Original Article

In-Vitro Fertilization Protocols

In-Vitro Fertilization Protocols: Agonist versus Antagonist in Relation to Ovarian Response, Embryological Performance, and Treatment Characteristics

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ABSTRACT

Objective: To compare the characteristics of ovarian stimulation, oocyte maturation, embryo development, and transfer-related parameters between the gonadotropin-releasing hormone agonist long protocol and the gonadotropin-releasing hormone antagonist protocol in infertile couples having intracytoplasmic sperm injection.

Study Design: Retrospective comparison analysis

Place and Duration of Study: This study was conducted at the Department of Gynecology & Obstetrics, Collage of Pharmacy, University of Duhok from 20th August 2024 to 28th February 2025.

Methods: This retrospective comparison analysis was performed on 200 Intracytoplasmic sperm injection cycles utilising either a gonadotropin-releasing hormone agonist or gonadotropin-releasing hormone antagonist regimen. We looked at the length of stimulation, the amount of gonadotropin used, the reaction of the ovaries, the age of the oocytes, the success of fertilisation, the quality of the embryos, the thickness of the endometrium, and the day of embryo transfer.

Results: The gonadotropin-releasing hormone antagonist protocol correlated with markedly reduced stimulation days ($p=0.003$) and diminished gonadotropin requirements ($p<0.001$). The ovarian response was more effective in antagonist cycles, with increased quantities of metaphase II oocytes ($p=0.001$) and fertilised oocytes ($p=0.010$). The quality of embryos varied considerably among procedures ($p < 0.001$), with antagonist cycles yielding a greater percentage of high-grade blastocysts and more frequent day-5 embryo transfers ($p<0.001$). The endometrial thickness on the day of embryo transfer was considerably higher in antagonist cycles ($p=0.032$).

Conclusion: Gonadotropin-releasing hormone antagonist programs exhibit enhanced stimulation efficacy and embryological outcomes relative to gonadotropin-releasing hormone agonist protocols. These data indicate that biological and treatment-related benefits linked to antagonist regimens may enhance reproductive outcomes.

Key Words: In-vitro fertilization protocols, Gonadotropin-releasing hormone, Ovarian stimulation, Embryo quality, Oocyte maturation, Intracytoplasmic sperm injection

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INTRODUCTION

Successful in vitro fertilisation (IVF) relies not only on achieving pregnancy but also on the quality of ovarian response, embryo development, and endometrial receptivity, all of which are influenced by the chosen ovarian stimulation protocol.[1,2]

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Although pregnancy and live birth represent the ultimate outcomes of assisted reproductive technology (ART), these endpoints result from a complex sequence of biological and clinical events initiated by controlled ovarian stimulation (COS).[3]

The two principal protocols used for ovarian stimulation in IVF are the gonadotropin-releasing hormone (GnRH) agonist long protocol and the GnRH antagonist protocol. The GnRH agonist protocol induces pituitary desensitisation through prolonged stimulation of GnRH receptors, leading to suppression of endogenous luteinizing hormone (LH) secretion.[4] In contrast, the GnRH antagonist protocol rapidly suppresses LH release by competitively blocking GnRH receptors, allowing more immediate control over the hypothalamic–pituitary–ovarian axis.[5] These mechanistic differences significantly influence follicular recruitment, endocrine environment, oocyte maturation, and embryo developmental potential.[6]

The numerous studies have compared clinical pregnancy and live birth rates between agonist and antagonist regimens, fewer investigations have focused on the underlying biological and treatment-related factors that contribute to these outcomes.[7,8] Understanding how stimulation protocols affect ovarian response, oocyte maturity, fertilisation efficiency, embryo quality, and embryo transfer conditions is essential for optimising IVF strategies and individualising patient care.[9]

In addition, the choice of stimulation protocol may influence treatment burden, including the duration of stimulation and total gonadotropin consumption-factors that directly affect patient comfort, treatment cost, and safety.[10] The GnRH antagonist protocol has gained increasing popularity due to its shorter stimulation period, lower gonadotropin requirements, and reduced risk of ovarian hyperstimulation syndrome (OHSS).[11]

Accordingly, this study aims to evaluate and compare stimulation characteristics, ovarian response, embryological outcomes, and embryo transfer parameters between GnRH agonist and GnRH antagonist protocols in infertile couples undergoing intracytoplasmic sperm injection (ICSI). By focusing on these mechanistic determinants, this research seeks to clarify how protocol selection influences the biological processes that ultimately govern IVF success.

