

CASE REPORT

Live birth in a 46-year-old woman using microdose GnRH agonist flare-up protocol combined with GnRH antagonist: a case report

Hong Zhang¹, Ya-Qiong Liu^{2,3}, Guang-Xiu Lu^{1,3} & Fei Gong^{1,2,3}

¹Reproductive and Genetic Hospital of CITIC-Xiangya, Changsha, China

²Institute of Reproductive and Stem Cell Engineering, School of basic medicine, Central South University, Changsha, China

³Key Laboratory of Reproductive and Stem Cell Engineering, Ministry of Health, Changsha, China

Correspondence

Fei Gong, Institute of Reproductive and Stem Cell Engineering, Central South University, Changsha, Hunan Province 410008, China. Tel: +86 731 82355100 8401; Fax: +86 731 84497661; E-mail: gfdirector@126.com

Funding Information

National Natural Science Foundation of China, (81501328).

Received: 18 February 2016; Revised: 18 July 2016; Accepted: 8 August 2016

Clinical Case Reports 2016; 4(12): 1107–1111

doi: 10.1002/ccr3.693

Introduction

With advances in assisted reproductive technology, the percentage of women with advanced age seeking IVF treatment has increased substantially in recent years. According to a report published by the CDC [1] in 2005, approximately 12% of the women receiving embryo transfers (ETs) using fresh, autologous oocytes were ≥ 41 years of age. Female fecundity and fertility begins to decline significantly after 32 years of age and decreases substantially after 37 years of age [2]. Very few successful pregnancies after the age of 45 years have been reported. The Bologna criteria [3] define patients of advanced age (≥ 40 years) with an abnormal ovarian reserve test (ORT), regardless of a previously performed controlled ovarian hyperstimulation (COH), as poor responders (PORs). Their follicular depletion decides the ovarian reserve and is the main cause for the poor response [4]. Although various protocols and adjuvants have been used to improve the clinical outcomes for PORs, few retrieved oocytes, high rates of cycle cancellation, and very few

Key Clinical Message

Few successful pregnancies after age 45 years with low ovarian reserve have been reported. We report a 46-year-old woman with basal FSH 20.36 mIU/mL and an antral follicle count of four obtained two embryos and delivered a healthy infant with IVF using a microdose GnRH-a flare-up protocol combined with GnRH-ant.

Keywords

Advanced maternal age, GnRH agonist, GnRH antagonist, ovarian stimulation, poor responder.

successful pregnancy outcomes are frequently observed. There is no standard treatment for PORs, and choosing an optimal stimulation protocol remains a challenging task for clinicians.

Several studies have reported successful pregnancies in women of advanced reproductive age (≥ 45 years). Dal Prato et al. [5] were the first to report the case of a 46-year-old woman whose ovaries were stimulated using clomiphene citrate and three oocytes were retrieved. Recently, Trolice reported a case of a 46-year-old woman with anti-mullerian hormone (AMH) level < 0.16 who conceived through IVF [6]. The stimulation protocol comprised a combination of a GnRH antagonist (GnRH-ant) and letrozole. More recently, Rani et al. [7] reported the case of a 50-year-old woman who conceived through IVF, and the standard long protocol was followed. However, her ovarian reserve analysis revealed a serum FSH level of 9.18 IU/L, AMH level of 1.74 ng/mL, and an antral follicle count (AFC) of 5. These findings suggest that treatment protocols in patients with advanced reproductive age are usually decided based on their ovarian

reserve and previous clinical experiences of the assisted reproductive technology (ART) centers.

Here, we present a novel protocol of a microdose GnRH agonist (GnRH-a) flare-up strategy combined with a GnRH-ant, which resulted in live birth in a 46-year-old woman with abnormal ORT, using fresh autologous oocytes through IVF.

Case Report

The patient was a 46-year-old pluripara with secondary infertility for 3 years. She previously had two children, after which she underwent tubal ligation for birth control. Unfortunately, she lost her children in an accident when she was 43 years old. To restore fertility, the patient underwent a salpingostomy; however, she failed to conceive. A hysteroscopy examination conducted 3 years later revealed that both of her fallopian tubes were obstructed, after which she decided to undergo IVF treatment.

