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Impact of single vs. multiple GnRH-agonist doses for luteal phase support in antagonist ICSI cycles: a three-arm randomized controlled trial

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Abstract. *Background:* All stimulated ART cycles had inadequate luteal phase (LP), according to many studies. Current LPS regimens utilizing progesterone alone may not always be enough to promote implantation. The novel use of gonadotropin-releasing hormone agonists (GnRH-a) is increasing, aiming to enhance reproductive outcomes. Most studies evaluating the efficacy of GnRH-a for LPS used the long GnRH-a protocol with conflicting results. This study was conducted to assess whether administration of single or multiple doses of GnRH-agonists in the luteal phase improves clinical outcomes in intracytoplasmic sperm injection cycles (ICSI) using the GnRH antagonist protocol. *Methods:* In a single-centre, parallel, open-label, three-arm randomized controlled trial, 75 infertile women undergoing ICSI cycles were randomized into three groups according to luteal phase support (LPS) regimens. The study involved adding a single subcutaneous injection of 0.1 mg triptorelin on the 6th day after oocyte retrieval (group I; n = 25), multiple doses of triptorelin on the 5th, 7^{th,} and 9th days after oocyte retrieval (group II; n = 25), or a control group with no added triptorelin (group III; n = 25) to routine LPS with progesterone. *Results:* No statistical differences were detected between the groups regarding clinical pregnancy, implantation, multiple pregnancy rates or β-hCG concentration. The clinical pregnancy rate (CPR) was slightly higher in the multiple-dose group (72%), vs. 60% and 52% for the single-dose group and control group, respectively (p = 0.334). *Conclusions*: Compared to progesterone alone, the addition of mid-luteal GnRH agonists did not significantly improve implantation or CPR. However, the multiple-dose addition showed promisingly higher CPR, warranting further research.

Keywords: Gonadotropin-releasing hormone agonists, luteal phase support, ICSI; pregnancy outcome.

Introduction

Several lines of evidence indicate that the luteal phase (LP) in all stimulated ART cycles is inadequate.(1) To address this issue, additional luteal phase support (LPS) has been consistently used to improve pregnancy outcomes by inducing progesterone release with exogenous human chorionic gonadotropin (hCG) or directly supplementing with progesterone.(2) Currently, progesterone is the

standard and safe method for LPS in hCG-triggered ART cycles. However, the pregnancy outcomes with current LPS regimens using progesterone alone are still not optimal for some women.[3]

The novel use of gonadotropin-releasing hormone agonists (GnRH-a) is increasing, aiming to enhance reproductive outcomes. In 1993, Balasch et al. reported that the inadvertent use of mid-luteal GnRH-a improved implantation.[4] Subsequent studies revealed significant improvements in clinical and ongoing pregnancies, as well as live births.[1] These findings suggest that relying solely on progesterone for LPS may not always be enough to promote implantation. Therefore, alternative methods can be explored.[5]

As a true agonist, GnRH-a first increases gonadotropin production; subsequently, receptors are down-regulated. When injected during the mid-luteal phase, an initial flare-up of gonadotropins results, with the high LH levels contributing to enhanced progesterone production from the corpus luteum (CL) and stronger LPS.[6] There is a suggestion that GnRH-a directly affects the endometrium or embryo.[7]

Various studies have investigated the potential of GnRH-a for LPS, but a limited number of studies have compared single and multiple doses of GnRH-a in the mid-luteal phase; therefore, the most effective administration of GnRH-a has yet to be discovered.[8] Most studies evaluating the efficacy of multiple doses of GnRH-a for LPS used the long GnRH-a protocol, while fewer used the GnRH-antagonist (GnRH-ant) protocol.[5, 8, 9] This study was conducted to evaluate the impact of single and multiple mid-luteal GnRH-a on clinical outcomes in ICSI cycles using the antagonist protocol.

