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An Evaluation and Comparison of Various Stimulation Protocols for in Vitro Fertilization

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Abstract: *As a result of genetic, epigenetic, or both influences, infertility is a significant medical concern in the Western world, prompting ongoing research and developments in assisted reproductive technology. A wide range of stimulation protocols are accessible for the purpose of achieving controlled ovarian hyperstimulation (COH) during in vitro fertilization (IVF). The agonist extended protocol, antagonist protocol, and minimal stimulation protocol are compared in this article. The minimal stimulation and gonadotropin-releasing hormone (GnRH) antagonist protocol reduces gonadotropin use and treatment duration. The extended protocol for GnRH agonists improves folliculogenesis and pregnancy rate, which is the primary objective of COH. Notwithstanding its expensive and protracted methodology, the GnRH agonist long protocol has yielded favorable outcomes for the majority of women. Conversely, individuals who have inadequate ovarian reserve may benefit more from the implementation of a minimal stimulation protocol. To reach more conclusive results, it is obviously essential to conduct larger-scale studies with more targeted comparisons that account for additional confounding variables and variations in patients' response criteria.*

Keywords: Stimulation protocols, In vitro fertilization (IVF), Ovarian stimulation

I. INTRODUCTION

Controlled ovarian hyperstimulation (COH) has been part of IVF since the 1970s (1). Assisted reproduction procedures (IVF) have evolved to suit low, middle, and high-responsiveness patients. Gonadotropin-releasing hormone (GnRH) analogues and oestradiol inhibitors, such as clomiphene citrate (CC), have increased assisted reproduction options and improved in vitro fertilization (2,3). Scientific literature has more ovarian stimulation regimens. CC, FSH, LH, GnRH analogues, Gn, and CC (1,4) are used in these regimens.

The use of a GnRH agonist or antagonist analogue determines whether an IVF treatment is agonist or antagonist. A minimum stimulation regimen uses CC and FSH or Gn (1,4-6). This article compares the pros and cons of the three IVF protocols.

GnRH agonist long protocol and antagonist protocol

GnRH agonist and antagonist regimens use GnRH analogues. GnRH receptors interact with decapeptides modeled after human GnRH. These analogues feature gonadotropin amino acid sequence changes that improve their half-lives and effectiveness relative to natural hormones (2,7,8). GnRH agonists maintain gonadotropin secretion, whereas antagonists mediate chemical hypophysectomy (9). Both analogues are extensively used in IVF to stimulate folliculogenesis by preventing natural LH surge and timing oocyte retrieval (10-12). Triptorelin, leuprorelin, deslorelin, goserelin, nafarelin, and cetrorelix and ganirelix are agonistic and antagonistic counterparts used in clinical practice (9). Since its discovery in the 1980s, the extended GnRH agonist regimen has been the gold standard in IVF (10,13). Recently developed GnRH antagonists provide an alternate IVF therapy.

The GnRH long agonist regimen (Figure 1) begins with 0.1 mg triptorelin on cycle day 21 and 150-225 IU of gonadotropin on cycle day 2. Gonadotropin dose depends on follicular development. GnRH agonist and gonadotropin are given continuously until human chorionic gonadotropin (HCG) injections begin 14 days after the GnRH agonist regimen is stopped or when follicles reach 16–18 mm.

