Reproductive Medicine; *Endocrinology and Infertility*

Different Timing of Adjuvant Low Dose hCG and GnRH Agonist Trigger Protocol, in OHSS High-Risk Patient with Peak E2 Level <4000 pg/mL

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ABSTRACT

OBJECTIVE: The aim of the study is to compare the live birth rates between 1,500 I.U. of Human chorionic gonadotropin at the time of Gonadotropin-releasing hormone agonist trigger day or 35-36 h later on the oocyte pick-up day, without affecting the risk of significant ovarian hyperstimulation syndrome development in high-risk patients with peak E2 level <4,000 pg/mL

STUDY DESIGN: This single-center prospective cohort study encompassed the period from March 2016 to March 2018 year. A total of 216 patients entered for final analysis, underwent a flexible antagonist protocol, intracytoplasmic sperm injection, and embryo transfer on the 3rd or 5th day in autologous cycles. Patients were randomized in one of two groups: Group A- Dual trigger group - 1,500 IU of Human chorionic gonadotropin at the time of Gonadotropin-releasing hormone agonist trigger day and Group B- 1,500 IU of Human chorionic gonadotropin 35-36 h later, on the oocyte pick-up day. To compare the two groups, we used nonparametric and parametric statistical tests. Significant differences were considered all values of $p<0.05$.

RESULTS: There is no significant difference between the two (A vs B) groups according to the average number of retrieved oocytes (13.08 vs 14.41 $p=0.08$), M II oocytes (10.5 vs 10.95 $p=0.46$), GV (1.24 vs 1.52 $p=0.09$, the fertility rate (68.46% vs 64.04% $p=0.07$). The dual trigger group (A) had a significantly higher live birth rate (62.29% vs 42.37% $p<0.05$) compared with the Gonadotropin-releasing hormone-a trigger group (B). There were no cases of moderate or severe ovarian hyperstimulation syndrome in both groups.

CONCLUSION: Our study shows that in hyper responders where the E2 peak is <4,000 pg/mL, the two approaches to the final oocyte maturation trigger have a correct outcome of the results, both in terms of the results from the in vitro fertilization and the low risk of ovarian hyperstimulation syndrome appearance.

Keywords: Controlled ovarian stimulation, Gonadotropin-releasing hormone agonist trigger, Live birth, Low 1,500 IU Human chorionic gonadotropin, Ovarian hyperstimulation syndrome

Gynecol Obstet Reprod Med 2020;26(3):203-209

Introduction

Ovarian hyperstimulation syndrome (OHSS) is a serious iatrogenic complication as a result of a response to gonadotropin and hCG application as a trigger. The incidence of moderately and severe hyperstimulation is generally 3.1 to 8%, but in high-risk responders, women with young age, low body mass index (BMI) and polycystic ovarian syndrome (PCOS), the percentage is over 20% accompanied by high morbidity and even patient mortality in some cases (1,2). The introduction of the antagonist protocol with agonist (GnRH-a) as a trigger for oocyte maturation has dramatically reduced this incidence (3). The advantage of this type of trigger (GnRH agonist) eliminates the risk for OHSS because of its short half-life in IVF cycles, but there is controversy regard-

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Submitted for :Publication *26.07.2020*
Revised for: Publication *23.10.2020*
Accepted for Publication: *07.11.2020*

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Quick Response Code:	Access this article online
	Website: www.gorm.com.tr e- mail: info@gorm.com.tr
	DOI:10.21613/GORM.2020.1133

How to cite this article: Petanovska Kostova EZ. Sotiroska V. Dimitrov G. Stojkovska S. Hadzi Lega M. Stojanovska D. Stamenkovska N. Simjanovska L. Petanovski Z. Different Timing of Adjuvant Low Dose hCG and GnRH Agonist Trigger Protocol, in OHSS High-Risk Patient with Peak E2 Level <4000 pg/mL.Gynecol Obstet Reprod Med. 2020;26(3):203-209