Materials and methods

Study design and participants

This was a single-centre, parallel, open-label, three-arm randomized controlled trial (RCT) in a private IVF institution in Alexandria, Egypt. The study evaluated the impact of adding a mid-luteal single-dose or multiple-dose GnRH-a to standard LPS in GnRH-ant-treated ICSI cycles. We used CONSORT reporting guidelines.[10]

A total of 75 infertile women participated. The study protocol was completely explained to all participants, and written informed consent was obtained. Infertile women aged ≤ 38, BMI ≤ 30, basal FSH ≤ 10 IU/L, and AMH ≤ 5 ng/ml were eligible. Endometriosis, polycystic ovarian syndrome, uterine pathology or anomaly, and medical comorbidities were excluded.

Randomization

Patients were randomly assigned in a 1:1:1 allocation ratio, according to a computer-generated randomization list, into three LPS groups: Group I: received single

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mid-luteal GnRH-a; Group II: received multiple mid-luteal GnRH-a; Group III: received only routine LPS. The randomization sequence was concealed using an opaque sealed envelope technique. Participants were about their allocated arm on the day of embryo transfer (ET) by the principal investigator. Figure 1 shows the study consort flow chart.

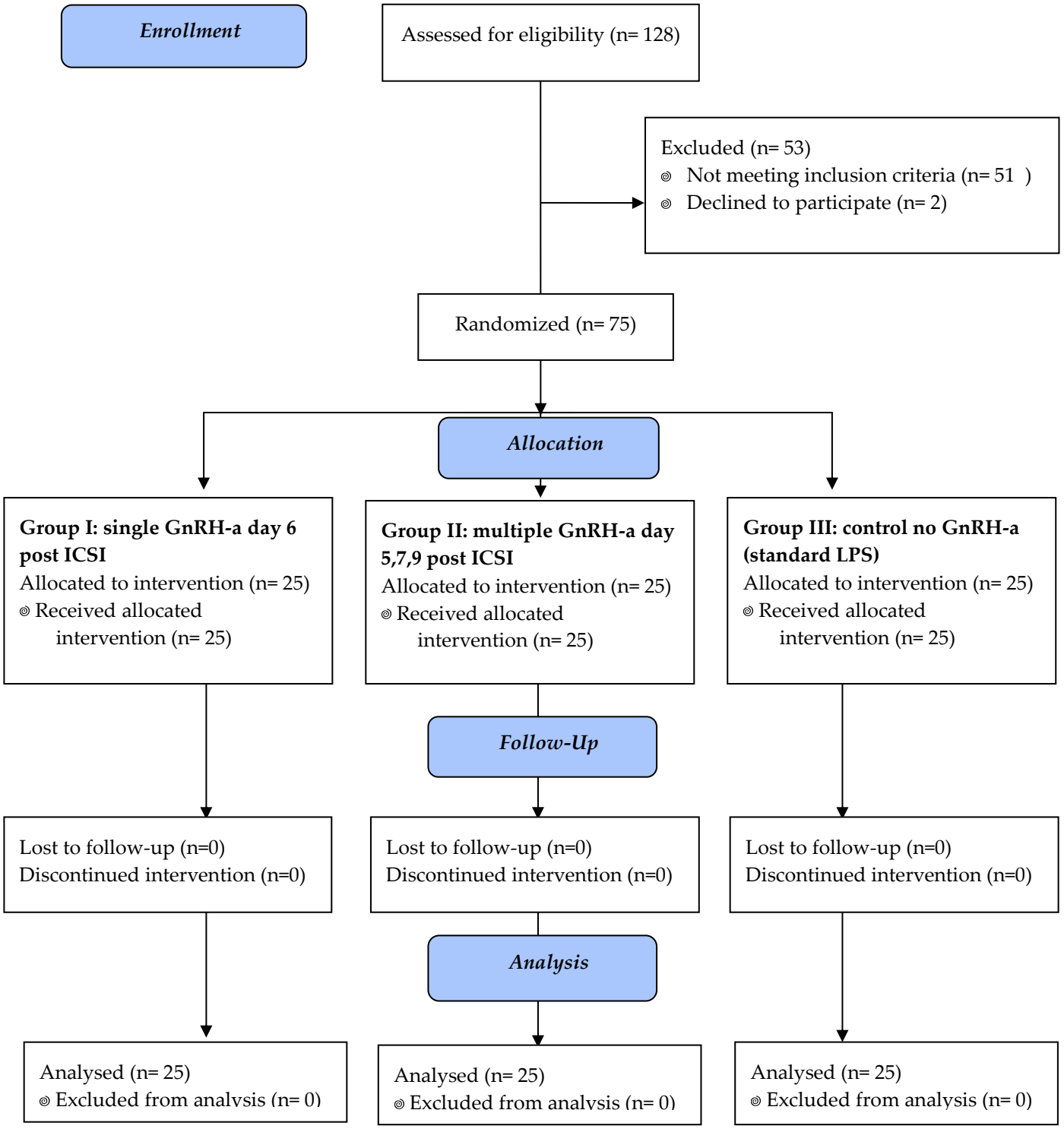

Figure 1. Study Consort Flow Diagram.

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Controlled ovarian stimulation (COS)

On day 2 of the menstrual cycle, ovarian stimulation was initiated with 300 IU gonadotropins, recombinant FSH (Gonal-f®; Serono, Switzerland), and human menopausal gonadotropin (HMG) for 5 days (Merional®; IBSA, Switzerland). Patients received a daily subcutaneous (SC) injection of GnRH-ant, cetrorelix acetate 0.25 mg (Cetrotide®; Geneva, Switzerland), starting on the sixth day of stimulation or when the leading follicle reached 14 mm, until the day of the ovulation trigger. Transvaginal ultrasound (TVS) and estradiol levels were used for cycle monitoring, and gonadotropin doses were adjusted based on individual responses. When at least three leading follicles reached 18 mm, follicular maturation was triggered with hCG 10,000 IU IM (Choriomon® 5000 IU; IBSA). Ovum pick-up (OPU) was scheduled 35–36 hours later (Figure 2).

