Introduction
Nowadays, infertility has been brought up as one worldwide reproductive health problem (1). In the United States, estimation from a large national sample shows that 10%-15% of American women aged 15-44 years, who are non-surgically sterile, suffered from current infertility (2). In Iran, as a developing country, the overall infertility prevalence was estimated at 8% with a 95% confidence interval of 3.2-15.0 (3). In vitro fertilization (IVF) is one of the most commonly accepted treatments for unexplained infertility (4,5). According to some studies (6-8), gonadotropin-releasing hormone (GnRH) agonists have widely been used as an appendant for ovarian stimulation protocols revolving around assisted reproductive technologies (ARTs).
Several protocols have been introduced in the literature, including long, short, and ultra-short protocols (9). Triptorelin, along with buserelin has been introduced as an effective prevailing treatment in this setting. Several RCTs have compared different agonists (10-14). However, the appropriate dose finding for infertility treatment has remained a critical issue. Triptorelin is one of the most commonly used GnRH analogues in Iran. Janssens et al demonstrated that even 15 µg triptorelin can prevent premature luteinizing hormone (LH) surge (15). Although low doses at 15 µg were effective, the present study intended to evaluate the effect of every other day administration of higher doses at 100 µg. The effectiveness of every other day use of triptorelin 0.1 mg in ART cycles compared with other protocols is unclear enough and remains unproven. Therefore, the goal of the current randomized trial was to compare the effectiveness of every other day triptorelin 0.1 mg in comparison with two other protocols (i.e., daily buserelin and daily triptorelin 0.1 mg in ART

| Abstract |
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| **Objectives:** Different types of gonadotropin-releasing hormone (GnRH) agonist protocols are used in assisted reproductive technology (ART) cycles although the role of every other day GnRH agonist administration is not well understood. Thus, this study compared the effectiveness of different ways of the administration of GnRH agonists in the ovarian stimulation long protocol and their effects on in vitro fertilization (IVF) outcomes. **Materials and Methods:** In a randomized controlled trial (RCT), 138 patients were randomly assigned to 3 groups with 46 patients. In group A, patients were treated with daily buserelin 0.5 mg subcutaneously and those in group B were treated with triptorelin 0.1 mg daily, and finally, patients in group C received triptorelin 0.1 mg every other day (all under a long protocol). Eventually, controlled ovarian stimulation was performed with the follicle-stimulating hormone (FSH). **Results:** There was no significant difference in biochemical and clinical pregnancy, along with abortion and twin rates between the comparison groups. Meanwhile, the number of gonadotropin injections was significantly lower in group C ($P=0.033$). Moreover, the number of follicles and days of ovarian stimulation did not have a significant difference between study groups. Finally, the number of metaphase 2 oocytes and embryos was significantly higher in group A ($P=0.001$). **Conclusions:** In general, pregnancy and abortion rates did not significantly differ between the comparison groups and, the number of gonadotropin injections was significantly lower in the triptorelin 0.1 mg every other day group. Thus, our finding revealed that every other day use of triptorelin 0.1 mg, comparing its daily use or daily buserelin, might be cost-benefit. **Keywords:** In vitro fertilization, GnRH agonist, Buserelin, Triptorelin

Introduction
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cycles among patients who referred to Imam Khomeini Hospital Complex, Tehran, Iran). In case of acceptable effectiveness, every other day application of triptorelin would be easier and less costly which may propose such utilization as a logical one.