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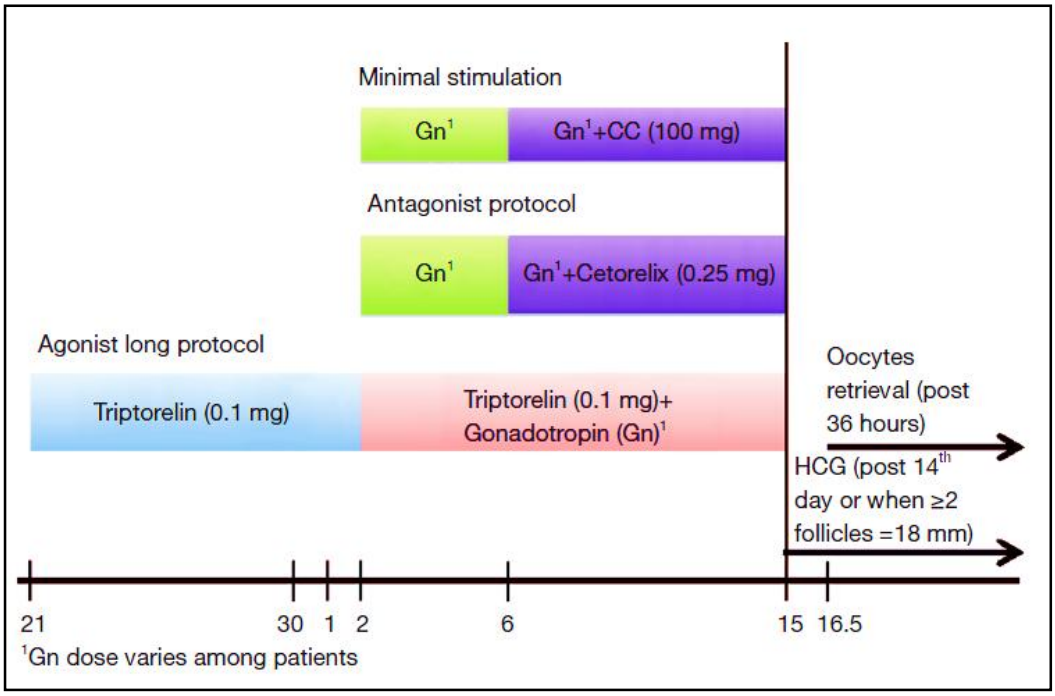

Figure 1 Stimulation procedures for agonist long, antagonist and minimal stimulation protocols for IVF. GN, gonadotropin mixtures; CC, clomiphene citrate; IVF, in vitro fertilization.

The GnRH antagonist regimen (Figure 1) begins daily gonadotropin injection at 150–225 IU after follicle diameter evaluation on cycle-day 2/3 for each patient. Gonadotropin dose depends on follicular response. At 14 mm follicular size, or six days following gonadotropin injection, subcutaneous GnRH antagonist (e.g., cetrorelix) is given. Patients are regularly assessed using transvaginal sonography (TVS) and hormonal profiling, which measures FSH, LH, estrogen, and progesterone. Mature oocytes are retrieved 34–36 hours after HCG administration. Patients with increased LH and estrogen levels need further monitoring to avoid premature ovulation. With an increased risk of ovarian hyperstimulation syndrome (OHSS), stopping cycles is crucial.

Minimal stimulation protocol

CC, an estrogen receptor modulator and competitive oestradiol inhibitor, has been used in fertility therapy for 40 years (3). The main drawback of CC is its anti-estrogenicity. The antiestrogenic property may block the early surge of LH, which is essential for folliculogenesis (4). CC with human menopausal gonadotropin (HMG) in the minimum stimulation regimen is more effective (46% vs. 25.9%) than HMG alone (3,4,14). Figure 1 shows that CC is given from the sixth day, or sooner if LH levels rise, until HCG is given. IVF and mature oocyte retrieval follow.

For minimum stimulation methods, clomiphene-resistant individuals may use letrozole, an aromatase inhibitor. In the first two to three days of menstruation, 2.5 mg letrozole is given with gonadotropin for five days. Letrozole, created to treat metastatic breast cancer, has not been approved for ovulation induction.

Criteria for IVF protocol selection

Physicians decide whether to utilize lengthy agonist, antagonist, or minimum stimulation protocols on each patient. The pros and cons of each therapy and, most crucially, patient response determine the choice. Patients who react to gonadotropin stimulation are classified as high, middle, or poor (15,16). FSH, oocyte number, cycle cancellation rate, gonadotropin dosage, and E2 levels are usually utilized to define inadequate ovarian response (15). However, specialists may define poor responders differently. Over time, screening tests such ovarian reserve, CC challenge, GnRH, GnRH agonist, AMH, and AFC have been added (15,17). Low ovarian response with adequate stimulation occurred in 9-24% of IVF cycles (18). Malmusi et al. defined poor responders as those with fewer than 4 oocytes and

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no ovarian response with FSH above 300 IU (19). Poor response is linked to advanced maternal age, which lowers oocyte quality and follicle counts. Some young patients have experienced this, although the reasons are unknown (15,17). Despite several studies determining which treatment is best for patients in each response group, there is no agreement since each procedure has pros and downsides.