ing the pregnancy rates (because of the luteal phase defect). Over the years, the agonist (GnRH-a) trigger protocol has been supplemented with application of intensive steroid luteal support (4) raising the results of in vitro fertilization and reducing the incidence of OHSS up to 0.72% in some studies (5). On the other hand, the results of some studies show conflicting results despite the intense luteal support. The use of drugs for intensive luteal support gives better results such as higher pregnancy rates but only in a sub-group of patients (estradiol peak ≥4,000 pg/mL), not in all of the patients triggered with GnRH agonist. The hypothesis is that patients with high levels of estradiol peak ≥4,000 pg/mL have higher serum levels of luteinizing hormone (LH) causing solid restitution of luteal function (6). On the other hand, the second group requires LH support, so adding a low bolus dose of LH or hCG (which has the same LH effect) on the day of follicular aspiration results in optimal steroid secretion (7). To improve the results of IVF, an alternative approach is adding a small bolus dose of hCG combined with GnRH-a, which improves oocyte maturation and provides more sustained support for the corpus luteum (8). Even though the bolus dose of hCG is very low, it still increases the risk of OHSS occurrence, however, cases of moderate and severe OHSS occurrence have only been reported in studies where the dual trigger was administered at peak estradiol ≥4,000 pg/mL or patients with a large number of aspirated oocytes (9). Therefore, it is essential to select patients who will benefit from dual triggering. The aim of this study is to compare the quality and quantity of the oocytes, pregnancy outcomes, live birth, and safety of OHSS occurrence on fresh transfer in hyper responders with a peak of estradiol <4,000 pg/mL and restitution of luteal phase when added a small bolus dose of hCG.

Material and method

This is a single-center prospective cohort study encompassed the period from March 2016 to March 2018 year realized on patients underwent in vitro fertilization at In Vitro Fertilisation Centre, First Private General Hospital Re-Medika, Skopje, North Macedonia.

The initial inclusion criteria were: Young women ≥18 years and ≤35 years, with FSH <10 mIU/mL, AMH ≥3.5 ng/mL and AFC ≥12 antral follicles on basal ultrasound including PCO patients according to The Rotterdam ESHRE/ASRM diagnostic criteria (10), own oocytes and fresh embryo transfer. To minimize the bias, only the first cycle for each patient in that period was analyzed. Patients with a history of high response to COH and or history of OHSS were also included in the study. All the patients provided written informed consent before their enrollment in the study. Approval for this study was obtained from the Institutional Review Board of First Private General Hospital Re-Medika, Skopje, North Macedonia (N 03-799/16). The final inclusion criteria were: All patients must have ≥14 follicles of over 11mm diameter, (11) and peak estradiol level <4,000pg/mL, on trigger day. Randomization was performed on the day of triggering, respecting variables that may additionally influence the final results such as BMI, smoking status, age, and sperm analysis. Patients with peak estradiol level ≥4,000 pg/mL on trigger day, more than 18 oocytes retrieved, patients recognized with significant risk for developing OHSS, were excluded from the final analysis. A total of 216 patients, who met the final requirements, entered the study.

Patients were randomized into one of two groups: Group A- Dual trigger group-1,500 IU of hCG at the time of GnRH agonist trigger day and Group B-1,500 IU of hCG 35-36 h later, on the OPU day. Controlled ovarian stimulation (COS) was achieved with short antagonist stimulation protocol used in all patients. The initial gonadotropin dose of human menopausal gonadotropin (hMG; Menopur; Ferring Pharmaceuticals, Parsippany, NJ, the United States) in a total dose of 150-225 IU per day, was based on BMI, antral follicle count (AFC), AMH, age, and prior response to gonadotropins. We used transvaginal ultrasound monitoring for follicular measurement and we also examined the estradiol, progesterone, and LH levels. When the leading follicle reached ≥14 mm in diameter or serum level of estradiol reached >350 pg/mL, GnRH antagonist (Cetrorelix, Cetrotide; Merck Healthcare KGAA, Darmstadt, Germany) was started at 0.25 mg subcutaneously once per day and continued until the day of oocyte maturation trigger. When at least 3 follicles reached ≥17 mm in mean diameter group (A) subjects were triggered subcutaneously with Triptorelin 0.2 mg (Decapeptile, Ferring GmBh, Kiel, Germany) and 1,500 IU of hCG i.m. (Choriomon, IBSA Institute Biochemique S.A, Lugano, Switzerland), while group (B) subjects were triggered with subcutaneous Triptorelin 0.2 mg and after 35-36 hours were given 1.500 IU hCG (administrated at OPU day). Serum LH, levels were assessed the day after trigger to ensure adequate LH surge response to the GnRH agonist trigger. Transvaginal, ultrasound-guided, oocyte retrieval was performed 35-38 h after the trigger. All meta-phase II oocytes were injected with a 35 degree angled ICSI pipette (Humagen, CooperSurgical, Trumbull, CT, USA). Embryo transfer was performed on day 3 (cleavage stage) or day 5 (blastocyst stage) after oocyte retrieval, based on embryo quality. Evaluation of embryo quality on Day 3 was based on blastomeres number, fragmentation rate, multinucleation, and early compaction. The selection criteria of Day 3 embryos were early cleavage on Day 1, four cells on Day 2, and eight cells or early compaction on Day 3, with minimal fragmentation and no multinucleation. After applying the selection criteria, transfer was only performed using Grade I (excellent quality) embryos (with at least eight blastomeres on Day 3, of equal size, with <10% of fragmentation, and no multinucleation) and Grade II (good quality) embryos (6-10 blastomeres, equal or moderate in size, with <15-20% of fragmentation, and no multinucleation). Grade III (fair quality) and Grade IV (bad quality) embryos were not transferred (12), the minimum conditions re-