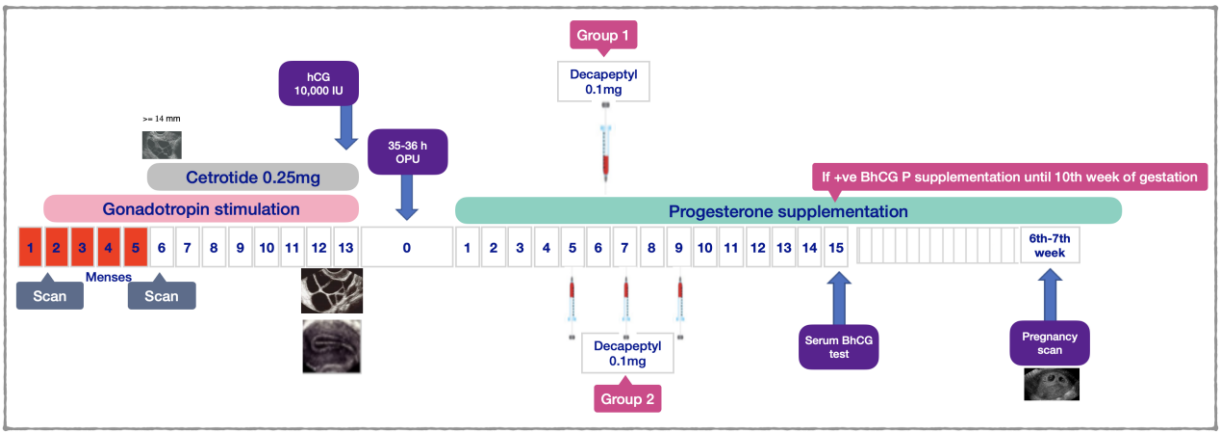

Figure 2. Diagrammatic Representation of the Study Controlled Ovarian Stimulation and Luteal Phase Support. On the second day of menses, patients receive ovarian stimulation with 300 IU gonadotropins. On the 6th day of stimulation, or when the leading follicle reached 14 mm, cetrotide 0.25 mg was initiated till the day of oocyte pickup (OPU). For all patients, luteal support started the day after OPU with progesterone and continued if pregnant until the 10th week of gestation. On the sixth day post-OPU, group 1 receives an additional single-dose decapeptyl 0.1 mg. Group 2 receives three additional doses of decapeptyl 0.1 mg on the 5th, 7th, and 9th post-OPU. Test for pregnancy on the day 15 post OPU and a vaginal scan for pregnancy on week 6-7 of gestation. hCG, human chorionic gonadotropin.

Assisted reproductive technique

One hour after retrieval, oocytes were denuded, and each mature oocyte (metaphase II; MII) was immediately injected by the partner's sperm. On day 3 (67–72 hours) following OPU, embryonic cleavage stage development was assessed and quality graded. Depending on the available embryos, a maximum of two of the best-quality day 3 or 5 embryos were transferred. Surplus embryos were vitrified.

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Luteal phase support

All patients received routine LPS in the form of 400 mg vaginal progesterone pessaries twice a day (Cyclogest®, Actavis UK, Ltd.) starting the day after OPU until the pregnancy test and, if pregnant, for 10 weeks. Participants in groups I and II received additional LPS with a mid-luteal SC injection of triptorelin acetate (Decapeptyl® 0.1 mg, Ferring, Germany) as follows: Group I (single dose, 25 patients) received one decapeptyl injection on the 6th day post-OPU; Group II (multiple doses, 25 patients) received decapeptyl injections on the 5th, 7th, and 9th days post-OPU; and Group III (control, 25 patients) received the routine LPS without GnRH-a. To match time, participants administered GnRH-a at 11:00 p.m.

Outcome measures

Pregnancy was diagnosed by positive serum β-hCG on day 15 after ET. Clinical pregnancy was defined by an intrauterine gestational sac with embryonic cardiac activity at 6-7 weeks of pregnancy. A multiple pregnancy is a gestation with more than one fetus.

The clinical pregnancy rate (CPR)—the number of clinical pregnancies divided by ET procedures—was the primary outcome measure. Secondary outcome measures included implantation rate (number of gestational sacs detected by TVS/number of transferred embryos), multiple pregnancy rate (percentage of pregnancies with more than one fetus), and serum β-hCG concentration (mIU/ml) on day 15 post-ICSI.

Statistical analysis

We determined the sample size using Power Analysis and Sample Size Software (PASS 2020). The calculation was based on the reference paper's parameters.8 In ICSI/ET cycles, the clinical pregnancy rate for the multiple-dose GnRH agonist group was 50%, compared to 22.5% for the single-dose GnRH agonist group.8 We needed at least 75 eligible female patients undergoing ICSI to test the hypothesis that there was a proportional difference in the effects of adding a single-dose or multiple-dose GnRH agonist to the routine luteal phase support. This was done with a 95% confidence level and 80% power using the Chi-square test.

The study used IBM SPSS software. The Shapiro-Wilk test verified distribution normality, while the chi-square test compared categorical variables. We used ANOVA for normally distributed quantitative variables, Kruskal-Wallis for abnormally distributed variables, and Dunn's multiple comparisons test for pairwise comparisons. Results were considered significant at the 5% level.