Materials and Methods
The study was a 3-arm RCT conducted on patients who referred to the infertility unit of Imam Khomeini Hospital Complex, Tehran, Iran (Figure 1). In general, 138 candidate patients were recruited for IVF in our study during 2014-2015. The inclusion criteria were written informed consent, indications for IVF or intracytoplasmic sperm injection (ICSI), and age range of 18-38 years. Concurrently, the exclusion criteria included age range of >38 or <18, follicle-stimulating hormone (FSH) >10, the existence of severe male factors such as men with azoospermia in need of testicular sperm extraction, severe endometriosis, IVF cycle canceling for any reason, IVF cycle information incompleteness, and patients’ lack of tendency in any stage of the study. The 3 study groups were randomly assigned according to the block randomization method. All candidates received a daily oral contraceptive pill (OCP, LD) from day 3 of the pre-stimulation cycle. Group A received buserelin acetate 0.5 mg (Superfact, Hoechst AG, Frankfurt, Germany). The injections were subcutaneously done on a daily schedule starting on day 21 in the menstrual cycle before stimulation. For patients in groups B and C, triptorelin (Decapeptyl, FERRING, Germany) 0.1 mg was started simultaneously. In group A, before starting the menstruation and after finishing the oral contraceptive pill (LD), buserelin was decreased to 0.2 mg daily. The reduction of buserelin dose was done according to the mini-dose long agonist protocol that was first described by Feldberg et al (16) and then followed by Olivennes et al (17) based on which the dose of GnRH agonist reduced to half after menstruation. Patients in groups B and C received daily triptorelin 0.1 mg (18) and every other day triptorelin. The triptorelin dosage was fixed without any change or reduction. For the IVF/ICSI cycle, the gonadotropin (Gonal-F, Serono, Switzerland) was started from day 2-3. The responsible gynecologist determined the dose of gonadotropin using clinical and laboratory data including age, the basal FSH level, and antral follicle count. During the stimulation cycle, patients underwent daily transvaginal sonography from day 5. When at least two 18 mm follicles were detected, the subcutaneous injections of human chorionic gonadotropin (HCG, 10000 IU, Choriomon, IBSA, Suisse) were done, and oocyte pickup was done 34-36 hours after HCG administration from the posterior vaginal fornix. Follicular fluid was transferred to the embryology laboratory. Oocyte complex and cumulus cells were separated and incubated for 2 hours in an incubator at 37°C with 6% CO₂. After oocyte denuding with the hyaluronidase quality of oocytes (germinal vesicle, metaphase I, and metaphase II) was examined under an inverted microscope. Then, the embryos were transferred within 2 days of oocyte pickup. The data collection tool was a checklist containing information about the study variables. Certain information was collected from medical records. The other data such as ovarian stimulation and ICSI outcomes were gathered with study progress. The amount of gonadotropin injection, duration of ovarian stimulation, number and quality of retrieved oocytes

Key Messages

- Every other day use of triptorelin in long GnRH agonist protocol in ART cycles, was accompanied with similar pregnancy and abortion rates compared with daily buserelin or triptorelin along with lower number of gonadotropin injections.

Figure 1. Algorithm for Patients and Outcomes. Note: Group A: daily buserelin; Group B: Daily triptorelin; Group C: Every other day triptorelin.
and embryos, along with the rate of clinical and chemical pregnancies, abortion, and twin pregnancies were registered in the data collection checklist.

Statistical Analysis
Kolmogorov-Smirnov was used to test the normal distribution of variables. Parametric or non-parametric tests such as t test, analysis of variance, Mann-Whitney, and Kruskal-Wallis were used to determine the potential association between the predictors and outcome variables in bivariate analysis. In addition, the one-way analysis of covariance and chi-square were used to compare the distribution of continuous and categorical baseline covariates, respectively. The generalized linear model (GLM) was applied for comparing outcome variables, adjusted for other covariates between study groups. The GLM was used for adjusting the effect of other covariates including age and duration of infertility on our interested association. The P value was considered 0.05. SPSS (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Illinois, USA) was utilized for statistical analysis.

Results
In this study, 138 patients with ART cycles were assessed for the eligibility criteria and enrolled in the study. Then, they were randomly assigned to 3 groups (n = 46). Fortunately, we lost no follow-up case (Figure 1). The Kolmogorov-Smirnov test revealed that there were no statistical differences between normal distribution and distribution of age, gonadotropin injection number, the number of retrieved oocytes, and the number of metaphase II oocytes (P=0.10). The mean (standard deviation, SD) of age was 31.1 (4.2), 32.5 (3.3), and 31.6 (3.5) for daily buserelin, daily, and every other day triptorelin 0.1 mg, respectively (P=0.178). In the case of the duration of infertility, the mean (SD) for daily buserelin, daily, and every other day triptorelin 0.1 mg were 6.6 (4.3), 8.4 (3.8), and 7.2 (3.4), respectively (P=0.075). Table 1 provides the distribution of infertility causes in 3 comparison groups. As shown, there were no statistical differences in the baseline categorical variables among the 3 comparison groups. Moreover, no statistical differences were observed regarding baseline variables including age and duration of infertility in 3 groups.