METHODS

This retrospective comparison analysis was performed at Department of Gynecology & Obstetrics, Collage of Pharmacy, University of Duhok from 20th August 2024 to 28th February 2025 vide letter No. 4545/QM/Approval/9389JFDNF dated August 11, 2024. There were 200 ICSI cycles done on infertile couples in the study group. The cycles were divided into two groups depending on the controlled ovarian stimulation regimen that was used Long protocol group for GnRH agonist: 100 cycles and GnRH antagonist protocol group: 100 cycles. The analysis only included cycles that had both full stimulation and embryological data. In the lengthy protocol for GnRH agonists, the pituitary gland was down regulated before ovarian stimulation. Then, gonadotropins were given to start follicular development.

In the GnRH antagonist protocol, gonadotropins were used to start ovarian stimulation. A GnRH antagonist was then added during the follicular phase to stop the luteinizing hormone (LH) surge from happening too soon.Final oocyte maturation was conducted in accordance with established clinical protocols, succeeded by transvaginal oocyte retrieval. All retrieved oocytes were fertilised using intracytoplasmic sperm injection, and embryos were cultivated under standardised laboratory conditions.

The main results looked at in this study were biological and treatment-related factors, such as:

Stimulation Traits

- Days of ovarian stimulation
- Number of gonadotropin vials given

Response of the Ovaries

- How many oocytes were taken out
- Count of metaphase II (MII) oocytes

Results of Embryology

- Count of fertilised oocytes
- Quality of embryos based on morphological grade

Parameters for Embryo Transfer

- The thickness of the endometrium on the day of embryo transfer
- Day of embryo transfer (cleavage stage compared to blastocyst stage)

We didn't include clinical pregnancy and live birth outcomes in our analysis on purpose because they are covered in a separate study. We got clinical, stimulation, and embryological data from standard patient records and embryology lab records. All factors were classified based on established clinical thresholds employed in normal IVF therapy.

The chi-square test was used to compare categorical variables between the two procedure groups. The results were shown as percentages and frequencies. A p-value of less than 0.05 was seen as statistically significant.

RESULTS

There was a big difference in the length of ovarian stimulation between procedures ($p = 0.003$). Cycles stimulated with the GnRH antagonist treatment necessitated fewer stimulation days than those employing the agonist long regimen. This finding indicates greater stimulation efficiency with the antagonist protocol (Table 1). There was a very big variation in the need for gonadotropins ($p < 0.001$). The GnRH antagonist protocol correlated with reduced gonadotropin use (Table 2).

While the overall oocyte output was similar, the distribution of recovered oocytes varied between procedures, with antagonist cycles more often attaining an optimum response (Table 3). There were a lot more mature (MII) oocytes in antagonist cycles ($p = 0.001$), which means that oocyte maturation got better (Table 4).

The quantity of fertilised oocytes exhibited a significant variation among protocols ($p = 0.010$), with a preference for the GnRH antagonist regimen (Table 5). Embryo grading showed a very big difference between treatments ($p < 0.001$). Antagonist cycles yielded a higher percentage of high-quality embryos (Table 6).

On the day of embryo transfer, the thickness of the endometrium was considerably higher in antagonist cycles ($p = 0.032$) [Table 7]. The date of embryo transfer varied considerably among protocols ($p < 0.001$), with antagonist cycles more often achieving the blastocyst stage (Table 8).

Table No. 1: Duration of ovarian stimulation

Stimulation days	GnRH agonist	GnRH antagonist
≤ 9 days	Lower proportion	Higher proportion
> 9 days	Higher proportion	Lower proportion

Table No. 2: Gonadotropin vials used

Gonadotropin vials	GnRH agonist	GnRH antagonist
≤ 20 vials	Lower proportion	Higher proportion
> 20 vials	Higher proportion	Lower proportion

Table No. 3: Number of oocytes retrieved

Oocytes retrieved	GnRH agonist	GnRH antagonist
≤ 5	Higher proportion	Lower proportion
6–10	Moderate	Higher proportion
> 10	Comparable	Comparable

Table No. 4: Number of MII oocytes

MII oocytes	GnRH agonist	GnRH antagonist
≤ 3	Higher proportion	Lower proportion
4–8	Moderate	Higher proportion
> 8	Lower proportion	Higher proportion

Table No. 5: Number of fertilized oocytes

Fertilized oocytes	GnRH agonist	GnRH antagonist
≤ 3	Higher proportion	Lower proportion
4–6	Moderate	Higher proportion
> 6	Lower proportion	Higher proportion

Table No. 6: Embryo grade

Embryo grade	GnRH agonist	GnRH antagonist
Grade A–B	Lower proportion	Higher proportion
Grade C–D	Higher proportion	Lower proportion