quired for the decision to apply prolonged embryo cultivation and blastocyst embryo transfer were: at least 3 embryos that were in eight-cell stages or early compaction, and ≤6 viable embryos Grade I (excellent quality) or Grade II (good quality) on Day 3 (13,14). In all patients from the OPU day, the luteal phase was supported with the subcutaneous application of progesterone, 50mg daily (Prolutex, IBSA Institute Biochemique S.A., Lugano, Switzerland) or vaginal progesterone gel, 90 mg daily (Crinone 8%; Central Pharma, Bedford, United Kingdom) according to market availability of the drugs, and E2 tablets, 4-6 mg per day (Estrofem, NovoNordisk, Denmark), until a negative pregnancy test or 8 to 10 weeks of gestation. After 14 days of embryo transfer, pregnancy tests were made with measuring serum level of hCG. Detection of at least one gestational sac and embryo with a positive heartbeat is considered the confirmation of clinical pregnancy, so we made an additional vaginal ultrasound examination after two weeks from the positive pregnancy test to make sure of the results. After embryo transfer, all patients were continuously monitored anamnestic, biochemical, and by ultrasound according to the ratio of the occurrence of OHSS. The diagnosis and classification of OHSS were as mild, moderate, and severe, based on the described criteria by Fiedler and Ezcurra (15) and adapted as Mild (abdominal discomfort, nausea/vomiting, diarrhea, enlarged ovaries); Moderate (Mild features + Elevated hematocrit (>41%), ultrasonographic evidence of ascites, elevated WBC >15,000, hypoproteinemia), Severe (Mild and moderate features + hemoconcentration - Hct >55%, clinical evidence of ascites WBC >25,000, hydrothorax, oliguria/anuria, Na+ 5 mEq/L, tense ascites, elevated liver enzymes, low blood/central venous pressure, rapid weight gain >1 kg in 24 hours, syncope, severe abdominal pain, venous thrombosis).

Statistical analysis

For group comparison were used non-parametric and parametric statistical tests (Pearson Chi-square test, Fisher exact test, Student t-test for independent samples, Mann-Whitney test).

Significant differences were considered all values of $p<0.05$.

Results

A total of 216 patients entered the final analysis of this study respecting the inclusion and exclusion criteria. Out of a total of 270 patients who passed the basic criteria for admission to the study and reached the trigger day criteria and randomization, 54 (20%) of the patients underwent the "freeze all protocol" due to the above criteria as estradiol higher than >4,000 pg/mL, (23/54; 42.59%) on the trigger day as the main criterion, more than 18 retrieved oocytes (18/54; 33.33%) in the group of estradiol <4,000 pg/mL on trigger day and 13 patients underwent" freeze all" according to other criteria as they had a history of OHSS, very low BMI, and patients recognized with significant risk for developing OHSS. Fresh embryo transfer was realized in 216 patients and they entered the final analysis. Both groups had typical markers for high ovarian reserve, which included all patients in the risk group for OHSS. The mean AMH in the group (A) was 6.76±3.8 ng/mL vs 7.56±3.8 ng/mL in Group (B) while the percentage of PCO was not significantly higher in the group (A) in comparison to the group (B). (Table I) The duration of ovarian stimulation and the total dose of gonadotropin required were comparable in both groups. (Table II). Even though both of the groups are homogenous, regarding several sensitive to OHSS variables: age, BMI, AMH, PCOS, and duration of COS (Table I), which makes both groups comparable, peak estradiol levels during COS and on trigger day were significantly higher in group B (2,851.3 vs 2,262.9 pg/mL, $p<0.05$), which indicates a higher risk for OHSS and therefore this group contains a slightly higher number of patients (triggered with GnRH-agonist)