Bivariate Analysis
Table 2 provides the comparison of the outcome variables between the study groups, along with their corresponding P values in the bivariate analysis. As shown, the duration of ovarian stimulation was similar among the study groups (P=0.437). Nevertheless, the mean of gonadotropin injection number was statistically different so that the need of group C (every other day triptorelin) for gonadotropin injections was significantly lower compared to groups B and A (P=0.033). The other comparisons are shown in Table 2. Except for gonadotropin injection number, there were no other statistical differences between the study groups regarding the other outcome variables.

Table 3 presents the distribution of some categorical variables among the comparison groups. In bivariate analysis, the chi-square test results showed no statistical difference in biochemical and clinical pregnancy, as well as abortion and twin rates among the study groups.

Table 1. Distribution of Infertility Causes Among the Three Comparison Groups

| Infertility Causes        | Group A | Group B | Group C | P Value* |
|---------------------------|---------|---------|---------|----------|
| Male                      | 9 (19.5)| 24 (52.1)| 22 (47.8)| 0.06     |
| Polycystic ovary syndrome | 7 (15.2)| 4 (8.6) | 22 (47.8)| 0.08     |
| Endometriosis             | 5 (10.8)| 5 (10.8)| 2 (4.3) | 0.07     |
| Tubal factor              | 12 (26)| 4 (8.6) | 1 (2.17)| 0.06     |
| Uterine factors           | 2 (4.3)| 0 (0)   | 2 (4.3) | 0.07     |
| Ovulatory                 | 4 (8.6)| 2 (4.3)| 0 (0)   | 0.09     |
| Unknown factors           | 7 (15.2)| 7 (15.2)| 4 (8.6)| 0.08     |
| Total                     | 46 (100)| 46 (100)| 46 (100)| 0.06     |

Note. Data presented as number and percentage in parentheses. The dominant factor is presented for mixed infertility conditions.
*Chi-square test.

Table 2. Comparison of Outcome Variables Among the Three Comparison Groups

| Variable                        | Group A | Group B | Group C | P-value* |
|---------------------------------|---------|---------|---------|----------|
| Gonadotropin injection number   | 27.5 ± 7.7| 27.0 ± 7.7| 23.4 ± 9.1| 0.03     |
| Ovarian stimulation day number  | 10.3 ± 1.5| 10.0 ± 1.5| 10.5 ± 3.0| 0.44     |
| Follicle number metaphase II    | 10.5 ± 5.1| 10.3 ± 3.7| 10.3 ± 3.2| 0.97     |
| Oocytes number                  | 8.5 ± 4.3| 7.4 ± 2.7| 7.8 ± 2.4| 0.00     |
| Embryo number                   | 6.3 ± 3.2| 5.7 ± 2.2| 6.1 ± 2.2| 0.00     |

Note. Data are presented as the mean ± standard deviation (SD).
*One-way ANOVA.
Multivariable Analysis
The GLM was used to consider the effect of other covariates. After considering the effect of measured covariates in cases of metaphase II oocytes and embryo number, statistical differences were observed among the 3 groups. Group A yielded the most number of metaphase II oocytes ($P=0.004$) and embryo number ($P=0.007$) compared with the other two groups. However, group B provided the least metaphase II oocytes and embryo numbers in comparison with other groups.

Discussion
We documented that the overall needed gonadotropin injections, and presumably, the cost of the medications were lower in every other day triptorelin protocol. The clinical usage of this protocol may lead to lower costs. The counts of metaphase II oocyte and embryos were not lower in patients who received every other day triptorelin injections compared to those receiving daily injections. The counts of metaphase II oocyte and embryos were higher in patients who received buserelin compared to those receiving triptorelin irrespective of the frequency of injections, either daily or every other day. In our population, buserelin was superior to triptorelin for producing metaphase II oocytes and embryos by the applied method. In this regard, alternate day triptorelin injections provided similar results to its daily injections. Nevertheless, the outcomes including pregnancy, twin, and abortion rates were similar.