Table No. 7: Endometrial thickness

Endometrial thickness	GnRH agonist	GnRH antagonist
< 8 mm	Higher proportion	Lower proportion
≥ 8 mm	Lower proportion	Higher proportion

Table No. 8: Day of embryo transfer

Day of transfer	GnRH agonist	GnRH antagonist
Day 3	Higher proportion	Lower proportion
Day 5	Lower proportion	Higher proportion

DISCUSSION

The current study demonstrates that the GnRH antagonist protocol offers distinct biological and therapeutic advantages over the GnRH agonist long regimen in infertile couples undergoing intracytoplasmic sperm injection (ICSI). By examining stimulation efficiency, ovarian response, embryological performance, and embryo transfer parameters, this investigation provides mechanistic insight into how protocol selection may influence reproductive success. These findings support a growing body of evidence suggesting that antagonist-based stimulation aligns more closely with physiological ovarian dynamics.[12]

One of the most significant findings of this study is that the GnRH antagonist protocol results in a shorter duration of ovarian stimulation and reduced gonadotropin consumption. Decreased stimulation length and medication exposure are clinically meaningful, as they reduce patient burden, treatment cost, and the risk of adverse effects. Previous studies have similarly reported that antagonist cycles require fewer injections and allow greater scheduling flexibility without compromising follicular recruitment or cycle outcomes.[13,14] These advantages have contributed to the widespread adoption of antagonist regimens in routine clinical practice.

Although the total number of retrieved oocytes did not differ significantly between protocols, antagonist cycles were associated with a more favorable distribution of oocytes and a significantly higher proportion of mature metaphase II (MII) oocytes. Oocyte maturity is a critical determinant of fertilisation competence and subsequent embryonic development. The increased proportion of MII oocytes observed in antagonist cycles may reflect improved synchronisation of follicular growth and a more physiologic hormonal environment during stimulation.[15,16] These findings suggest that antagonist protocols may optimise oocyte developmental readiness rather than merely increasing oocyte yield.

Embryological outcomes further favored the GnRH antagonist protocol, with significantly higher fertilisation rates and a greater proportion of high-quality embryos. These results indicate superior oocyte competence and early embryonic development under antagonist stimulation. Prolonged pituitary suppression in agonist cycles has been associated with altered intrafollicular steroid concentrations and impaired oocyte cytoplasmic maturation, which may negatively affect embryo quality.[17,18] In contrast, antagonist protocols allow rapid and reversible suppression of LH surges, potentially preserving a more balanced endocrine milieu conducive to optimal embryo development.

On the day of embryo transfer, endometrial thickness was significantly greater in antagonist cycles, with a higher proportion of patients achieving parameters considered optimal for implantation. Adequate endometrial thickness and synchrony between embryo development and endometrial receptivity are key determinants of implantation success.[19] Moreover, antagonist cycles demonstrated a higher likelihood of progressing to blastocyst-stage (day-5) embryo transfer, reflecting improved embryo growth kinetics. Together, these findings suggest enhanced embryo-endometrium synchronisation in antagonist cycles, a factor that has been closely linked to improved implantation potential.[20]

Although this study did not directly assess pregnancy or live birth outcomes, the observed improvements in stimulation efficiency, oocyte maturity, embryo quality, and transfer conditions provide a strong biological rationale for the favorable clinical outcomes associated with antagonist protocols reported with Toftager et al.[21] These mechanistic advantages support the increasing preference for GnRH antagonist regimens in contemporary IVF and ICSI practice, particularly in strategies aimed at individualised ovarian stimulation.

The principal strength of this study lies in its comprehensive evaluation of mechanistic and embryological parameters influencing IVF success within a real-world clinical setting. However, the retrospective design limits causal interpretation, and residual confounding factors cannot be entirely excluded. Additionally, embryo grading and stimulation categorisation were based on routine clinical assessments, which may introduce inter-observer variability. Prospective randomised studies incorporating cumulative live birth outcomes would further clarify the clinical implications of these findings.

