, and patients can be monitored for a more extended period to determine pregnancy outcomes.
- It is suggested that a study should be designed and performed to investigate the possible molecular mechanisms involved in the effect of GnRH agonist on increasing ovarian stimulation, increasing embryo implantation, and endometrial receptivity.
- Different doses of GnRH agonists can be used simultaneously in a study to obtain the best and most effective dose
- It is also possible to study the expression pattern of different genes involved in embryo implantation before and after using the dual Trigger method in patients.

Conclusions

Dual trigger with GnRH agonist plus declined hCG dose increased the number of retrieved oocytes, implantation, and pregnancy rates and decreased the risk of OHSS compared to standard hCG trigger. The gynecologists should select the best strategy based on the patient's condition.

Authors' Contribution

NN conceived and designed the study and critically revised the manuscript. FV performed the statistical analysis and interpretation of data, and helped to draft the manuscript. All authors had full access to the data, read and approved the final manuscript, and takes responsibility for the integrity of the data and the accuracy of the analysis.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

The present study was approved by the Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran (Code: IR.TBZMED.REC.1397.594). The study protocol has been registered in the Iranian Registry of Clinical Trials (identifier: IRCT20101227005485N7). All stages of the work were fully explained to the participants and all of them signed an informed consent before participating in the study.

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References

- Zegers-Hochschild F, Adamson GD, de Mouzon J, et al. The international committee for monitoring assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary on ART terminology. *Fertil Steril.* 2009;92(5):1520-1524. doi:10.1016/j.fertnstert.2009.09.009
- Practice Committee of tAmerican Society for Reproductive Medicine. Definitions of infertility and recurrent pregnancy loss. *Fertil Steril.* 2008;89(6):1603. doi:10.1016/j.fertnstert.2008.03.002
- World Health Organization (WHO). WHO international classification of diseases, 11th Revision (ICD-11). WHO; 2019.
- Mascarenhas MN, Flaxman SR, Boerma T, et al. National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. *PLoS Med.* 2012;9(12):e1001356: 1-12. doi:10.1371/journal.pmed.1001356
- Shankardass MK. Looking back, looking forward: A profile of sexual and reproductive health in india. *JSTOR.* 2006;55(2):338-340.
- Barbieri RL. Female infertility. *Yen Jaffe's Reprod Endocrinol.* 2019;1:556-581. doi:10.1016/B978-0-323-47912-7.00022-6.
- Nosrati R, Graham PJ, Zhang B, et al. Microfluidics for sperm analysis and selection. *Nat Rev Urol.* 2017;14(12):707-730. doi:10.1038/nrurol.2017.175
- Shrestha D, La X, Feng HL. Comparison of different stimulation protocols used in in vitro fertilization: A review. *Ann Transl Med.* 2015;3(10):1-7. doi:10.3978/j.issn.2305-5839.2015.04.09
- Humaidan P, Nelson S, Devroey P, et al. Ovarian hyperstimulation syndrome: Review and new classification criteria for reporting in clinical trials. *Hum Reprod.* 2016;31(9):1997-2004. doi:10.1093/humrep/dew149
- Orvieto R. Triggering final follicular maturation-hCG, GnRH-agonist or both, when and to whom? *J Ovarian Res.* 2015;8(1):1-6. doi:10.1186/s13048-015-0187-6.
- Castillo JC, Humaidan P, Bernabéu R. Pharmaceutical options for triggering of final oocyte maturation in ART. *BioMed Research International.* 2014;2014:580171. doi:10.1155/2014/580171
- Humaidan P, Kol S, Papanikolaou E. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? *Hum Reprod Update.* 2011;17(4):510-524. doi:10.1093/humupd/dmr008
- Kol S, Humaidan P. GnRH agonist triggering: recent developments. *Reprod Biomed Online.* 2013;26(3):226-230. doi:10.1016/j.rbmo.2012.11.002