GnRH agonist long protocol versus GnRH antagonist protocol

Ovarian cysts, menopause, longer treatment duration, and more gonadotropin ampoules are the primary downsides of GnRH agonist extended protocol. Follicular production decreases with antagonists (20). Antagonists reduce LH and estrogen production, decreasing pregnancy and implantation (21). Patients using the agonist method had greater oocyte retrieval and mature production ($P < 0.05$) compared to antagonist treatment, despite comparable cycle cancellation rates (7,19,22). Prolonged agonist treatment increased oocytes and embryo production. GnRH agonist prolonged protocol may boost cumulative pregnancy rate. Rabinson et al. found no difference in efficacy between the two regimens in individuals with high BMI (>40), suggesting that BMI may affect gonadotropin beginning dose and therapy (23). The agonist regimen was chosen by normal BMI patients. High-BMI people need high-dose gonadotropin for ovarian stimulation (23). Poor response, PCOS, and other response characteristics were studied.

Another contested research in the same year found that antagonist therapy increased oocyte counts ($P=0.022$) in poor GnRH extended protocol responders (24). Due to its fast gonadotropin suppression (shorter cycle length, low estrogen level on HCG injection day, and fewer oocytes than agonist), the antagonist approach may prevent moderate or severe ovarian hyperstimulation syndrome (OHSS), especially in PCOS patients (25,26). Similar studies cautioned against concluding that the antagonist protocol prevents OHSS better than the agonist long regimen owing to a lack of larger randomized trials with acceptable sample size and standardized OHSS grade criteria. The two therapies prevented OHSS equally except for severe instances (27). After HCG, Alama et al. discovered GnRH agonists prevented OHSS (28). GnRH agonist reduces OHSS risk as a last oocyte maturation trigger. Only after discovering the GnRH antagonist was its trigger revealed. Even if antagonist treatment is superior, HCG ultimate maturation still causes OHSS. Endothelial growth factor enhances granulosa cell fluid shifting and vascular permeability (28). Finish oocyte maturation using GnRH agonist to avoid this. GnRH agonists diminish VEGF, inhibin B, and steroidogenesis gene expression, lowering OHSS risk (29).

GnRH antagonists worsened Beckwith-Wiedemann syndrome, naevus, skin tags, torticollis, pyloric stenosis, and asymmetry (30). Overall, GnRH agonist prolonged therapy generated better developed follicles with fewer risks than antagonist-controlled ovarian stimulation.

Effectiveness of the minimal stimulation protocol

Antiestrogenic CC stimulates follicular development and reduces LH surges. The minimal stimulation method is practical and employs few gonadotropin ampoules. The agonist requires 25 gonadotropin ampoules; this therapy uses 5.7. Because of this method, oocytes are less developed, lowering saved embryo viability. Equal pregnancy and transplanting rates were seen with agonist therapy (4,31). This regimen is cheaper than antagonistic or agonistic treatments for elderly women or those with minimal ovarian reserve. The minimum stimulation regimen generated fewer oocytes than the GnRH agonist but had comparable transplanting and pregnancy rates (32,33). This technique may be ideal for weak ovarian response due to cost-effectiveness and reduced OHSS risk (34).

IVF with gonadotropins and CC raised the likelihood of multiple pregnancies, which might cause early delivery, growth retardation, and miscarriage. The couple's infertility may complicate ovarian stimulation and low birth weight (35). High gonadotropin levels produce chromosomal aneuploidy in developing oocytes due to improper meiotic division (36). In mice, ovarian stimulation of zygotes caused female pronucleus chromosomal aberrations (25). Similar research observed higher mosaicism and aneuploidy in in vitro-fertilized embryos (37,38). Baart et al. discovered that low-dose FSH stimulation generated fewer mitotic segregation mistakes, mosaicism, and malformed embryos than high-dose FSH stimulation (38). IVF patients with CC had ventricular septal defect, cardiac defect, and chromosomal abnormality (30).

