Introduction

Infertility is considered as a devastating issue that can cause suffering to any couple, with a prevalence of 15% in the first year of marriage (1). The gonadotropin-releasing hormone (GnRH) agonist has been a major asset in the assisted reproductive technology since its development in the 1980s (2,3). In more than 2 decades, the GnRH agonist became the “gold standard” in ovarian stimulation and thus was used in the long protocol with the purpose of an initial increase in the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. Accordingly, these changes cause a reduction in the stimulation of ovary, the suppression of folliculogenesis, and a decline in circulatory estrogen to the menopausal level within three weeks by maintaining the continuous administration of the GnRH agonist leading to the down-regulation of GnRH receptors and consequently the lowering of LH and FSH (4-7). The prevention of the early LH surge leads to a reduction in the risk of ovarian hyperstimulation syndrome (8), which is related to the ability of the GnRH agonist to desensitize the pituitary gland (9).

According to some studies (10-12), the GnRH antagonist was first used in the late 1990s aiming at reaching more friendly in vitro fertilization (IVF). In addition, the GnRH antagonist offered several advantages such as reducing the total number of the applied gonadotropins, the risk of ovarian hyperstimulation syndrome, and the costs, as well as having low side effects and shorter treatment durations (6).

The binding of GnRH agonist to a receptor on pituitary causes the desensitization of pituitary and consequently the down-regulation of gonadotropin secretion (13,14). Further, the GnRH agonist initially has stimulatory effects, followed by subsequent reductions in gonadotropin levels (14). On the other hand, the GnRH antagonist produces the immediate inhibition of gonadotropin secretions thus it can be given after the initiation of gonadotropins in IVF protocols (15).

This study compared two different protocols of controlled ovarian hyperstimulation (COH) in order to determine which protocol leads to better outcomes in terms of pregnancy rate and identify the predictors of the higher rate of pregnancy. Finally, the study sought to examine the quality of oocytes (i.e., number, fertilization, and the like), as well as the embryo retrieval rate and quality between these two protocols.

Abstract

Objectives: The comparison of gonadotropin-releasing hormone (GnRH) agonist against GnRH antagonists regarding the pregnancy rate and effect of various predictors on pregnancy outcomes.

Materials and Methods: This prospective comparative study involved 189 women who underwent intracytoplasmic sperm injection (ICSI) cycles and were divided into agonist (107 patients) and antagonist arm (82 patients) groups. The chemical and clinical pregnancy rate was the main outcome and the other outcomes included the number and quality of oocyte measurement and pregnancy outcomes.

Results: Based on the results, the agonist protocol showed a higher rate of pregnancy (32.7%, 95% CI: 23.9-42.4%) compared to the antagonist protocol (22.0%, 95% confidence interval (CI): 13.6-32.5%) with the odds ratio (OR) of (95% CI) = 1.73 (0.89-3.35). The results further revealed that the count of retrieved oocytes, count of M2 oocytes, count of fertilized oocytes, count of embryos, and the fertilization percent out of total retrieved oocytes were higher in the agonist arm compared to the antagonist arm.

In the multivariate analysis after adjusting for the confounders, the agonist protocol had higher odds of a successful pregnancy compared to the antagonist protocol by 57% (partial OR = 1.57, P value = 0.23).

Conclusions: In general, the agonist protocol offers a favourable outcome in comparison to the antagonist arm, and there seems to be an intrinsic benefit for the agonist protocol, which is not explained by the higher number of transferred embryos.

Keywords: GnRH agonist, GnRH antagonist, Pregnancy rate, In vitro fertilization, ICSI
Materials and Methods

Study Design
This prospective comparative study including 226 IVF cycles was conducted at the Reproductive Medical Center of Fertility and IVF cycle in Kamal AL-Samaria Hospital (Baghdad/Iraq) from November 2014 to November 2015. In this study, 122 women were given the agonist protocol while 104 others received the antagonist protocol. Of those women, 15 and 22 cases discontinued the program from each group, respectively (Figure 1).