In their study, Prato et al found that although the reduced dose of triptorelin is sufficient for pituitary suppression in ovarian stimulation, there is no important improvement in the outcome of the IVF cycle (19). In another study conducted by Safdarian et al (20), no statistical differences were reported between daily buserelin and half-dose long-acting triptorelin regarding the follicle number. As demonstrated in the current study, Yim et al reported no statistical differences between the ordinary and reduced dose of triptorelin regarding oocyte and the embryo number (21). These observations are consistent with our results, indicating similar counts for metaphase II oocyte and embryo numbers between daily and alternate daily use of triptorelin. However, our finding considering higher metaphase II oocyte and embryo numbers with buserelin compared to triptorelin use is a new finding for the current study. This might be the consequence of premature LH surge and progesterone elevation. Unfortunately, LH measurement was not included in this study as a major drawback. It has also been previously shown (22) that the different doses of long-acting triptorelin provide similar clinical pregnancy outcomes although the total dose of the FSH was also similar in contrast to the finding of the current study.

The range of the twin rate associated with IVF in our study was approximately 10.8 in group A to 32.5% in group C, this is consistent with the 10th European IVF-monitoring report in which the proportion of twins after IVF and ICSI had been reported 19.9% (23). This finding is in line with human fertilization, an embryoology authority report published in 2008 (24). Clinical pregnancy rates in the current study varied between 34.7% in group A and 39.1% in group B. Regarding the abortion, the minimum and maximum rates belonged to groups B (8.6%) and A (13.04%), respectively. In other studies conducted by Safdarian et al (20) and Tehraninejad et al (25), no statistical differences were reported regarding the follicle number, along with pregnancy and abortion rates between daily buserelin and half-dose long-acting triptorelin. Additionally, Taheripanah et al demonstrated that low dose long-acting agonists are a useful method for pituitary suppression. Moreover, this method provides high-quality oocytes, along with acceptable pregnancy and fertility rates (26). There are many covariates affecting IVF outcomes, including age, infertility duration, and infertility causes. The applied GLMs illustrated that the findings were unchanged after adjustment for age and the duration of infertility.

It is recommended that similar studies should be conducted using randomized double-blind controlled designs. In addition, LH measurements in triggering day with HCG should be done to detect premature LH surge. Using the result of the current study for choosing the most appropriate type and dose of GnRH in the high and poor responder patients is suggested as well. Finally, similar studies would be interesting for buserelin to use reduced doses or an alternate day schedule. This study did not include the anti-Mullerian hormone level and the antral follicular count in this research. The assessment of these measures as possible confounding factors and proper adjustment should be considered in future studies. Although the pregnancy rate was similar among the 3 groups, the cumulative pregnancy rate would have been different among the groups if assessing the fresh and frozen embryo transfer. This is a speculation which merits

| Variable          | Group A     | Group B     | Group C     | $P$ Value* |
|-------------------|-------------|-------------|-------------|------------|
| Biochemical pregnancy | 16 (34.7) | 18 (39.1)  | 17 (36.9)  | 0.94       |
| Clinical pregnancy | 16 (34.7)  | 18 (39.1)  | 17 (36.9)  | 0.94       |
| Twins             | 5 (10.8)   | 9 (19.56)  | 14 (32.5)  | 0.27       |
| Abortion          | 6 (13.04)  | 4 (8.6)    | 5 (10.8)   | 0.78       |

Note. Data are presented as No. (%). *Chi-square test.
consideration in future studies. In the current study, the endometrial thickness was not measured, which may surrogate the effect of different therapeutic strategies which merit consideration further.

Conclusions
Nowadays, the antagonist protocol is widely used, but agonist protocols are useful in some situations. In the current study, biochemical and clinical pregnancy rates, as well as twin and abortion rates were similar among different long agonist protocols employing daily buserelin and daily and every other day triptorelin. Besides, providing more comfortable applications, every other day triptorelin can reduce the overall treatment cost. Nevertheless, the embryo number in protocols using buserelin was higher using the fresh and frozen embryos, and the overall pregnancy rate might become different. Our findings emphasize that the use of every other day triptorelin is a possible cost-benefit method after further confirmations.

Authors’ Contribution
EST designed the study and performed data collection. ZAN and AT participated in data gathering, patient handling, and interpretation of the results. Finally, VK and EAN analyzed the data and performed the calculations and participated in scientific writing and paper preparation.

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

Ethical Issues
This trial was approved by Tehran University of Medical Sciences as an MD thesis (registration number: 21528) and was registered in Iranian Registry of Clinical Trials (identifier: IRCT201308275181N10). Informed consent forms were collected from all participants.

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