Table I: *Basal patient characteristics between patients triggered by GnRH agonist + hCG 1500 IU (DUAL TRIGGER), group (A), and those triggered by GnRH agonist as a trigger and hCG 1500 IU, 35-36h latter on OPU day, group (B).*

	(A) Dual Trigger n=98	(B) GnRh-a+hCG (OPU day) n=118	*p*
Women's age (y) (mean±SD)	31.34±3.1	30.75±3.6	0.20
Man's age (y) (mean±SD)	35.48±4.9	34.54±4.9	0.09
Women smokers (n) (%)	28 (28.57)	32 (27.12)	0.81
Man smokers (n) (%)	37 (37.76)	58 (49.15)	0.09
Woman's BMI(kg/m^2) mean±SD)	23.28±3.3	23.81±3,7	0.28
Previous IVF (n) (%)	54 (55.67)	69 (63.3)	0.26
AM hormone (ng/mL) (mean±SD)	6.76±3.8	7.56±3.8	0.34
AFC > 12 follicles (n) (%)	65 (66.33)	84 (71.19)	0.42
PCO (n) (%)	33 (33.67)	34 (28.81)	0.44
Normospermia Oligospermia	64 (65.31)	72 (61.02)	0.053
Severe Oligospermia (<4m/cm^3)	30 (30.61)	30 (25.42)	
(n) (%)	4 (4.08)	16 (13.56)	

(Table II). There is a significant difference in the percentage of oocytes obtained according to the punctured follicles- "oocyte yield" in dual trigger group (A) (57.79±14.6 % vs 47.09±17.9%; $p<0.005$) (Table II). There is no significant difference between the two (A vs B) groups according to the average number of retrieved oocytes (13.08 vs 14.41 $p=0.08$), M II oocytes (10.5 vs 10.95 $p=0.47$) and GV (1.24 vs 1.52 $p=0.09$). Group (A), even if it had a lower mean number of obtained oocytes and MII oocytes, had a better maturation rate, (80.7±15.3 vs 77.4±18.0 $p=0.15$) and a better percentage of patients who had more than 75% of mature oocytes per aspiration (71.43% vs 62.71% $p=0.18$) (Table III). The dual trigger group (A) had a non-significant but better fertility rate (68.46 % vs 64.04 % $p=0.07$). The number of embryos transferred was similar in the two groups. The dual trigger group had a non-significantly but higher percentage of patients with Blastocyst Embryo Transfer (74.49% vs 62.72% $p=0.065$) (Table III). The general occurrence of OHSS was 3.2%. All patients had a mild form of OHSS and there were no significant differences between the groups in terms of the incidence of hyperstimulation syndrome (2.05% vs 4.24%, $p=0.37$). There were no cases of moderate or severe OHSS and no hospitalized patients in both groups (Table III). The dual trigger group (A) had a significantly higher ongoing pregnancy rate (64.29% vs 45.76% $p<0.05$) and live birth rate (62.29 % vs 42.37% $p<0.05$), compared to the GnRH-a trigger group (B).

Discussion

An added dose of 1,500 IU hCG applied on the day of aspiration of the oocytes has already been established in antagonist protocol with GnRH as a trigger, in support of the luteal phase and successfully elevates serum progesterone levels (16,17) especially in the group of patients with serum E2 <4,000 pg/mL, who have a lower pregnancy rate compared to women with peak E2 over 4000 pg/mL, despite intensive steroid hormone support (5).

