Introduction

Ovarian hyperstimulation syndrome (OHSS) is the most serious complication in in vitro fertilization (IVF) cycles, with an incidence of 3%-6% for moderate and 0.1%-2% for severe cases (1). Thus, many studies have focused on avoiding this iatrogenic complication using various strategies (2). It is well established that this syndrome almost always requires the exogenous administration of human chorionic gonadotropin (hCG) or endogenous pregnancy-derived hCG stimulation (3). However, due to its luteinizing hormone (LH) homology, extended half-life, and simple manufacturing process, hCG is an excellent trigger for final oocyte maturation (3). Nevertheless, the introduction of gonadotropin-releasing hormone (GnRH) antagonist protocols has allowed the utilization of a GnRH agonist (a) to induce final oocyte maturation. The GnRH agonist displaces the GnRH antagonist in the pituitary gland, activating the GnRH receptor and resulting in a surge of gonadotropins (flare-up) similar to the natural midcycle surge (3). Moreover, GnRH agonist triggering has been shown to retrieve more metaphase II (MII) oocytes compared with hCG triggering (4). Kol and Humaidan hypothesized that this finding may be related to the endogenous follicle-stimulating hormone (FSH) surge elicited along with the LH surge after GnRH agonist triggering (4, 5).

Several studies have investigated the optimal dose of urinary or recombinant (r) hCG to induce oocyte maturation in IVF cycles (6, 7). The minimum optimal dose of hCG was first recommended by Abdalla et al. (8). They compared the effect of 2000-, 5000-, and 10,000-IU hCG doses for successful oocyte recovery and, as a result, recommended at least 5000 IU hCG for an adequate follicular response (8). Later, several studies concluded that the clinical outcome was similar between females receiving 5000 or 10,000 IU of urinary hCG (7). A recent study found that the optimal dose of r-hCG to induce final oocyte maturation in oocyte donors was 250 µg and that a double dose of r-hCG was not associated with a higher number of retrieved MII oocytes or higher pregnancy rates among recipients (6). In contrast, the preferred GnRH agonist doses for triggering oocyte maturation have been found to be 0.2 mg for triptorelin, 0.5 mg for buserelin, and 1
mg for leuprolide acetate (9). Several trials have explored different doses and types of GnRHa (GnRH agonists) in terms of their induction of final follicular maturation in IUI (10-12) and egg donor cycles (13). However, there are limited data in the literature about the optimal minimum doses of GnRH agonists for inducing final oocyte maturation in IVF cycles. In this report, we present a case series to show the efficacy of a low-dose GnRH agonist (0.1 mg triptorelin) for final oocyte maturation in females undergoing assisted reproductive treatment (ART).

Material and Methods

This retrospective analysis used the data of a small case series of patients (n=9) undergoing GnRH antagonist cycles using 0.1 mg triptorelin for final oocyte maturation between March 2012 and May 2013. The study was approved by the institutional review board and informed consent was provided by each of the couples. As there is no established dose of GnRH agonist for inducing final oocyte maturation in our center, individual attending physicians determined the dose of GnRH analog and luteal phase support according to their own preference. One of the physicians (BG) in the department preferred a lower triggering dose of GnRHa (0.1 mg triptorelin) for inducing final oocyte maturation, based on the successful results of previous IUI studies (10-12). Therefore, the current study analyzed these cases retrospectively and compared the results (fresh transfers) with a control group (n=14) on the basis of the study group.

Ovarian stimulation was initiated with recombinant FSH (Puregon; Organon, Turkey and Gonal-f; Merck Serono, Turkey) from Day 2 or 3 of the cycle and continued until the day of ovulation induction. Cycles were monitored using ultrasound scanning. A GnRH antagonist, ganirelax (Orgalutran; Organon, Turkey) or cetrorelax (Cetrotide; Merck Serono, Turkey), was administered when the leading follicle reached a maximum diameter of 14 mm. When at least two follicles had reached a diameter of 17 mm, final oocyte maturation was triggered by administering 0.1 mg triptorelin (Decapeptyl; Ferring, Turkey).