Inclusion Criteria
Women within the age range of 18-45 years undergoing their 1st or 2nd cycle of IVF regardless of their indication for infertility were included in this study.

Sample Size Calculation
The sample size was computed 91 women in the two arm groups based on a previous study by Orvieto et al (16) in which they reported 41.2% and 25.3% pregnancy rates in agonist and antagonist arms, respectively, as well as the types I and II error of 10% and 20%.

GnRH Agonist Protocol of COH
Decapeptyl 0.1 mg (Ferring, Kiel Germany), given subcutaneous on day 21 of the previous cycle, was given daily for 14 days.

GnRH Antagonist Protocol of COH
FSH (Gonal F Merck Serono or Puregon MSD) was given on the 2nd day with the dominant follicle size ≤14 mm and the GnRH antagonist (Cetrotide, Asta Medica AG, Frankfurt, Germany) was administered daily.

Criteria of Human Chorionic Gonadotropin Admiration
Two ampules of Ovitrelle 250 μg/0.5 mL (Merck Serono S.p.A, Italy) were given in the presence of ≥3 follicles (≥18 mm in diameter), and continuous elevations in the serum estradiol concentration.

Oocyte Aspiration
It was performed 34 to 36 hours after human chorionic gonadotropin injection guided by vaginal ultrasound. Moreover, intracytoplasmic sperm injection (ICSI) was performed using standard procedures as described by Rubino et al (17), with embryo transfer proceeded 2 or 3 days later. The maturation and morphological features of oocyte were investigated immediately before ICSI. Then, the fertilization rate, early embryo development, and transfer were documented and studied as well.

Statistical Analysis
All analyses were carried out using IBM SPSS 21.0 (Chicago, IL). An independent t test was used to compare between two continues variables Then, partial regression analysis was used to calculate the odds ratio and its 95% confidence interval.

Results
The incidence of chemical pregnancy was higher in women who received the agonist protocol with an odds ratio of 1.73 although it did not reach statistical significance (Table 1).

As shown in Table 5, using the agonist protocol increased the odds of having a successful chemical pregnancy by 57% after adjusting for confounding the effect of the M2 count, age, and transferred embryos compared to those using the antagonist protocol. Nonetheless, this estimate failed to reach a statistically significant level. There seems to be an intrinsic added benefit for the agonist protocol, which is not explained by a higher number of M2 oocytes or the higher number of transferred embryos.

Key messages
- The agonist protocol showed higher pregnancy rates compared to antagonist protocol.
- The agonist protocol showed higher Embryonic outcomes.
- The agonist protocol showed higher successful pregnancy rates compared to antagonist protocol.

Table 1. Incidence Rate of Chemical Pregnancy by the Type of the Ovulation Induction Protocol Among Subjects Who Completed the Treatment Schedule

| Protocol used       | Total (N) | Chemical Pregnancy (Pregnancy Test) | 95% CI          |
|---------------------|-----------|------------------------------------|-----------------|
|                     | N         | No. | %                      |                |
| Agonist protocol    | 107       | 35  | 32.7 (23.9% to 42.4%)   |
| Antagonist protocol | 82        | 18  | 22.0 (13.6% to 32.5%)   |
| Total               | 189       | 53  | 28.0 (21.7% to 35.0%)   |

Note. P (Chi-square) = 0.10 [NS]; NS: Not significant; The odds ratio for chemical pregnancy in the agonist group compared to the antagonist group = 1.73 (95% CI = 0.89-3.35).
There was a significant direct correlation between the counts of the retrieved oocytes and serum FSH in the agonist protocol, but it was not statistically significant in the antagonist protocol. Moreover, a significant direct correlation was observed between the age and serum FSH in the antagonist protocol although it was not statistically significant in the agonist protocol (Table 6).