The use of low doses of hCG at the time of GnRH agonist trigger (dual trigger) may be advantageous since it may ensure the same correct luteal support as 1,500 IU of hCG 35-36 h

Table II: *Controlled ovarian stimulation*

	(A) Dual Trigger n=98	(B) GnRh-a+hCG (OPU day) n=118	*p*
Total dose of gonadotropins (IU) (mean±SD)	1853±432	1635±531	0.59
Duration of COS (days) (mean±SD)	9.5±1.4	9.57±1.5	0.7
Serum basal LH (mIU/mL) (mean±SD)	6.56±4.1	6.51±3.3	0.74
Serum E2-day of antagonist start (mIU/mL) (mean±SD	506.16±334.4	671.6±417.9	0.002^{sig*}
Serum LH -day of antagonist start (mIU/mL) (mean±SD)	4.43±4.5	4.36±3.7	0.9
Serum E2 - trigger day (mIU/mL) (mean±SD)	2262.9±1406.1	2581.3±1496.6	0.023^{sig*}
Serum LH - trigger day (mIU/mL) (mean±SD)	2.17±1.79	2.55±2.5	0.35

Table III: *Embryologic outcomes*

	(A) Dual Trigger n=98	(B) GnRh-a + hCG (OPU day) n=118	*p*
% of oocytes / punctured follicles (mean±SD)	57.79±14.6	47.09±17.9	0.00012^{sig*}
Oocytes (mean±SD)	13.08±3.614	14.41±6.7	0.08
Mature oocytes (mean±SD)	10.5±3.310	10.95±5.2	0.46
GV - oocytes (mean±SD)	1.24±1.7	1.52±1.6	0.09
Proportion of mature oocytes (%)	80.7±15.3	77.4±18.0	0.15
≥75% of mature oocytes (%)	70 (71.43)	74 (62.71)	0.18
<75% of mature oocytes (%)	28 (28.57)	44 (37.29)	
ICSI - fertilization of oocytes (mean±SD)	11.11±3.4	11.58±5.4	0.45
Fertilized oocytes (mean±SD)	7.51±3.2	7.45±3.8	0.89
Fertilization rate (%)	68.46±17.9	64.04±17.0	0.07
3rd-day transfer	25 (25.51)	44 (37.29)	0.065
Blastocyst Embryo Transfer	73 (74.49)	74 (62.72)	0.065
Blastocyst transfer (mean±SD)	2.56±3.1	2.56±2.5	0.26
Cryo embryos (mean±SD)	1.02±2.1	1.24±2.4	0.96
OHSS - mild form (n), (%)	2 (2.05)	5 (4.24)	0.37
OHSS - moderatte/severe, hospitalisation	0	0	

later on the OPU day, but also it can be potentially helpful to minimize the risk of suboptimal response to GnRH-agonist trigger (post-trigger day LH less than 15 IU/l) (18). This failure can range from empty follicle syndrome (19), lower oocyte yield (20) and less retrieved oocytes and mature oocytes (21). In our study, we didn't have patients with empty follicle syndrome which is connected with the application of contraceptive pills for a longer period and low BMI (20). This is not included in our everyday practice for patients undergoing IVF protocols. In every patient, the levels of LH on post-trigger day were >15 IU/l. The percentage of oocytes obtained according to the number of punctured follicles was significantly higher in the group (A). (Table III) In the group of Dual trigger (A) we obtained not statistically significant lower average number of oocytes, (13.08 vs 14.41 p=0.08), but what it was really interesting that the trend has changed in relation to a higher percentage of oocyte maturation and fertilization rate, compared to the Dual trigger group. (Table III) The dual trigger group (A) resulted in a significantly higher ongoing pregnancy rate and live birth rate (Table IV)

The main question is "What is the hCG dose on the day of application of the GnRH trigger which is powerful enough to bring oocyte maturity and luteal support, on one side, and is safe in terms of the appearance of OHSS in cycles with the fresh transfer?". To date, there is no consensus on this issue. In our study, we compare the same fixed-dose of hCG (1,500 IU) in the two protocols based on 1) Our observation of different dosage of additional hCG and GnRH agonist trigger day in our everyday practice with Dual trigger and we chose the most frequent Dual trigger dosage of hCG-1500 IU, 2) Data from studies about the safe doses of additional hCG: retrograde analysis of 10,427 cycles of IVF-ICIS showed that dual trigger from 1,500 IU brings the low risk of OHSS with an incidence of 0.13% of moderate OHSS and a severe OHSS form in 0.03% of the population surveyed, which makes these doses safe for OHSS occurrence (22).