Oocyte pick-up was performed 35 h and 30 min after triggering. ICSI was performed in all patients. Embryos were evaluated on the second and third days, and up to two embryos per patient were transferred. For luteal phase support, all patients received micronized progesterone 90 mg vaginally (Crinone 8% vaginal gel; Merck Serono, Turkey) or cetrorelix (Cetrotide; Merck Serono, Turkey), was administered when the leading follicle reached a maximum diameter of 14 mm. When at least two follicles had reached a diameter of 17 mm, final oocyte maturation was triggered by administering 0.1 mg triptorelin (Decapeptyl; Ferring, Turkey).

Results

a. Cycle characteristics of Group 1, including fresh and thawed transfers (n=9)

The demographic and cycle characteristics of each patient in Group 1 (n=9) are shown in Table 1. Seven patients had fresh cycle embryo transfers. The other two patients had frozen embryos; embryo transfer was performed during a subsequent artificial cycle in these patients due to OHSS risk. Seven patients in Group 1 conceived, one of whom (case 1) had a preterm delivery of twins at Week 24 due to preterm premature rupture of membranes (PPROM), one patient had a single term delivery (case 2), one patient had a term twin delivery (case 3), two patients had an ongoing pregnancy (case 4-Week 25 and case 6-Week 21), while two pregnancies ended as miscarriages (case 5 and case 9). The reproductive outcomes for these patients (including both fresh and thawed transfers) were as follows: the clinical pregnancy rate was 77.7%, the ongoing pregnancy+birth rate was 55.5%, implantation rate was 52.9%, and the abortion rate was 22.2% (n=2).

b. Comparison of fresh transfers (n=7) of Group 1 with Group 2 (oocyte and age-matched control group; n=14)

Only fresh embryo transfers were included in the comparative analysis; therefore, the results of patient 3 and patient 4 in group 1 were not included in the statistical analysis. The patients’ characteristics and treatment outcomes are shown in Table 2. The mean ages of patients in groups 1 and 2 were 29.89±4.48 (24-37) and 28.92±3.54 (23-36) years, respectively (p>0.05). There were no significant differences between the groups in mean duration of infertility, previous IVF attempts, duration of stimulation, or total dose of gonadotropins required (p>0.05).

There were also no significant differences in the mean number of aspirated follicles, mean number of retrieved oocytes, or mean number of metaphase II oocytes between the groups (p>0.05). Immature oocytes (MI) were significantly more numerous in group 2 than in group 1 (p<0.05). Fertilization rates were similar in both groups (72% in group 1 and 73% in group 2). The mean number of embryos was 10.11±5.86 (4-21) in group 1 and 10.79±3.14 (7-18) in group 2. The numbers of transferred embryos were 1.85±0.37 (1-2) and 1.36±0.49 (1-2) in groups 1 and 2, respectively (p<0.05). There were no significant differences between the groups in implantation rate (46.1% vs. 57.8%), clinical pregnancy rate (71.4% vs. 57.1%), and ongoing pregnancy rates (42.8% vs. 42.8%) (p>0.05). There was no case of OHSS in any patient in either group.