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Results

We included a total of 75 participants (25 per group); all of them received the allocated treatment, and none of them lost out on follow-up. Table 1 shows baseline and cycle characteristics for all participants. Demographic data showed no significant differences across groups I, II, and III, with the mean female age being 31.44 ± 6.16, 31.80 ± 5.26, and 30.56 ± 4.94, respectively (p = 0.714). Male factors implicated infertility in the majority of patients (56%, 68%, and 60% in groups I, II, and III, respectively). We identified no significant difference in infertility type or aetiology across study groups (p = 0.686 and MCp = 0.907, respectively). All groups had similar hormonal profiles. Mean AMH values were 2.06 ± 1.54, 2.41 ± 1.64, and 2.12 ± 1.69 ng/ml in groups I, II, and III, respectively, p = 0.671. Stimulation cycle characteristics were also comparable between all groups (Table 1). According to the embryo laboratory data, there were no significant differences between the three groups in the number of day 3 embryos (p = 0.766) or grade A embryos (p = 0.627). The majority received day 3 ET, only 16% of group III had day 5 ET, and 4% of groups I and II had day 5 ET (MCp = 0.721), as shown in Table 1.

Table 2 compares the clinical outcomes. The mean β-hCG for groups I, II, and III was 429.4 ± 246.9, 398.4 ± 189.0, and 355.9 ± 178.5 mIU/ml, respectively (p = 0.698); these levels were also comparable when assessed for singleton pregnancies. The interventional groups had a higher CPR than the control, with the multiple dosage group (group II) having a 72% CPR compared to the single dose group (group I) (60%), and the control group (group III) (52%), although it didn't reach statistical significance (p = 0.344). Implantation and twin pregnancies were similar across the study groups; all p values were greater than 0.05 (Table 2). The implantation rate was 51% in group II and 44% in groups I and III. In group II, the twin pregnancy rate was 28%, and in groups I and III, it was 24%.

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Table 1: Baseline characteristics, ovarian stimulation and laboratory parameters in patients randomized to receive triptorelin or control.

	Group I (Single-dose triptorelin)	Group II (Multiple-dose triptorelin)	Group III (Control)	*P*-value
Subjects (n)	25	25	25	
Age (y)	31.44 ± 6.16	31.80 ± 5.26	30.56 ± 4.94	0.075
Male age (y)	38.28 ± 7.63	37.48 ± 6.45	35.08 ± 4.54	0.185
Body mass index (kg/m2)	26.64 ± 2.65	26.30 ± 3.08	25.50 ± 3.32	0.400
Duration of infertility (y)	5.94 ± 4.95	5.0 ± 2.87	3.91 ± 2.58	0.348
Type of infertility				
Primary (%)	60.0 (15/25)	48.0 (12/25)	56.0 (14/25)	0.686
Secondary (%)	40.0 (10/25)	52.0 (13/25)	44.0 (11/25)	
Cause of infertility				
Male factor (%)	32.0 (8/25)	40.0 (10/25)	24.0 (6/25)	
Ovarian factor (%)	16.0 (4/25)	12.0 (3/25)	16.0 (4/25)	MCp= 0.907
Male and ovarian factors (%)	24.0 (6/25)	28.0 (7/25)	36.0 (9/25)	
Unexplained (%)	28.0 (7/25)	20.0 (5/25)	24.0 (6/25)	
AMH (ng/ml)	2.06 ± 1.54	2.41 ± 1.64	2.12 ± 1.69	0.671
TSH (mIU/L)	2.11 ± 1.07	2.14 ± 0.92	1.97 ± 0.97	0.799
PRL (ng/ml)	17.68 ± 5.80	16.53 ± 6.12	16.36 ± 6.47	0.711
Days of stimulation	10.48 ± 1.39	10.52 ± 1.33	9.96 ± 0.89	0.178
Days of cetrotide treatment	5.08 ± 1.19	5.36 ± 1.08	5.12 ± 0.88	0.560
Endometrial thickness (mm)	9.78 ± 1.16	9.78 ± 1.44	10.4 ± 0.99	0.109
E2 level on hCG day (pg/ml)	2322.9 ± 1463.7	2392.5 ± 1263.9	2377.3 ± 1509.5	0.967
P4 level on hCG day (ng/ml)	0.63 ± 0.30	0.78 ± 0.32	0.65 ± 0.35	0.255
No. of MII oocytes	10.04 ± 6.94	11.36 ± 6.76	9.56 ± 5.91	0.518
No. of day 3 embryos	7.68 ± 5.38	8.60 ± 4.85	7.68 ± 5.17	0.766
No. of grade A embryos on day	5.84 ± 4.41	6.72 ± 3.92	5.68 ± 3.90	0.627
No. of transferred embryos	2.0 ± 0.0	1.96 ± 0.20	2.0 ± 0.0	0.373
Day of embryo transfer				
Day 3 (%)	92.0 (23/25)	92.0 (23/25)	84.0 (21/25)	MCp=0.721
Day 5 (%)	8.0 (2/25)	8.0 (2/25)	16.0(4/25)	