A new single-center prospective study demonstrates no significant differences in pregnancy rates between the two protocols comparing 1,000 vs 1,500 IU of hCG, with no statistically significant higher incidence of OHSS in the group of hCG applied on OPU day compared to the group of Dual trigger. This demonstrates that either protocol may be a reliable option for patients at risk of OHSS who desire a fresh transfer (23).

The main strength of this study is that it is a single study center and the compactness of a small team that worked according to the same embryological protocol for all patients (ICSI), all activities during COS and decisions for type of trigger, "freeze all" as well as follow-up of patients after OPU was under the supervision of one senior gynecologist.

When it comes to OHSS, there were no hospitalized patients with a moderate or severe form of OHSS. Out of all the milder forms of OHSS, there were 5 cases in agonistic trigger with 1500 IU hCG and two cases in dual trigger group, which gives us the right to say that both protocols are with low risk of OHSS occurrence.

How risky a small additional dose of hCG can be for the occurrence of OHSS is shown by one study with a significantly high OHSS rate (8,6%) in dual trigger used in hyper responders in contrast to other studies where the incidence of OHSS was below 1% (24). We have to highlight that more of the patients, in that study, with a severe form of OHSS, on the trigger day had high estradiol >4000 pg/ml and more than 18 oocytes aspirated, both of them as main indicators, used as a criterion, were excluded from our study and replaced with a freeze all protocol. On the other hand, even 1500 IU HSC applied on the day of oocyte aspiration also carries a risk for the occurrence of hyperstimulation syndrome (25). This only shows that the strict selection of patients is necessary to reduce the risk of OHSS.

An additional benefit in reducing OHSS is a technique called segmentation of the protocol: "freeze all" oocytes or embryos in the current cycle and their transfer in one of the following cycles (26). Anyways, this approach is not possible when the patients insist on embryo transfer in the same cycle for various reasons, economical, religious, etc. On the other hand, a new study shows that even the approach of the GnRH-agonist trigger alone and the application of the "freeze all" technique, does not completely avoid the risk of OHSS (27).

Table IV: *Outcome of IVF process*

	(A) Dual Trigger n=98	(B) GnRh-a + hCG (OPU day) n=118	*p*
Biochemical Pregnancy (n) (%)	6 (6.12)	10 (8.47)	0.5
Clinical pregnancy rate (n) (%)	68 (69.39)	59 (50)	0.23
Early pregnancy lost (n) (%)	5 (5.1)	5 (4.24)	0.76
Ongoing pregnancy rate (n) (%)	63 (64.29)	54 (45.76)	0.0065[sig]
Pregnancy lost after 12 weeks (n) (%)	2 (2.04)	4 (3.39)	0.55
Live birth rate (n) (%)	61 (62.24)	50 (42.37)	0.0036[sig]

Conclusion

Our study showed that 1,500 IU dual trigger gives better quality of oocytes and embryos with correct luteal support, the same as the second group. We believe that the dual trigger protocol takes precedence over luteal support hCG because in daily practice it covers certain, although small, percentages of the agonist suboptimal endogenic response as a trigger since it may ensure retrieval of an appropriate number of mature oocytes.

The adjuvant low dose of hCG (1,500 IU) at the time of GnRH agonist trigger day improves clinical pregnancy and live birth rates in hyper responders with peak E2 <4,000 pg/mL without increasing the risk of clinically significant OHSS. The protocol of dual trigger and optional freezing all oocytes or embryos in patients with a high risk of developing OHSS, with peak E2 <4,000 pg/ml or more than 18 oocytes aspirated, is a promising technique in everyday practice. However, it is still important to timely identify the high-risk patients and high-risk situations for developing OHSS.

Our study should be further investigated. Prior to the dual trigger's routine implementation, further large prospective studies are needed to examine the role of dual triggers in the high ovarian responders in GnRH-antagonist protocols.