The patient had regular menstrual cycles and her BMI was 24.99 kg/m². Her early follicular phase serum FSH and luteinizing hormone (LH) concentrations before treatment were 20.36 and 5.71 mIU/mL, respectively. Transvaginal ultrasound showed an AFC of 4, and left and right ovarian volumes of 28 × 18 × 20 mm and 30 × 19 × 28 mm, respectively. The semen analysis of her husband revealed teratozoospermia.

Based on the age, basal hormone level, and AFC of the patient, the microdose flare-up agonist protocol was selected. On day 2 of her menstrual cycle, triptorelin acetate (triptorelin; Ipsen Pharma Biotech, Paris, France) was started at 50 µg sc/Qd, which was continued until the day of hCG injection. HMG (Menotropins for Injection; Livzon Pharmaceutical Group Inc, Zhuhai, China) at 300 IU per day was started on day 3 of the cycle and continued for 9 days. On day 4 of stimulation, the concentrations of serum estradiol (E2), LH, and progesterone (P) were 143.9 pg/mL, 8.05 mIU/mL, and 0.42 ng/mL, respectively; the diameter of the leading follicle was 10 mm and the endometrial thickness was 5.1 mm. Because of the slight

rise of LH and low level of E2, 0.25 g/day of a GnRH-ant, cetrorelix acetate (Cetrotide; Serono, Geneva, Switzerland), was started. On day 7 of stimulation, the concentrations of serum E2, LH, and P were 505.2 pg/mL, 6.09 mIU/mL, and 0.47 ng/mL, respectively; the endometrial thickness reached 9.7 mm, and there were two leading follicles with mean diameters of 15 and 13.5 mm. No LH surge was observed in the urine LH on day 7 after stimulation and thereafter. On day 10 after stimulation, the concentrations of serum E2, LH, and P were 985.1 pg/mL, 6.95 mIU/mL, and 0.61 ng/mL; two follicles measuring 19 mm in diameter were observed in the right ovary and the endometrial thickness was 11.4 mm. Human chorionic gonadotropin (HCG, 8500 IU, Profasi; Serono, Geneva, Switzerland) was administered intramuscularly on the same day. The duration of administration of GnRH agonist was 11 days, the total dosage of Gn was 2700 IU, and the length of stimulation was 9 days. Figure 1 shows a scheme for this protocol.

Thirty-six hours later, two oocytes were retrieved transvaginally under ultrasound guidance and inseminated by conventional IVF. Eighteen hours after insemination, each of the oocytes was fertilized with two pronuclei. On day 3 after fertilization, two cleavage-stage embryos (grade 2; 8-cell and 7-cell) were transferred under abdominal ultrasound guidance. Luteal phase support was achieved using 90 mg of 8% micronized natural progesterone gel (Crinone; Serono, Geneva, Switzerland) and HCG (2000 IU) was administered intramuscularly every other day from day 1 to day 9 after oocyte retrieval. On the day of ET, methylprednisolone (5 mg/day) and aspirin (50 mg/day) were also administered.

Obstetric ultrasound 27 days post-ET revealed a single viable intrauterine pregnancy. Because of financial constraints, our patient declined preimplantation genetic screening before ET, but prenatal screening and periodic prenatal visits were recommended to her. The patient consented to prenatal screening for Down syndrome in the second trimester and was found to have a low risk for it, trisomy 18, and neural tube defects. Moreover, no

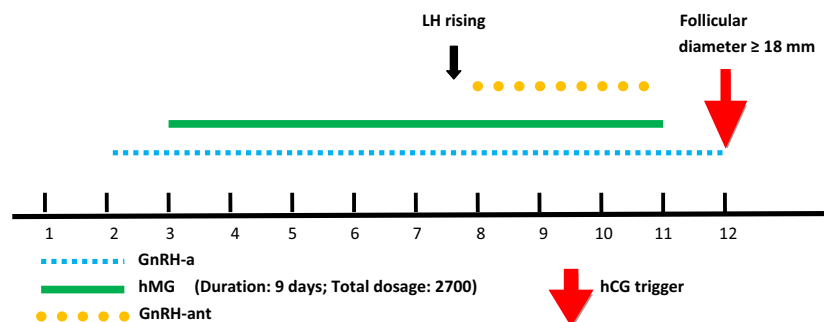


Figure 1. The microdose GnRH-a flare-up combined with GnRH-ant protocol used in this case.

pregnancy-related complication was observed during her antenatal period, and a healthy male infant weighing 3400 g was delivered via Cesarean section at 38 weeks' gestation.