- Engmann L, Benadiva C. Ovarian hyperstimulation syndrome prevention strategies: luteal support strategies to optimize pregnancy success in cycles with gonadotropin-releasing hormone agonist ovulatory trigger. *Semin Reprod Med.* 2010;28(6):506-512. doi:10.1055/s-0030-1265678
- Haas J, Bassil R, Samara N, et al. GnRH agonist and hCG (dual trigger) versus hCG trigger for final follicular maturation: A double-blinded, randomized controlled study. *Hum Reprod.* 2020;35(7): 1648-1654. doi:10.1093/humrep/deaa107
- O'Neill KE, Senapati S, Maina I, et al. GnRH agonist with low-dose hCG (dual trigger) is associated with higher risk of severe ovarian hyperstimulation syndrome compared to GnRH agonist alone. *J Assist Reprod Genet.* 2016;33(9):1175-1184. doi:10.1007/s10815-016-0755-8
- Al-Mousawi BJ, Al-Azzam MA, Al-Zahawi B, et al. Agonist versus antagonist in intracytoplasmic Sperm Injection Cycles: Which Is the Best? *Int J Women Health Rep Sci.* 2020;8(3):285-289. doi:10.15296/ijwhr.2020.46
- Schieve LA, Devine O, Boyle CA, et al. Estimation of the contribution of non-assisted reproductive technology ovulation stimulation fertility treatments to US singleton and multiple births. *Am J Epidemiol.* 2009;170(11):1396-1407. doi:10.1093/aje/kwp281
- Fabris AM, Cruz M, Legidos V, et al. Dual triggering with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin in patients with a high immature oocyte rate. *Reprod Sci.* 2017;24(8):1221-1225. doi:10.1177/1933719116682873
- De Oliveira SA, Calsavara VF, Cortés GC. Final oocyte maturation in assisted reproduction with human chorionic gonadotropin and gonadotropin-releasing hormone agonist (dual trigger). *JBRA Assist Reprod.* 2016;20(4):246-250. doi:10.5935/1518-0557.20160047.
- Lu X, Hong Q, Sun L, et al. Dual trigger for final oocyte maturation improves the oocyte retrieval rate of suboptimal responders to gonadotropin-releasing hormone agonist. *Fertil Steril.* 2016;106 (6):1356-1362. doi:10.1016/j.fertnstert.2016.07.1068
- Griffin D, Feinn R, Engmann L, et al. Dual trigger with gonadotropin-releasing hormone agonist and standard dose human chorionic gonadotropin to improve oocyte maturity rates. *Fertil Steril.* 2014; 102:405-409. doi:10.1016/j.fertnstert.2014.04.028.
- Şükür YE, Ulubaşoğlu H, İlhan FC, et al. Dual trigger in normally-responding assisted reproductive technology patients increases the number of top-quality embryos. *Clin Exp Reprod Med.* 2020; 47(4):300-305. doi: 10.5653/ceerm.2020.03804.
- Castillo JC, Haahr T, Martínez-Moya M, et al. Gonadotropin-releasing hormone agonist for ovulation trigger—OHSS prevention and use of modified luteal phase support for fresh embryo transfer. *Ups J Med Sci.* 2020;125(2):131-137. doi:10.1080/03009734.2020.1736696
- Cerrillo M, Pacheco A, Rodríguez S, et al. Effect of GnRH agonist and hCG treatment on VEGF, angiopoietin-2, and VE-cadherin: trying to explain the link to ovarian hyperstimulation syndrome. *Fertil Steril.* 2011;95:2517-2519. doi:10.1016/j.fertnstert.2010.12.054
- Steures P, Berkhout JC, Hompes PG, et al. Patients' preferences in deciding between intrauterine insemination and expectant management. *Hum Reprod.* 2005;20(3):752-755.

- doi:10.1093/humrep/deh673
27. Shapiro BS, Daneshmand ST, Garner FC, et al. Gonadotropin-releasing hormone agonist combined with a reduced dose of human chorionic gonadotropin for final oocyte maturation in fresh autologous cycles of in vitro fertilization. *Fertil Steril*. 2008;90(1):231-3. doi:10.1016/j.fertnstert.2007.06.030
 28. Soares SR, Gómez R, Simón C, et al Targeting the vascular endothelial growth factor system to prevent ovarian hyperstimulation syndrome. *Hum Reprod Update*. 2008;14(4):321-333. doi:10.1093/humupd/dmn008.
 29. Chuderland D, Ben-Ami I, Kaplan-Kraicer R, et al. The role of pigment epithelium-derived factor in the pathophysiology and treatment of ovarian hyperstimulation syndrome in mice. *J Clin Endocrinol Metab*. 2013;98(2):E258-E626. doi:10.1210/jc.2012-3037.
 30. Miller I, Chuderland D, Ron-El R, et al. GnRH agonist triggering modulates PEDF to VEGF ratio inversely to hCG in granulosa cells. *J Clin Endocrinol Metab*. 2015;100(11):E1428-E1436. doi:10.1210/jc.2015-2312.

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