Acknowledgments: We would like to thank MD Prof. Beti Zafirova for her supervision of the data collection process and statistical analysis. Also, we would like to thank Iva Kolevska and Vase Nikovski for their technical support.
Funding: No grants for this research.
Conflict of interest: No conflict of interest.
Declarations Ethics approval and consent to participate: the research was approved by the Ethics Committee of the First Private Hospital Re Medika Skopje, North Macedonia.
Informed consent: Both verbal and written consent have been taken from the study participants and the study was conducted in accordance with the Declaration of Helsinki.
Authors' contribution: EZPK: Designed the concept, data collecting, processing, writing the article, VS: Design, review GD: consultancy SS: Consultancy, MHL: Consultancy, DS: Data collecting, data analyses, statistics. NS: Data collecting. LS: Critical review, ZP: Supervision of the concept, data collecting, processing, data analysis, and involved in the revision of the final manuscript.

References

1. Humaidan P, Quartarolo J, Papanikolaou EG. Preventing ovarian hyperstimulation syndrome: guidance for the clinician. Fertil Steril. 2010;94(2):389-400. Doi: 10.1016/j. fertnstert.2010.03.028
2. Delvigne A, Rozenberg S. Epidemiology and prevention of ovarian hyperstimulation syndrome (OHSS): a review. Hum Reprod Update. 2002;8(6):559-77. Doi: 10.1093/humupd/8.6.559
3. Itskovitz J, Boldes R, Levron J, Erlik Y, Kahana L, Brandes JM. Induction of preovulatory luteinizing hormone surge and prevention of ovarian hyperstimulation syndrome by gonadotropin-releasing hormone agonist. Fertil Steril. 1991;56(2):213-20. PMID: 1906406
4. Humaidan P, Bredkjaer HE, Bungum L, Bungum M, Grøndahl 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(5):1213-20. Doi: 10.1093/humrep/deh765
5. Iliodromiti S, Lan VT, Tuong HM, Tuan PH, Humaidan P, Nelson SM. Impact of GnRH agonist triggering and intensive luteal steroid support on live-birth rates and ovarian hyperstimulation syndrome: a retrospective cohort study. J Ovarian Res. 2013;6(1):93. Doi: 10.1186/1757-2215-6-93
6. Kummer N, Benadiva C, Feinn R, Mann J, Nulsen J, Engmann L. Factors that predict the probability of a successful clinical outcome after induction of oocyte maturation with a gonadotropin-releasing hormone agonist. Fertil Steril. 2011;96(1):63-8. Doi: 10.1016/j. fertnstert. 2011. 04.050
7. Humaidan P, Ejdrup Bredkjaer H, Westergaard LG, Yding Andersen C. 1,500 IU human chorionic gonadotropin administered at oocyte retrieval rescues the luteal phase when gonadotropin-releasing hormone agonist is used for ovulation induction: a prospective, randomized, controlled study. Fertil Steril. 2010;93(3):847-54. Doi: 10.1016/j.fertnstert.2008.12.042
8. Griffin D, Benadiva C, Kummer N, Budinetz T, Nulsen J, Engmann L. Dual trigger of oocyte maturation with gonadotropin-releasing hormone agonist and low-dose human chorionic gonadotropin to optimize live birth rates in high responders. Fertil Steril. 2012;97(6):1316-20. Doi: 10.1016/j.fertnstert.2012.03.015
9. Mahajan N, Sharma S, Arora PR, Gupta S, Rani K, Naidu P. Evaluation of dual trigger with gonadotropin-releasing hormone agonist and human chorionic gonadotropin in improving oocyte maturity rates: A prospective randomized study. J Hum Reprod Sci. 2016;9(2):101-6. Doi: 10.4103/0974-1208.183506
10. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod. 2004;19(1):41-7. Doi: 10.1093/humrep/deh098
11. Papanikolaou EG, Pozzobon C, Kolibianakis EM, Camus M, Tournaye H, Fatemi HM et al. Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles. Fertil Steril. 2006;85(1):112-20. Doi: 10.1016/j.fertnstert.2005.07.1292

12. Sotiroska V, Petanovski Z, Dimitrov G, Hadji-Lega M, Shushleski D, Saltirovski S, et al. The day of embryo transfer affects delivery rate, birth weights, female-to-male ratio, and monozygotic twin rate. Taiwan J Obstet Gynecol. 2015;54(6):716-21. Doi: 10.1016/j.tjog.2015.06.011