Letrozole causes ovulation in clomiphene-resistant women. Unlike clomiphene, it prevents estrogen receptor depletion throughout the body and does not affect the hypothalamic-pituitary-ovarian axis' negative feedback mechanism,

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enabling monofollicular development and minimizing multiple pregnancies (39). Letrozole enhances PCOS ovulation and pregnancy more than clomiphene. The chemical is not licensed for ovulation induction and has been linked to congenital defects (40,41).

Cost-effective low stimulation is preferred by physicians and patients. Compared to the other two treatments, less stimulation increases risk.

Effect of stimulation protocols on prenatal outcome

In general, imprinting defects are caused by disruptions in the methylation of differentially methylated regions (DMRs) brought about by ovarian stimulation. The most significantly impacted genes were those that are imprinted during the later stages of oocyte development, according to studies. The presence of imprinted genes is critical for placental function and the growth and development of embryos (37,42,43). Methylation defects are likely to be the cause of a number of genetic diseases, including Angelman syndrome (deficiency at DMRs of SNRPN), Silver-Russell syndrome (deficiency in PEG1/MEST), and Beckwith-Wiedemann syndrome (deficiency of methylation at DMRs of KCNQ1OT1). DNA methylation is an essential mechanism for regulating gene imprints. Indelions in imprinting have been detected in offspring conceived through the use of assisted reproductive technologies (43).

II. SUMMARY

This review synthesizes the efficacy of three in vitro fertilization (IVF) protocols: minimal stimulation, GnRH agonist long, and minimal stimulation. This comprehensive examination of patients across all response criteria confirms that each strategy has a cost-benefit ratio and risks epigenetic alterations. Cost-benefit analysis shows that the agonist prolonged protocol is better for in vitro fertilization (IVF) stimulation than the antagonist and minimum stimulation procedures. COH blocks LH flow to increase mature follicles. The long GnRH agonist treatment generates more mature follicles than the others. Due to this protocol's long duration and high gonadotropin doses, many doctors prefer minimum stimulation and antagonist treatments. The extended approach including GnRH agonists yields more viable embryos and oocytes, which may be cryopreserved and used for frozen embryo transfer in elderly patients with very high BMIs.

Due to its equivalent pregnancy and transplanting rates, lower cost, and gonadotropin ampoules, the minimum stimulation procedure using CC and gonadotropin is a better option to the GnRH agonist protocol. This approach has fewer daily monitoring visits and ultrasonography than the usual procedure, which may assist patients with low ovarian reserve and poor responders. However, this approach reduces follicular growth in healthy ovaries. This study recommends bigger, more patient-based trials with stronger response definition and screening criteria to reach a more rational conclusion. Future comparisons of these regimens must include epigenetic factors that affect perinatal outcomes.

REFERENCES

[1]. Williams SC, Gibbons WE, Muasher SJ, et al. Minimal ovarian hyperstimulation for in vitro fertilization using sequential clomiphene citrate and gonadotropin with or without the addition of a gonadotropin-releasing hormone antagonist. Fertil Steril 2002;78:1068-72.

[2]. Itskovitz-Eldor J, Kol S, Mannaerts B. Use of a single bolus of GnRH agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report: short communication. Hum Reprod 2000;15:1965-8.

[3]. Zhang J, Chang L, Sone Y, et al. Minimal ovarian stimulation (mini-IVF) for IVF utilizing vitrification and cryopreserved embryo transfer. Reprod Biomed Online 2010;21:485-95.

[4]. Ibrahim AE. The Minimal Stimulation Protocol for ICSI: An Alternative Protocol for Ovarian Stimulation. N Y Sci J 2014;7:19-23.

[5]. Marci R, Caserta D, Lisi F, et al. In vitro fertilization stimulation protocol for normal responder patients. Gynecol Endocrinol 2013;29:109-12.

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[6]. Mohsen IA, El Din RE. Minimal stimulation protocol using letrozole versus microdose flare up GnRH agonist protocol in women with poor ovarian response undergoing ICSI. Gynecol Endocrinol 2013;29:105-8.