Note: AMH, anti mullerian hormone; **TSH**, thyroid stimulating hormone; **PRL**, prolactin; **E2**, estradiol; **P4**, progesterone; **MII**, metaphase two; **MC**, Monte Carlo test.

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Table 2. Clinical outcomes in patients randomized to receive triptorelin or control.

	Group I (Single-dose triptorelin)	Group II (Multiple-dose triptorelin)	Group III (Control)	*P*-value
Clinical pregnancy (%)	60.0 (15 / 25)	72.0 (18 / 25)	52.0 (13 / 25)	0.344
β-hCG concentration (mIU/L) all pregnancies	429.4 ± 246.9	398.4 ± 189.0	355.9 ± 178.5	0.698
β-hCG concentration (mIU/L) singleton pregnancies	405.8 ± 171.5	386.6 ± 179.4	293.9 ± 152.4	0.413
Implantation rate (%)	44.0 (22/50)	51.0 (25/49)	44.0 (22/50)	0.722
Multiple (twin) pregnancies	24.0 (6 / 25)	28.0 (7 / 25)	24.0 (6 / 25)	0.932

Discussion

Although the relationship between GnRH-a as LPS and pregnancy is still an enigma,[11, 12] administering mid-luteal single or multiple boluses of GnRH-a in several conventional IVF protocols has gained popularity in recent years. The reported benefit of a single mid-luteal GnRH-a bolus suggests that multiple boluses may improve its effects.[5, 8, 13]

This three-arm RCT compared LPS with progesterone alone to two GnRH-a addition methods: single mid-luteal (day 6) and multiple mid-luteal doses (days 5, 7, and 9) in women undergoing GnRH-ant ICSI-ET. Both interventional groups demonstrated non-statistically significant higher CPR than the control group after adding subcutaneous mid-luteal GnRH-a for LPS. β-hCG concentrations, implantation rates, and twin pregnancies were similar in all groups.

Similar to our work, Eftekhar et al. observed that the addition of multiple mid-luteal doses of GnRH-a (0.1 mg triptorelin on days 0, 3, and 6 after ET) did not improve embryo implantation or pregnancy in antagonist ICSI cycles compared to standard LPS with progesterone alone.[9] Yıldız et al. found that adding leuprolide acetate 1 mg as a single-dose (day 6 post-OPU) or double-dose (on days 6 and 9 post-OPU) resulted in non-statistically significant improvement in implantation and clinical pregnancy compared to the control group, which agrees with our results. However, contrary to our findings, leuprolide addition increased the multiple pregnancy rate compared to control. This study was also a three-arm RCT, but in women undergoing COS with the

long GnRH-a protocol. All women received vaginal progesterone (600 mg/day) and estradiol (4 mg) from the day of OPU.[14] In line with this, in the RCT by Inamdar and Majumdar, the addition of multiple mid-luteal doses of leuprolide 1 mg SC injections (on the 6th, 7th, and 8th days after OPU) for LPS using the long GnRH-a protocol resulted in similar implantation, CPR, and multiple pregnancy rates compared to progesterone-only LPS.[15]