13. Milki AA, Hinckley MD, Fisch JD, Dasig D, Behr B. Comparison of blastocyst transfer with day 3 embryo transfer in similar patient populations. Fertil Steril. 2000;73(1):126-9. Doi: 10.1016/s0015-0282(99)00485-9

14. Sills ES, Palermo GD. Human blastocyst culture in IVF: current laboratory applications in reproductive medicine practice. Rom J Morphol Embryol. 2010;51(3):441-5. PMID: 20809018

15. Sills ES, Palermo GD. Human blastocyst culture in IVF: current laboratory applications in reproductive medicine practice. Rom J Morphol Embryol. 2010;51(3):441-5. PMID: 20809018

16. Humaidan P, Kol S, Papanikolaou EG; Copenhagen GnRH Agonist Triggering Workshop Group. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update. 2011;17(4):510-24. Doi: 10.1093/humupd/dmr008

17. Fatemi HM, Polyzos NP, van Vaerenbergh I, Bourgain C, Blockeel C, Alsbjerg B, et al. Early luteal phase endocrine profile is affected by the mode of triggering final oocyte maturation and the luteal phase support used in recombinant follicle-stimulating hormone-gonadotropin-releasing hormone antagonist in vitro fertilization cycles. Fertil Steril. 2013;100(3):742-7. Doi: 10.1016/j.fertnstert. 2013.05.028

18. 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(5):1351-6. Doi: 10.1093/humrep/des049

19. Castillo JC, Garcia-Velasco J, Humaidan P. Empty follicle syndrome after GnRHa triggering versus hCG triggering in COS. J Assist Reprod Genet. 2012;29(3):249-53. Doi:10.1007/s10815-011-9704-8

20. Meyer L, Murphy LA, Gumer A, Reichman DE, Rosenwaks Z, Cholst IN. Risk factors for a suboptimal response to gonadotropin-releasing hormone agonist trigger during in vitro fertilization cycles. Fertil Steril. 2015;104(3):637-42. Doi: 10.1016/j.fertnstert.2015.06.01

21. Chang FE, Beall SA, Cox JM, Richter KS, DeCherney AH, Levy MJ. Assessing the adequacy of gonadotropin-releasing hormone agonist leuprolide to trigger oocyte maturation and management of inadequate response. Fertil Steril. 2016;106(5):1093-1100.e3. Doi: 10.1016/j.fertnstert.2016.06.013

22. Gunnala V, Melnick A, Irani M, Reichman D, Schattman G, Davis O, et al. Sliding scale HCG trigger yields equivalent pregnancy outcomes and reduces ovarian hyperstimulation syndrome: Analysis of 10,427 IVF-ICSI cycles. PLoS One. 2017;12(4):e0176019. Doi: 10.1371/journal.pone.0176019

23. Engmann LL, Maslow BS, Kaye LA, Griffin DW, DiLuigi AJ, Schmidt DW, et al. Low dose human chorionic gonadotropin administration at the time of gonadotropin releasing-hormone agonist trigger versus 35 h later in women at high risk of developing ovarian hyperstimulation syndrome - a prospective randomized double-blind clinical trial. J Ovarian Res. 2019;12(1):8. Doi: 10.1186/s13048-019-0483-7

24. O'Neill KE, Senapati S, Maina I, Gracia C, Dokras A. 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):1175-84. Doi: 10.1007/s10815-016-0755-8

25. Seyhan A, Ata B, Polat M, Son WY, Yarali H, Dahan MH. Severe early ovarian hyperstimulation syndrome following GnRH agonist trigger with the addition of 1500 IU hCG. Hum Reprod. 2013;28(9):2522-8. Doi: 10.1093/humrep/det124

26. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. Hum Reprod. 2011;26(10):2593-7. Doi: 10.1093/humrep/der251

27. Fatemi HM, Popovic-Todorovic B, Humaidan P, Kol S, Banker M, Devroey P, et al. Severe ovarian hyperstimulation syndrome after gonadotropin-releasing hormone (GnRH) agonist trigger and "freeze-all" approach in GnRH antagonist protocol. Fertil Steril. 2014;101(4):1008-11. Doi: 10.1016/j.fertnstert.2014.01.019