[7]. Franco JG Jr, Baruffi RL, Mauri AL, et al. GnRH agonist versus GnRH antagonist in poor ovarian responders: a meta-analysis. Reprod Biomed Online 2006;13:618-27.

[8]. Daya S. Gonadotropin releasing hormone agonist protocols for pituitary desensitization in in vitro fertilization and gamete intrafallopian transfer cycles. Cochrane Database Syst Rev 2000:CD001299.

[9]. van Loenen AC, Huirne JA, Schats R, et al. GnRH agonists, antagonists, and assisted conception. Semin Reprod Med 2002;20:349-64.

[10]. Grow D, Kawwass JF, Kulkarni AD, et al. GnRH agonist and GnRH antagonist protocols: comparison of outcomes among good-prognosis patients using national surveillance data. Reprod Biomed Online 2014;29:299-304.

[11]. Kara M, Aydin T, Aran T, et al. Comparison of GnRH agonist and antagonist protocols in normoresponder patients who had IVF-ICSI. Arch Gynecol Obstet 2013;288:1413-6.

[12]. Khalaf M, Mittre H, Levallet J, et al. GnRH agonist and GnRH antagonist protocols in ovarian stimulation: differential regulation pathway of aromatase expression in human granulosa cells. Reprod Biomed Online 2010;21:56-65.

[13]. Lai Q, Zhang H, Zhu G, et al. Comparison of the GnRH agonist and antagonist protocol on the same patients in assisted reproduction during controlled ovarian stimulation cycles. Int J Clin Exp Pathol 2013;6:1903-10.

[14]. Ziadeh SM, Zakaria MR, Abu-Hieja A. Pregnancy rates using CC/hMG or hMG alone. J Obstet Gynaecol Res 1997;23:97-101.

[15]. Oehninger S. Poor responders in in vitro fertilization (IVF) therapy: the -challenge continues. Facts Views Vis Obgyn 2011;3:101-8.

[16]. Davis OK. IVF stimulation: protocols for poor responders. Methods Mol Biol 2014;1154:329-41.

[17]. Oudendijk JF, Yarde F, Eijkemans MJ, et al. The poor responder in IVF: is the prognosis always poor?: a systematic review. Hum Reprod Update 2012;18:1-11.

[18]. Tarlatzis BC, Zepiridis L, Grimbizis G, et al. Clinical management of low ovarian response to stimulation for IVF: a systematic review. Hum Reprod Update 2003;9:61-76.

[19]. Malmusi S, La Marca A, Giulini S, et al. Comparison of a gonadotropin-releasing hormone (GnRH) antagonist and GnRH agonist flare-up regimen in poor responders undergoing ovarian stimulation. Fertil Steril 2005;84:402-6.

[20]. Kim CH, You RM, Kang HJ, et al. GnRH antagonist multiple dose protocol with oral contraceptive pill pretreatment in poor responders undergoing IVF/ICSI. Clin Exp Reprod Med 2011;38:228-33.

[21]. A double-blind, randomized, dose-finding study to assess the efficacy of the gonadotrophin-releasing hormone antagonist ganirelix (Org 37462) to prevent premature luteinizing hormone surges in women undergoing ovarian stimulation with recombinant follicle stimulating hormone (Puregon). The ganirelix dose-finding study group. Hum Reprod 1998;13:3023-31.

[22]. Xiao J, Chang S, Chen S. The effectiveness of gonadotropin-releasing hormone antagonist in poor ovarian responders undergoing in vitro fertilization: a systematic review and meta-analysis. Fertil Steril 2013;100:1594-601.e1-9.

[23]. Rabinson J, Meltcer S, Zohav E, et al. GnRH agonist versus GnRH antagonist in ovarian stimulation: the influence of body mass index on in vitro fertilization outcome. Fertil Steril 2008;89:472-4.

[24]. Marci R, Caserta D, Dolo V, et al. GnRH antagonist in IVF poor-responder patients: results of a randomized trial. Reprod Biomed Online 2005;11:189-93.