Discussion

In the present case, because of the abnormal ovarian reserve and advanced age of the patient, at first, we intended to using the microdose GnRH-a flare-up protocol (Fig. 3) to flare up the level of endogenous Gn working synergistically with exogenous Gn to recruit more follicles. Some researchers have considered this as one of the most successful protocols for ovarian stimulation in PORs [8, 9]. In addition, we used hMG as the Gn, as patients older than 35 years [10] with basal FSH concentration >10 mIU/L have few active LH receptors and decreased activity of endogenous LH paracrined by ovarian follicles; hence, it is advisable to supply exogenous LH to COH with rFSH [11].

Although the protocol using hMG as Gn in a microdose GnRH-a flare-up regime helps enhance follicular recruitment, it may result in premature luteinization that cannot be inhibited by microdose GnRH-a. The increased serum LH levels, with a concomitant increase in serum progesterone and testosterone levels during early follicular maturation, may affect oocyte quality, in addition to compromising synchronization between the embryo and the endometrium [12], which may decrease the chance of fertilization and pregnancy, and increase the risk of spontaneous abortion [13]. But in this case, to inhibit the premature surge in LH, we added the GnRH-ant to rapidly block gonadotropin receptors without suppressing early follicular development.

The idea of the combination of GnRH-a flare-up and GnRH-ant protocol to minimize detrimental effects and combine the beneficial effects of these two stimulation protocols for PORs was first presented as a meeting abstract by Berger *et al.* [14] as a novel protocol, the

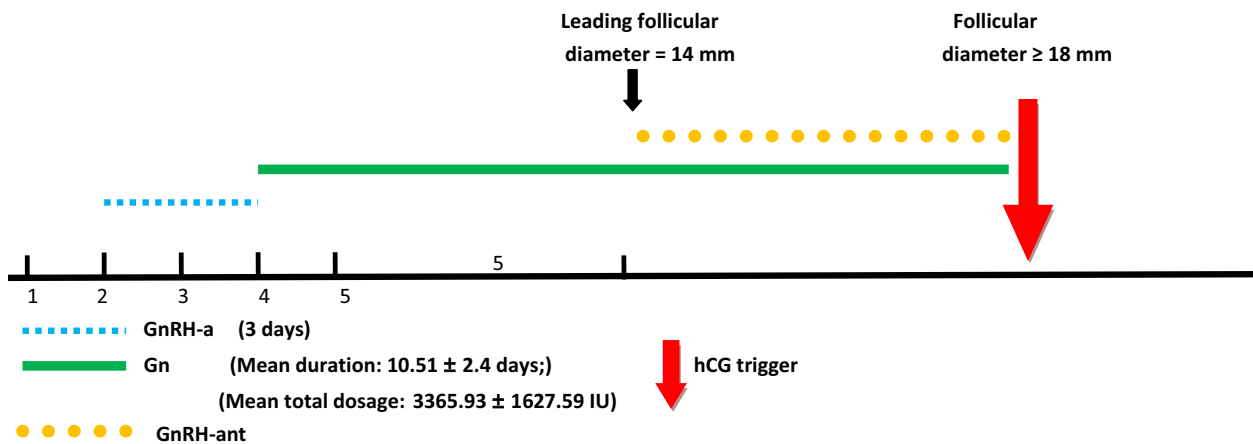


Figure 2. Schematic representation of the agonist-antagonist protocol.