Discussion

The major goal of studies in the field of assisted reproductive technologies is to improve the live birth rate while minimizing complications and the cost of treatment. Previous reports have claimed that GnRH agonist triggering in GnRH antagonist cycles is a new and effective modality for the most feared complication of controlled ovarian stimulation, OHSS (14). It has now been demonstrated that the flare-up effects of GnRH agonists with modified luteal support yield similar conceptual results as hCG in fresh IVF cycles (15, 16).
Although there are many reports about the optimal dose of hCG for inducing final oocyte maturation, there are limited data about the minimal optimal doses to trigger using GnRH agonists in IVF cycles (6, 7, 17, 18). Most previous studies have reported successful oocyte maturation with 0.2-0.3 mg triptorelin, 0.5 mg buserelin, and 1 mg leuprolide acetate (13). A similar clinical outcome was observed with 0.1 mg of triptorelin and 10,000 IU hCG in a GnRH antagonist protocol in a study that was presented during the 19th ESHRE meeting but has not yet been published (19). In a recent study of oocyte maturation using 0.1, 0.3, and 0.5 mg triptorelin, ovulation occurred in all IUI cycles (17). As there is no established dose of GnRH agonists for the induction of final oocyte maturation in IVF cycles, we hypothesized that lower doses of GnRH agonists may be sufficient for triggering. Herein, we report successful oocyte maturation using a lower dose of triptorelin acetate in a small case series.

As described in many reports, the main problem in GnRH agonist-triggered antagonist cycles is luteal phase support rather than...
oocyte maturation, since the decrease in gonadotropins that are released from the pituitary results in corpus luteum deficiency and a defective luteal phase (20). Do lower triggering doses of GnRH agonists negatively affect the luteal phase? In a recent report, an inadequate luteal phase was observed in 34.4% of the non-conceptional cycles of patients receiving triptorelin 0.1 mg to trigger ovulation in IUI cycles; however, increasing or repeating triptorelin did not restore the luteal phase or the pregnancy rate (17). Shalev et al. (11) compared the effects of 10,000 IU hCG and 0.1 mg triptorelin on ovulation after clomiphene citrate treatment. Interestingly, midluteal progesterone concentrations (>10 ng/mL) and the mean luteal phase duration were normal in both groups. Also, there were no significant differences in pregnancy and abortion rates between groups, which may have been related to the different dynamics at midcycle in clomiphene-stimulated cycles due to a direct hypothalamic effect (10, 21). Parneix et al. (12) also investigated the effect of different doses and modes of application of GnRH agonists for triggering ovulation, finding that although ovulation occurred in all groups, shorter and inadequate luteal phases were seen in all groups. According to these findings, higher doses and different modes of GnRH agonists for triggering do not appear to improve the luteal phase in non-IVF cycles.

Standard luteal phase support after GnRH agonist triggering has been reported to be associated with lower conception rates due to corpus luteum dysfunction (22). Therefore, intensive luteal phase supplementation is recommended to achieve optimal conception rates (22). Also, since excellent pregnancy rates were reported in patients undergoing frozen embryo transfer using GnRHa suppression protocols, Engmann et al. (22) suggested that LH may not be critical for implantation. Therefore, aggressive luteal support may be another beneficial approach in agonist trigger cycles. Engmann et al. (23) reported excellent implantation and on-going pregnancy rates with intensive luteal support using intramuscular progesterone daily and estradiol patches on alternate days. Although intramuscular administration (IM) of progesterone results in higher serum levels, some studies also support the use of vaginal progesterone gel (24). In our study, all patients received micronized estradiol hemihydrate 4 mg orally combined with progesterone 90 mg vaginally twice daily for luteal support. The main aim of this report was to demonstrate the effectiveness of lower doses of agonists to trigger final oocyte maturation, rather than pregnancy rates. Indeed, the rate of retrieved oocytes per follicle (89%) and fertilization rate (71%) seem to support the use of lower doses of GnRH agonists in clinical practice. These results also highlight the inadvertent administration of a lower dose (i.e., one instead of two ampoules) to the patients. Finally, clinicians should recognize that the cost of treatment can be reduced by using the minimum optimal dose of GnRH agonist for triggering.