**Discussion**

In the normal menstrual cycle, GnRH stimulates the production of both LH and FSH from the pituitary gland. More precisely, both LH and FSH regulate follicle development and the subsequent production of the dominant follicle while ovulation is induced through the mid-cycle LH surge. In addition, the administration of GnRH stimulates LH and FSH above the critical levels required for the stimulation of multiple follicles which will be subsequently harvested for the IVF cycle. Further, the administration of GnRH agonist and antagonist prevents premature LH surge (by their effects on the pituitary gland that affects the naturally produced LH and FSH). The final step in the process is achieved using human chorionic
gonadotropin bolus (GnRH agonist) and subsequent final oocyte maturation (18).

In the present study, the agonist arm showed a higher success of IVF outcomes with a 1.57 fold higher odds of achieving successful pregnancy compared to the antagonist arm. However, it did not reach the statistical significance, which is in agreement with the findings of other clinical studies like Orvieto et al (16) favoring the use of the GnRH agonist protocol. Similarly, the findings are in line with those of the meta-analysis by Kolibianakis et al (19) in which GnRH agonist had higher odds of achieving live birth although it did not reach statistical significance (OR: 0.86, 95% CI: 0.72 to 1.02, antagonist vs. agonist). In a more recent meta-analysis (20), the researchers examined 50 studies and found that the pregnancy rate in the GnRH antagonist arm had significantly lower odds compared to the GnRH agonist (relative risk: 0.89, 95% CI: 0.82-0.96), which corroborates with our findings.

Several possible explanations are available for these findings. According to Kolibianakis et al (21), the LH surge is not sufficiently suppressed by the GnRH antagonist. In one study, the LH surge occurred in 8% of antagonist arms compared to <1% in the agonist arm, and 80% of the surge in the antagonist arm occurred before the beginning of the antagonist therapy. Furthermore, low oocyte retrieval rates were obtained from the antagonist arm, which results in a lower pregnancy rate (22). This can be explained by the low suppression of endogenous FSH by the GnRH antagonist leading to asynchronous follicular development which is contrary to the appropriate suppression of FSH offered by the GnRH agonist resulting in better follicular development (23).

Conclusions
In general, the agonist protocol suggests better pregnancy outcomes compared to the antagonist protocol and the agonist protocol has an intrinsic benefit, which is not described by the higher number of transferred embryos.

Authors' Contribution
All author equally contributed to this work.

Conflict of Interests
Authors declare that they have no conflict of interests.

Ethical Issues
The Ethics Committee of Kamal AL-Samaria Hospital approved the study (ethical code: D201623). The purposes and procedures were explained to all participants and were given the right to participate or discontinue the study. Furthermore, written informed consent was taken, and patients were assured of data confidentiality and the lack of using data for other research objects according to the Declaration of Helsinki (as revised in Edinburgh, 2000).

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Table 5. Multiple Logistic Regression Model With the Odds of Successful Chemical Pregnancy as the Dependent (Outcome) Variable by the Type of the Protocol Used After Adjusting for Age and the Selected Count Predictor

| Variables | Partial OR | P value |
|-----------|------------|---------|
| Agonist protocol compared to antagonist | 1.57 | 0.23 [NS] |
| Count of M2 oocytes | 1.05 | 0.3 [NS] |
| Age | 1.03 | 0.36 [NS] |
| Count of transferred embryos | 1.88 | <0.001 |

Note. Overall predictive accuracy = 72.5%; P value (model) <0.001; NS: Non-significant; OR: odds ratio.

Table 6. The Relationship Between Serum FSH With Age, the Count of Doses, and the Count of Retrieved Oocytes

| Variables | Serum FSH |
|-----------|-----------|
| | Agonist Protocol | Antagonist Protocol |
| r | P Value | r | P Value |
| Count of doses | 0.048 | 0.71 [NS] | 0.13 | 0.41 [NS] |
| Count of retrieved oocytes | 0.258 | 0.047 | -0.29 | 0.06 [NS] |
| Age (years) | 0.219 | 0.09 [NS] | 0.474 | 0.002 |

Note. r: Correlation coefficient; FSH: follicle-stimulating hormone; NS: Non-significant.
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