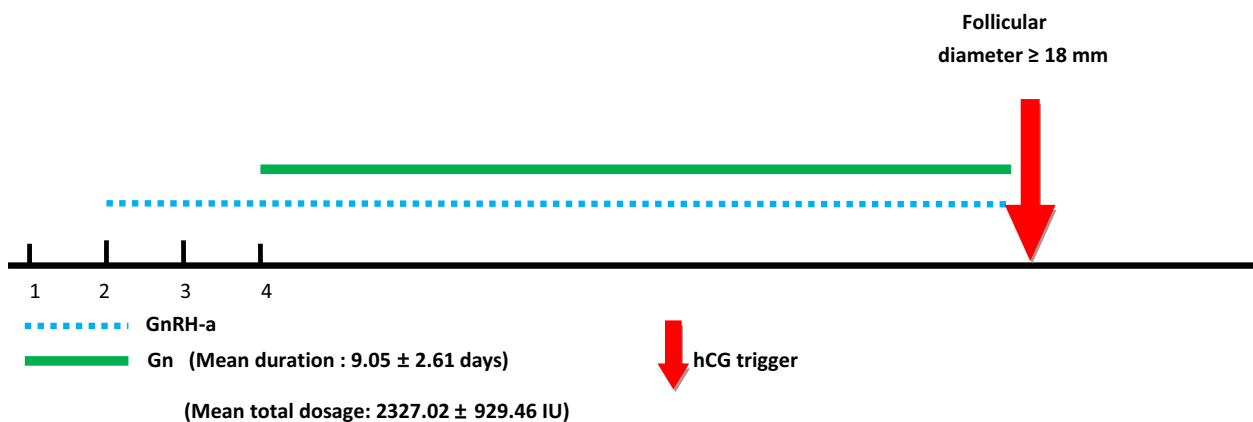


Figure 3. Schematic representation of the microdose GnRH-a flare-up protocol.

“agonist–antagonist protocol (AAP)”, which comprises a combination of a microdose flare-up GnRH agonist with a GnRH antagonist with oral contraceptive (OC) pretreatment. They claimed that AAP is a valuable novel protocol for PORs who have failed ≥ 2 previous IVF treatment cycles, and results in a clinical pregnancy rate $>13\%$, but no data are available regarding the stimulation characteristics. Two more studies were later conducted to compare AAP (ultrashort GnRH agonist/GnRH antagonist) (Fig. 2) with the microdose flare-up protocol in PORs. In 2007, Orvieto *et al.* [15] found that the ultrashort GnRH agonist/GnRH antagonist protocol resulted in greater oocyte retrieval and ET, and a reasonable clinical pregnancy rate. The length of GnRH agonist administration was 3 days, the total Gn dosage was 79 ± 35 of Gn ampules (5925 ± 2625 IU), and the length of stimulation was 11.3 ± 3.7 days. However, Berker *et al.* [12] performed a randomized clinical trial in 82 PORs and observed that modified AAP is not inferior to the microdose GnRH-a flare-up protocol but requires a higher dose of gonadotropins (3365.93 ± 1627.59 IU vs. 2327.02 ± 929.46 IU) and longer duration of stimulation (10.51 ± 2.4 days vs. 9.05 ± 2.61 days).

In the current case report, compared with the AAP protocol in previous studies [12, 15], we used a microdose GnRH agonist flare-up protocol combined with a GnRH antagonist (Fig. 1). A longer duration of GnRH-a administration (11 days in total), the use of hMG as Gn, and the early administration of the GnRH antagonist (because of a slight rise in LH) are the main differences from the aforementioned AAP (Fig. 2) and microdose flare-up GnRH agonist protocol (Fig. 3). The long administration of microdose GnRH-a agonist and administration of HMG as Gn has advantages such as less Gn administration and shorter stimulation time. Undeniably, the concentration of LH tends to rise initially in the microdose GnRH-a flare-up protocol, so the timely addition of GnRH-ant was a prerequisite for the success of this pregnancy. Therefore, further studies to determine the accurate time of GnRH-ant administration are warranted.

In conclusion, for PORs of advanced age, using HMG in a microdose agonist flare-up protocol combined with GnRH-ant at the appropriate time may be an effective stimulation protocol. Further studies, such as randomized clinical trials, on this stimulation protocol or studies to determine the most appropriate time to add GnRH-ant are recommended. Moreover, in clinical practice, elderly infertile couples (aged ≥ 45 years) should be adequately counseled regarding their probability of achieving a successful pregnancy outcome through IVF. Further, in order to relieve their financial, physical, and psychological stress, the options of oocyte donation, adoption, or discontinuation of the IVF treatment should be suggested.