Table 2. Comparison of matched 0.1 and 0.2 mg triptorelin-triggered groups for fresh transfer

| Variable                        | Group 1 (n=7)* | Group 2 (n=14) | p     |
|--------------------------------|--------------|---------------|------|
| Age (y)                        | 29.89±4.48 (24-37) | 28.92±3.54 (23-36) | 0.256 |
| BMI (kg/m²)                    | 27.78±4.98 (22-33.5) | 26.45±3.98 (21-32) | 0.289 |
| Duration of infertility (y)    | 5±3.42 (1-9) | 4.86±3.2 (1-14) | 0.787 |
| Number of Previous IVF attempts| 2.0±0.68 (1-7) | 1.43±0.64 (1-3) | 0.306 |
| Basal FSH (mIU/mL)             | 6.43±1.29 (4.1-8.1) | 5.78±1.39 (3.2-8.0) | 0.387 |
| Stimulation (days)             | 11±4.82 (7-23) | 10.38±1.39 (7-13) | 0.631 |
| Total dose of FSH (IU)         | 1936.11±1101.20 (800-3850) | 1727.8±755.45 (750-3250) | 0.481 |
| Aspirated follicles (n)        | 17.55±7.71 (11-35) | 18.34±6.87 (9-30) | 0.704 |
| Retrieved oocytes (n)          | 15.66±7.82 (6-32) | 17.04±4.0 (10-26) | 0.513 |
| Retrieved oocytes per aspirated follicles (%) | 89 | 92 | 0.906 |
| Metaphase II oocytes (MIII)     | 14±7.28 (6-30) | 14.0±3.50 (9-22) | 0.980 |
| Immature oocytes (MI-GV)       | 1.66±1.12 (0-6) | 3.04±2.32 (0-6) | <0.05 |
| Fertilization rate (%)          | 72            | 73            | 0.270 |
| 2PN (n)                        | 10.11±5.86 (4-21) | 10.79±3.14 (7-18) | 0.717 |
| Embryos transferred (n)        | 1.85±0.37 (1-2) | 1.36±0.49 (1-2) | <0.05 |
| Implantation rate per cycle n (%) | 6/13 (46.1) | 11/19 (57.8) | 0.471 |
| Clinical pregnancy rate per cycle n (%) | 5/7 (71.4) | 8/14 (57.1) | 0.290 |
| Ongoing pregnancy + birth rate per cycle n (%) | 3/7 (42.8) | 6/14 (42.8) | 0.433 |
| Abortion rate per cycle n (%)  | 2/7 (28.6) | 2/14 (14.2) | 0.517 |
| OHSS rate per cycle n (%)      | 0/7(0)       | 0/14 (0)      | ns   |

* Seven patients including fresh transfers.

IVF: in vitro fertilization; n: number; year; 2 PN: 2 pronucleus; MI: metaphase I; GV: germinal vesicle; IU: international unit; OHSS: ovarian hyperstimulation syndrome.
Another important aspect of agonist-triggered cycles is the incidence of empty follicle syndrome (EFS). The incidence of EFS has been reported as 0.6%-3.5% in GnRHa trigger cycles, which is similar to that reported (0.1%-3.1%) after an hCG trigger (12, 19, 25-29). Therefore, EFS is not an inherent and exclusive problem to the GnRHa trigger (25) but could be related to human error, abnormalities in the in vivo biological activity of some batches of commercially available GnRHa, hypothalamic dysfunction, or GnRH receptor mutations (23, 26, 28-30). Elucidating the relationship between lower doses of GnRHa and EFS will require further studies including a larger number of patients. However, in our case series, there were no EFS and no reduced number of retrieved oocytes. Also, the association between BMI and the required GnRHa dose is controversial. Although Kummer et al. (25) demonstrated that a higher BMI corresponded to less of an increase in LH and lower post-trigger progesterone level, they found that BMI did not predict the oocyte yield. However, it is possible that the excess subcutaneous tissue in obese patients interferes with the absorption of medication. Only three patients in the current study were obese, and there was no reduced number of mature oocytes or EFS in these patients. However, determining the optimal GnRHa dose according to BMI will require further research.