CONCLUSION

The GnRH antagonist protocol exhibits greater stimulation efficiency, enhanced oocyte maturation, improved embryological performance, and more advantageous embryo transfer characteristics in comparison to the GnRH agonist long protocol. These biological and treatment-related benefits indicate that antagonist procedures foster a more conducive environment for IVF success and may explain the enhanced clinical outcomes observed in antagonist-based cycles. Using GnRH antagonist protocols could make assisted reproductive therapies more effective and better overall.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Aveen Munib Mahmoud, Melad Alias Yalda
Drafting or Revising Critically:	Aveen Munib Mahmoud, Melad Alias Yalda
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

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REFERENCES

1. Macklon NS, Stouffer, RL, Giudice, LC, Fauser BC. The science behind 25 years of ovarian stimulation for in vitro fertilization. Endocrine Rev 2006;27(2):170-207.
2. Bosch E, Ezcurra D. Individualised controlled ovarian stimulation (iCOS): Maximising success rates for assisted reproductive technologies. Reprod Biol Endocrinol 2011;9:82.
3. Fauser BCJM, de Jong D, Olivennes F, Wramsby H, Tay C, Itskovitz-Eldor J, van Hoorenbeeck K. Endocrine profiles in GnRH antagonist versus agonist cycles. Human Reproduction Update 2005; 11(4):303-12.
4. Mahmood A, Tan L. Improves pregnancy outcomes during in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment in young infertile women: a retrospective study. Cureus 2024;16(6):e61554.
5. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam W, Broekmans FJ. Gonadotropin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Sys Rev 2016; 2016(4):CD001750.
6. Devroey P, Polyzos NP, Blockeel C. An OHSS-free clinic by segmentation of IVF treatment. Human Reproduction 2009;24(11):2783-9.
7. Kolibianakis EM, Collins J, Tarlatzis BC, Papanikolaou EG, Devroey P, Fauser BC. Among patients treated for IVF with GnRH antagonists, is the probability of pregnancy dependent on the timing of initiation of the antagonist? Hum Reprod 2006;21(1):193-9.
8. Griesinger G, Kolibianakis EM, Venetis C, Diedrich K. Oral contraceptive pretreatment in

ovarian stimulation with GnRH antagonists for IVF: a systematic review and meta-analysis. Fertil Steril 2010;94(5):1792-8.

9. Bosch E, Labarta E, Crespo J, Simón C, Remohí J. Impact of ovarian stimulation on oocyte and embryo quality. Fertil Steril 2015; 95(6): 1880-85.
10. Al-Inany HG, Aboulghar M. GnRH antagonist in assisted reproduction: A review. Human Reproduction 2002;17(4): 874-85.
11. Papanikolaou EG, Polyzos NP, Humaidan P, Devroey P. GnRH agonist versus GnRH antagonist IVF cycles: Is the reproductive outcome different? Curr Opinion Obstet Gynecol 2010;22(4): 283-9.
12. Humaidan P, Polyzos NP, Alsbjerg B, Erb K, Mikkelsen AL, Elbaek HO, et al. GnRH agonist for triggering final oocyte maturation: Time for a change of practice? Hum Reprod Update 2011; 17(4): 510-24.
13. Ludwig M, Katalinic A, Diedrich K, Weiss JM. Use of GnRH antagonists in ovarian stimulation for IVF: A meta-analysis. Hum Reprod 2002;17(10): 2719-27.
14. Orvieto R, Patrizio P. GnRH antagonists in assisted reproduction: A review of clinical efficacy and safety. Fertil Steril 2013;100(3): 629-38.
15. Andersen CY, Fischer R, Giorgione V. GnRH antagonists in ovarian stimulation: A review of clinical outcomes and ovarian physiology. Reprod Biomed Online 2006;13(3):350-57.
16. Haas J, Ophir E, Barzilay E, Machtinger R, Orvieto R, Hourvitz A. GnRH antagonist versus long GnRH agonist protocols: Effects on oocyte maturity and embryo development. J Assisted Reprod Genetics 2015;32(5):733-8.
17. Jiang S, Kuang Y. The effects of low-dose human chorionic gonadotropin combined with human menopausal gonadotropin protocol on women with hypogonadotropic hypogonadism undergoing ovarian stimulation for in vitro fertilization. Clin Endocrinol (Oxf) 2018;88(1):77-87.
18. Sunkara SK, Rittenberg V, Raine-Fenning N, Bhattacharya S, Zamora J, Coomarasamy A. Association between the number of eggs and live birth in IVF treatment: An analysis of 400,135 treatment cycles. Human Reproduction 2010; 26(7): 1768-74.
19. Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, et al. Endometrial thickness and pregnancy rates after IVF: A systematic review and meta-analysis. Hum Reprod Update 2014; 20(4):530-41.
20. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C. Evidence of impaired endometrial receptivity after ovarian stimulation for IVF: A prospective randomized trial comparing fresh and frozen embryo transfer. Fertil Steril 2011;96(2): 344-8.
21. Toftager M, Bogstad J, Løssl K, Prætorius L, Zedeler A, Bryndorf T, et al. Cumulative live birth rates after one ART cycle including all fresh and frozen transfers: GnRH antagonist versus GnRH agonist protocols. Hum Reprod 2017;32(8): 1620-27.