[25]. Al-Inany HG, Youssef MA, Aboulghar M, et al. GnRH antagonists are safer than agonists: an update of a Cochrane review. Hum Reprod Update 2011;17:435.

[26]. Lainas TG, Sfontouris IA, Zorzovilis IZ, et al. Flexible GnRH antagonist protocol versus GnRH agonist long protocol in patients with polycystic ovary syndrome treated for IVF: a prospective randomised controlled trial (RCT). Hum Reprod 2010;25:683-9.

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[27]. Pundir J, Sunkara SK, El-Toukhy T, et al. Meta-analysis of GnRH antagonist protocols: do they reduce the risk of OHSS in PCOS? Reprod Biomed Online 2012;24:6-22.

[28]. Alama P, Bellver J, Vidal C, et al. GnRH analogues in the prevention of ovarian hyperstimulation syndrome. Int J Endocrinol Metab 2013;11:107-16.

[29]. Haas J, Ophir L, Barzilay E, et al. GnRH agonist vs. hCG for triggering of ovulation--differential effects on gene expression in human granulosa cells. PLoS One 2014;9:e90359.

[30]. Tulandi T, Martin J, Al-Fadhli R, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril 2006;85:1761-5.

[31]. D'Amato G, Caroppo E, Pasquadibisceglie A, et al. A novel protocol of ovulation induction with delayed gonadotropin-releasing hormone antagonist administration combined with high-dose recombinant follicle-stimulating hormone and clomiphene citrate for poor responders and women over 35 years. Fertil Steril 2004;81:1572-7.

[32]. Weigert M, Krischker U, Pöhl M, et al. Comparison of stimulation with clomiphene citrate in combination with recombinant follicle-stimulating hormone and recombinant luteinizing hormone to stimulation with a gonadotropin-releasing hormone agonist protocol: a prospective, randomized study. Fertil Steril 2002;78:34-9.

[33]. Hwang JL, Huang LW, Hsieh BC, et al. Ovarian stimulation by clomiphene citrate and hMG in combination with cetrorelix acetate for ICSI cycles. Hum Reprod 2003;18:45-9.

[34]. Albuquerque LE, Tso LO, Saconato H, et al. Depot versus daily administration of gonadotrophin-releasing hormone agonist protocols for pituitary down regulation in assisted reproduction cycles. Cochrane Database Syst Rev 2013;1:CD002808.

[35]. Kapiteijn K, de Bruijn CS, de Boer E, et al. Does subfertility explain the risk of poor perinatal outcome after IVF and ovarian hyperstimulation? Hum Reprod 2006;21:3228-34.

[36]. Hodges CA, Ilagan A, Jennings D, et al. Experimental evidence that changes in oocyte growth influence meiotic chromosome segregation. Hum Reprod 2002;17:1171-80.

[37]. Katz-Jaffe MG, Trounson AO, Cram DS. Chromosome 21 mosaic human preimplantation embryos predominantly arise from diploid conceptions. Fertil Steril 2005;84:634-43.

[38]. Baart EB, Martini E, Eijkemans MJ, et al. Milder ovarian stimulation for in-vitro fertilization reduces aneuploidy in the human preimplantation embryo: a randomized controlled trial. Hum Reprod 2007;22:980-8.

[39]. Kar S. Current evidence supporting "letrozole" for ovulation induction. J Hum Reprod Sci 2013;6:93-8.

[40]. Begum MR, Ferdous J, Begum A, et al. Comparison of efficacy of aromatase inhibitor and clomiphene citrate in induction of ovulation in polycystic ovarian syndrome. Fertil Steril 2009;92:853-7.

[41]. Biljan MM, Hemmings R, Brassard N. The Outcome of 150 Babies Following the Treatment With Letrozole or Letrozole and Gonadotropins. Fertility and sterility 2005;84:S95.

[42]. Fortier AL, Lopes FL, Darricarrère N, et al. Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. Hum Mol Genet 2008;17:1653-65.

[43]. Lawrence LT, Moley KH. Epigenetics and assisted reproductive technologies: human imprinting syndromes. Semin Reprod Med 2008;26:143-52.