The major limitation of our study was definitely the low number of patients. Therefore, the power of the study was relatively low to make a precise comparative analysis. However, the aim of this study was to report the effectiveness of low-dose GnRH agonist triggering in these cases. In conclusion, the current study attempted to diagnose the effectiveness of low-dose GnRH agonist triggering in oocyte maturation. Our results support that 0.1 mg triptorelin acetate effectively induces final oocyte maturation in IVF cycles. However, as this was a small case series, larger randomized controlled studies are needed to determine the optimal dose for GnRH agonist triggering.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Instutional Review Board.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - G.B., G.F., I.A.; Design - G.B., G.F.; Supervision - G.B., G.F., I.A.; Resource - G.B., G.F., I.A., S.Z.; Materials - G.B., G.F., I.A.; Data Collection&or Processing - G.B., G.F.; Analysis&or Interpretation - G.B., G.F., I.Z.; Literature Search - G.F., G.B., I.Z.; Writing - G.B., G.F., I.Z.; Critical Reviews - G.B., G.F., I.Z.

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References

1. Aboulghar M. Agonist and antagonist coast. Fertil Steril 2012; 97: 523-6.
2. Meldrum DR. Preventing severe OHSS has many different facets. Fertil Steril 2012; 97: 536-8.
3. Kol S, Humaiden P. GnRH agonist triggering: recent developments. Reprod Biomed Online 2013; 26: 226-30.
4. Humaiden P, Papanikolaou EG, Kyrou D, Alsbjerg B, Polyzos NP, Devroye P, Faterni HM. The luteal phase after GnRHa-agonist triggering of ovulation: present and future perspectives. Reprod Biomed Online 2012; 24: 134-41.
5. Kol S, Humaiden P. LH(as hCG) and FSH surges for final oocyte maturation: sometimes it takes two to tango? Reprod Biomed Online. 2010; 21: 590-2.
6. Chua E, Martinez F, Tur R, Sanmartin P, Chueca A, Barri PN. Triggering ovulation with 250 ug ur 500 ug of r-hCG in oocyte donors treated with antagonist protocol has no effect on the number of mature oocytes retrieved: a randomized clinical trial. Gynecol Endocrinol 2012; 28: 678-81.
7. Tsoumpou I. Optimal dose of hCG for final oocyte maturation in IVF cycles: absence of evidence? Reprod Biomed Online 2009; 19: 52-8.
8. Abdalla HI, Ah-Move M, Brinsden P, Howe DL, Okonofua F, Craft I. The effect of the dose of human chorionic gonadotropin and the type of gonadotropin stimulation on oocyte recovery rates in an in vitro fertilization program. Fertil Steril 1987; 48: 958-63.
9. Papanikolaou EG, Humaidan P, Polyzos N, Kalatarioudou S, Kol S, Benediva C, Tournaye H, et al. New Algoritm for OHSS prevention. Reprod Biomed Online 2011; 9: 147.
10. Shalev E, Geslevich Y, Ben-Ami M. Induction of pre-ovulatory luteinizing hormone surge by gonadotrophin-releasing hormone agonist for women at risk for developing the ovarian hyperstimulation syndrome. Hum Reprod 1994; 9: 417-9.
11. Shalev E, Geslevich Y, Maltisky M, Ben-Ami M. Gonadotropin-releasing hormone agonist compared with human chorionic gonadotropin for ovulation induction after clomiphece citrate treatment. Hum Reprod 1995; 10: 2541-4.
12. Parneix I, Empereaire JC, Ruffie A, Parneix P. Comparison of different protocols of ovulation induction, by GnRH agonists and chorionic gonadotropin. Gynecol Obstet Fertil 2001; 29: 100-5.
13. Guillen JJ, Colodron M, Bodri D, Esteve C, Coll O, Vernaeve V. Exploring two different doses GnRH agonist for the induction of final oocyte maturation in GnRH antagonist-treated oocyte donor cycles: a retrospective comparison. ASRM 2011; p-514.
14. 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: 213-20.
15. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Comparison of ‘triggers’ using leuprolide acetate alone or in combination with low-dose human chorionic gonadotropin. Fertil Steril 2011; 95: 2715-7.
16. Iliodromitou S, Blockeel C, Tremellen KP, Fleming R, Tournaye H, Humaidan P, Nelson SM. Consistent high clinical pregnancy rates and low ovarian hyperstimulation syndrome rates in high-risk patients after GnRH agonist triggering and modified luteal support: a retrospective multicentre study. Hum Reprod 2013; 28: 2529-36.
17. Empereaire JC, Parneix I, Ruffie A. Luteal phase defects following agonist-triggered ovulation: a patient-dependent response. Reprod Biomed Online 2004; 9: 22-7.
18. Griesinger G, Diedrich K, Devroye P, Kolbianakis EM. GnRH agonist for triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update 2006; 12: 159-68.
19. Ossina E, Yavorovskykaya K, Kuzmichev L, Kornilov N, Belikov V, Belikova O, Samoilova A, et al. Triggering of final oocyte maturation in GnRH-antagonist IVF protocols: triptorolien 0.1 mg versus hCG. A randomized multicenter trial. Abstracts of the 19th annual meeting of the ESHRE, Berlin, Germany, p.1102 (abstract P-293).
20. Humaiden P, Papanikolaou EG, Tarlatzis BC. GnRHa to trigger final oocyte maturation: a time to reconsider. Hum Reprod 2009; 24: 2389-94.