Acknowledgments

The authors sincerely thank the patient and related staff at the Reproductive and Genetic Hospital of CITIC-Xiangya who participated in this study. We are grateful to Ge Lin for his assistance with writing this case report. This work was supported by a grant from the National Natural Science Foundation of China (No. 81501328).

Conflict of Interest

None declared.

References

1. Wright, V. C., J. Chang, G. Jeng, and M. Macaluso. 2008. Assisted reproductive technology surveillance—United States, 2005. *MMWR Surveill Sum* 57:1–23.
2. Faddy, M. J., R. G. Gosden, A. Gougeon, S. J. Richardson, and J. F. Nelson. 1992. Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Hum. Reprod.* 7:1342–1346.
3. Ferraretti, A. P., A. La Marca, B. C. Fauser, B. Tarlatzis, G. Nargund, and L. Gianaroli. 2011. ESHRE consensus on the definition of ‘poor response’ to ovarian stimulation for in vitro fertilization: the Bologna criteria. *Hum. Reprod.* 26:1616–1624.
4. Richardson, S. J., V. Senikas, and J. F. Nelson. 1987. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. *J. Clin. Endocrinol. Metab.* 65:1231–1237.
5. Dal Prato, L., A. Borini, M. Cattoli, M. S. Preti, L. Serrao, and C. Flamigni. 2005. Live birth after IVF in a 46-year-old woman. *Reprod. Biomed. Online* 11:452–454.
6. Trolice, M. P. 2014. Live birth from a 46-year-old using fresh autologous oocytes through in vitro fertilization. *Fertil. Steril.* 102:96–98.
7. Rani, G., S. Goswami, R. Chattopadhyay, S. Ghosh, B. Chakravarty, and A. Ganesh. 2015. Live birth in a 50-year-old woman following in vitro fertilization-embryo transfer with autologous oocytes: a rare case report. *Fertil. Steril.* 103:414–416.
8. Demiroglu, A., and T. Gurgan. 2009. Comparison of microdose flare-up and antagonist multiple-dose protocols for poor-responder patients: a randomized study. *Fertil. Steril.* 92:481–485.
9. Robab, D., A. Abbas, and A. Maryam. 2009. Clinical Effects of a microdose GnRH agonist flare regimen administered to poor responders undergoing ART cycles. *Acta Medica Iranica* 47:263–267.
10. Marrs, R., D. Meldrum, S. Muasher, W. Schoolcraft, L. Werlin, and E. Kelly. 2004. Randomized trial to compare the effect of recombinant human FSH (follitropin alfa) with or without recombinant human LH in women

- undergoing assisted reproduction treatment. *Reprod. Biomed. Online* 8:175–182.
11. Lisi, F., L. Rinaldi, S. Fishel, D. Caserta, R. Lisi, and A. Campbell. 2005. Evaluation of two doses of recombinant luteinizing hormone supplementation in an unselected group of women undergoing follicular stimulation for in vitro fertilization. *Fertil. Steril.* 83:309–315.
 12. Berker, B., C. I. Duvan, C. Kaya, R. Aytac, and H. Satiroglu. 2010. Comparison of the ultrashort gonadotropin-releasing hormone agonist-antagonist protocol with microdose flare -up protocol in poor responders: a preliminary study. *J. Turk. Ger. Gynecol. Assoc.* 11:187–193.
 13. Homburg, R., N. A. Armar, A. Eshel, J. Adams, and H. S. Jacobs. 1988. Influence of serum luteinising hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. *BMJ* 297:1024–1026.
 14. Berger, B., D. Ezcurra, and M. Alper. 2004. The agonist-antagonist protocol: a novel protocol for treating the poor responder. *Fertil. Steril.* 82(Suppl. 2):S126.
 15. Orvieto, R., J. Kruchkovich, J. Rabinson, E. Zohav, E. Y. Anteby, and S. Meltcer. 2008. Ultrashort gonadotropin-releasing hormone agonist combined with flexible multidose gonadotropin-releasing hormone antagonist for poor responders in in vitro fertilization/embryo transfer programs. *Fertil. Steril.* 90:228–230.