In contrast to our findings, Fusi et al. demonstrated that triptorelin addition in LP, as a single bolus or as five injections, increased CPR and delivery rate compared to LPS with 600 mg of vaginal progesterone alone. There were no differences in the outcome between a single injection or multiple boluses.[5] Indeed, the large sample size of 1344 patients powers this RCT. This study differs from ours in the dose and frequency of GnRH-a addition, they had a higher dose in the single mid-luteal group (0.2 mg triptorelin) and a frequent and earlier start in the multiple dose group, five injections every other day starting from the ET day (3rd day post-OPU), vs. only three doses every other day starting from day 5 in our study.

It is unclear whether this earlier GnRH-a start, on day 3 post-OPU, would enhance endometrial receptivity rather than CL function. Evaluating progesterone levels after daily GnRH-a during LP showed that day 14 after ICSI had significantly higher progesterone levels than control, but day ET levels were similar in both groups. This suggests that the very early CL (first 3 days after OPU) are not yet responsive to GnRH-a's action. Another possibility is that increasing CL progesterone secretion requires prolonged exposure to GnRH-a.[16] Longer or more frequent exposure to GnRH super-agonists can quickly result in GnRH receptor (GnRHR) saturation and pituitary desensitization. Therefore, GnRH-a dosage and frequency are crucial considerations.[17]

Preliminary studies suggest that buserelin 500 mg once or twice during the LP may have luteolytic effects for contraception.[18] However, there is no definitive evidence of a contraceptive effect. Pirard et al. discovered that low doses of GnRH-a stimulate the CL after mild IUI stimulation. In antagonist ART cycles, the frequency and dose needed for LPS exceeded 100 mg for IUI. Infrequent buserelin (every 2 days or once a day) treatment was associated with shorter LP durations and lower progesterone levels.[17] Geber and Sampaio (2013) retrospective study assessed three periods of agonist action using a long-acting depot formulation, finding similar CPR regardless of the duration of GnRH-a's effects (≤ 6 days, 7–12 days, and > 12 days).[19]

Recently, Qu and Li compared multiple-dose and single-dose GnRH-a addition to LPS in the second IVF attempt of patients with luteal phase defect (LPD) following standard LPS. The study found that the multiple-dose group had significantly greater CPR and LBR, while the single-dose group had lower progesterone levels. In contrast to our study, the multiple-dose group received decapeptyl daily for 14 days.[8] The beneficial effect of daily luteal GnRH-a in patients with LPD was previously reported in a matched case-control study, supporting the idea that prolonged GnRH-a use in the LP may solve this problem. The study also found that those who used luteal GnRH-a had almost three times higher progesterone levels on the 14th day following OPU.[20]

Unlike the current trial, these studies employed a '14-day luteal GnRH-a regimen',[8, 20] whereas our trial's multiple-dose group only received 3 doses, and our population was not definitively diagnosed with LPD. These discrepancies may explain the contradictory results, as other studies suggest that certain subsets of patients, including those with thin endometrium (≤7 mm) or a history of ET failure, could benefit more from luteal GnRH-a treatment.[21, 22]

Regarding the administration of a single midluteal GnRH-a, Tesarik et al. (2004) first evaluated the idea that a single SC midluteal (day 6) triptorelin injection improved embryo implantation. They divided the young donor sibling oocytes between two recipients, randomly assigning one to the GnRH-a treatment group. GnRH-a addition also increased twin pregnancies.[23] Subsequently, when the same group studied ICSI-ET with women's own gametes, they found similar results.[24] Unlike the current trial, Tesarik et al. added an hCG injection on ET day and 4 mg of E2 valerate daily for LPS. Other studies also found that implantation and CPR significantly improved with the addition of a single injection of 0.1 mg triptorelin[25] or 0.5 mg leuprolide[26] six days after ICSI to routine LPS using both long-agonist [25] and antagonist protocols.[26] Nevertheless, similar to our study, no differences in serum β-hCG levels were detected between pregnant women in the study group and the control group.[25]