21. Shoham Z, Schachter M, Lourmaye E, Weissman A, McNamee M, Insler V. The luteinizing hormone surge-the final stage in ovulation induction: modern aspects of ovulation triggering. Fertil Steril 1995; 64: 237-51.

22. Engmann L, Benediva C. Agonist trigger: what is the best approach? Agonist trigger with aggressive luteal support. Fertil Steril 2012; 97: 531-3.

23. Engmann L, DiLuigi A, Schmidt D, Nulsen J, Maier D, Benadiva C. The use of gonadotropin-releasing hormone (GnRH) agonist to induce oocyte maturation after cotreatment with GnRH antagonist in high-risk patients undergoing in vitro fertilization prevents the risk of ovarian hyperstimulation syndrome: a prospective randomized controlled study. Fertil Steril 2008; 89: 84-91.

24. Yanushpolsky E, Hurwitz S, Greenberg L, Racowsky C, Hornstein M. Crinone vaginal gel is equally effective and better tolerated than intramuscular progesterone for luteal support in in vitro fertilization-embryo transfer cycles: a prospective randomized study. Fertil Steril 2010; 94: 2596-9.

25. Kummer NE, Feinn RS, Griffin DW, Nulsen JC, Benadiva CA, Engmann LL. Predicting successful induction of oocyte maturation after gonadotropin-releasing hormone agonist(GnRHa) trigger. Hum Reprod 2013; 28: 152-9.

26. Zegers-Hochschild F, Fernande E, MackennaA, Fabres C, Allieri E, Lopez T. The empty follicle syndrome: a pharmaceutical industry syndrome. Hum Reprod 1995; 10: 2262-5.

27. Mesen TB, Yu B, Richter KS, Widra E, DeCherney AH, Segars JH. The prevalence of genuine empty follicle syndrome. Fertil Steril 2011; 96: 1375-7.

28. Quintans CJ, Donaldson MJ, Blanco LA, Pasqualini RS. Empty follicle syndrome due to human errors: its occurrence in an in-vitro fertilization programme. Hum Reprod 1998; 13: 2703-5.

29. 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.

30. Chevrier L, Guimiot F, Roux N. GnRH receptor mutations in isolated gonadotropin deficiency. Mol Cell Endocrinol 2011; 346: 21-8.