On the other hand, several studies concur with our results. Ata et al.[27] and Aghahosseini et al.[28] conducted double-blind RCTs, but they were unable to show any improvement in implantation, clinical pregnancy,[27, 28] or multiple pregnancies[27] when they administered a single 0.1 mg triptorelin on the 6th day following OPU, either in long GnRH-a[27] or GnRH-ant protocols.[28]

Similarly, Zafardoust et al. found a non-significant improvement in CPR with the addition of a single dose of 0.1 mg decapeptyl in LP in antagonist ICSI-ET cycles. In addition, no significant differences were found in β-hCG concentrations across groups. However, the group that received GnRH-a had a significant improvement in implantation rate.[22] It is important to mention that the demographics in this study are different from those of our patients. Specifically, the study included women who had experienced two or more failed IVF/ICSI-ET attempts. The potential benefits of GnRH-a LPS for this specific subgroup of IVF patients are currently uncertain.

The precise mechanism underlying the reported benefit of GnRH-a during LP remains unclear. There is a hypothesis that GnRH-a supports CL or stimulates endometrial GnRHRs. The presence of a functional LH receptor in the human uterus suggests that the consequences of endogenous LH release may differ from those of exogenous hCG. GnRH-a's direct action on the endometrium is based on the high expression of GnRH and GnRHRs in the human endometrium, and this exhibits a dynamic pattern with peaks during the LP.[7, 29]

The theory that GnRH-a directly affects the human embryo is supported by the finding of elevated levels of serum β-hCG after adding a single dose of mid-luteal GnRH-a.[24] Ata et al. doubt the direct embryonic effect of a subcutaneously injected GnRH-a on the mother because it must reach the endometrial cavity or be present in

maternal circulation during early uteroplacental circulation.[27]

Several meta-analyses found that single mid-luteal GnRH-a for LPS in fresh ART cycles enhances the implantation rate, CPR, and LBR. Ma et al. found no statistical difference in CPR between the multiple-dose protocol and the control protocol.[30] A recent pairwise and network meta-analysis confirmed that a multiple-dose regimen is the most effective and safe approach to increasing LBR and CPR. Nevertheless, this meta-analysis has various drawbacks.[12] Therefore, these results should be interpreted with caution.

The study's strengths include its randomized design and three-arm structure, allowing comparison of mid-luteal GnRH-a regimens to a control group. However, limitations include a broad group with different infertility diagnoses, a limited sample size, and the simultaneous comparison of three distinct groups, which made it difficult to achieve statistical significance. Future large multi-institutional RCTs are needed to investigate the impact of GnRH-a as LPS in various infertile groups, taking into account the diverse GnRH regimens, including daily luteal administration.

In conclusion, the current study found that adding 0.1 mg of triptorelin subcutaneously to routine LPS, either as a single injection or multiple injections during the mid-luteal phase, did not significantly increase the rates of implantation and clinical pregnancy compared to using LPS with progesterone alone. However, the clinically relevant increase in CPR in the interventional groups is promising and warrants further studies. Notably, prior research has exhibited inconsistencies in the timing, dosage, route, and duration of GnRH-a administration, as well as the ovarian stimulation protocol. To determine any potential beneficial effects of adjuvant GnRH-a on ICSI outcomes, a thorough investigation considering these different variables is necessary.

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No funding was received to perform the study.

Conflict of interest

None

Ethics Approval

The protocol for this study was approved by the Ethics Committee of Alexandria Faculty of Medicine (IRB approval number 0201599), and then it was registered on https://clinicaltrials.gov (No. NCT05286554). Recruitment ran from March 2022 to October 2023. Informed consent was obtained from all participants before data collection, following the Declaration of Helsinki.

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