The effect of letrozole versus artificial hormonal endometrial preparation on pregnancy outcome after frozen-thawed embryos transfer cycles: a randomized clinical trial

Azadeh Hosseini-Najarkolaei, Ashraf Moini, Ladan Kashani, Maryam Farid Mojtahedi, Elnaz Hosseini-Najarkolaee and Ensieh Salehi

Abstract

Background: Considering that clinical trial studies are limited in polycystic ovary syndrome (PCOS) patients, and there is no consensus on an optimum endometrial preparation protocol for frozen embryo transfer (FET), the present study was designed as a randomized clinical trial to compare the reproductive outcomes following stimulated cycles with letrozole plus human menopausal gonadotropin (HMG) for endometrial preparation compared with routine AC-FET.

Methods: This randomized controlled trial was carried out on infertile PCOS patients who underwent IVF/ICSI and FET cycles in Arash Women’s Hospital affiliated to Tehran University of Medical Sciences between September 2018 and January 2020. PCOS diagnosis was based on the Rotterdam criteria. Eligible patients were randomly allocated into two groups: stimulated cycle with letrozole plus (HMG) (intervention group) and routine artificial hormonal endometrial preparation (control group).

Results: One hundred seventy-seven infertile patients were recruited for participation in the study. Of these, 57 women were excluded due to non-eligibility for entering the study, and a total of 120 patients were randomly assigned to two study groups. After follow up, the cycle outcomes of 57 patients in the intervention group and 59 patients in the control group were compared. The data analysis showed that the two groups did not have significant differences in fundamental and demographic characteristics. After the intervention, there were no significant differences in implantation rate, chemical, ectopic, and clinical pregnancy rates between groups. Moreover, the rates of miscarriage and ongoing pregnancy were similar between groups (P > 0.05).

(Continued on next page)
Conclusions: We found similar pregnancy outcomes with two endometrial preparation methods. Noting that each treatment centre should select the most beneficial and cost-effective method with the least adverse effects for patients, letrozole preparations for FET could be incorporated into possible options; however, establishing this approach as first-line treatment is premature in light of current evidence, and future randomized clinical trials with larger sample sizes are required for widespread application.

Trial registration: The study was also registered in the Iranian Registry of Clinical Trials on March 20th, 2020. (IRCT2009052601952N12 at www.irct.ir, registered retrospectively).

Keywords: Frozen-thawed embryo transfer, Letrozole, Endometrial preparation, Artificial cycle, Ongoing pregnancy rate

Introduction
Polycystic ovarian syndrome (PCOS) is the most common cause of infertility in women at reproductive ages, with a prevalence of 8–13% [1]. Because there is an increased risk of ovarian hyperstimulation syndrome (OHSS) in these patients, the preferred strategy for retrieved oocytes is the freeze-all policy. Recent advances in vitrification techniques for cryopreservation of embryos, and increased acceptance and application of a selective single embryo transfer strategy, have led to a significant increase in the utilization of FET cycles [2].

The potential advantages of the freeze-all policy consisted of an increase in pregnancy rates, a reduction in treatment costs, and a decrease in OHSS incidences during treatment [3].

Different endometrial preparation regimes have been proposed to increase endometrial receptivity in frozen embryo transfer (FET) cycles; however, no superiority of any regimen in terms of clinical pregnancy or live birth rates has been found yet [4, 5]. Most previous studies focused on the optimal method of endometrial preparation in women with normal ovulatory functions, while few studies compare the different methods of endometrial preparation in women with ovarian dysfunction and PCOS diagnosis [2]. Artificial-cycle FET (AC-FET) is more commonly applied for this group of patients because of easier planning and patient convenience [2]. However, the main disadvantage of this method is the dangerous adverse effects of employed hormones, such as the risk of maternal thromboembolic events and genital malformations in male fetuses [2, 6].

In the first randomized clinical trial, Wright et al. compared the stimulated cycles using recombinant follicle stimulating hormone (rFSH) with artificial cycles and found the comparable pregnancy, implantation, cancellation rates and endometrial thickness [7]. Since then, several studies have compared the reproductive outcomes of endometrial preparation with stimulated cycles using gonadotropin and clomiphene or letrozole or both, with the artificial cycles. Notably, a number of studies have shown positive results involving ovarian stimulation (OS) for endometrial preparation in frozen embryo transfer cycles in PCOS patients [2, 8–12], and some studies have not found any differences compared with hormonal endometrial preparation [13, 14].

Letrozole is a third-generation aromatase inhibitor drug without antagonistic effects on the estrogen receptors and it induces mono-follicular development due to maintenance the normal central feedback [15]. Furthermore, a pilot study focusing on comparing letrozole and clomiphene citrate for ovulation induction in PCOS women concluded that letrozole positively influences several markers of endometrial receptivity compared with clomiphene citrate [16]. Recently, Zhang et al. performed a retrospective study in patients with PCOS undergoing FET and reported that letrozole administration for endometrial preparation was associated with higher live birth rates compared with artificial cycles after statistical adjustment for confounding factors; therefore, future prospective randomized studies are required to verify these findings [2]. The formation of corpus luteum and appropriate luteal phase support along with utilization of letrozole is another advantage of aromatase inhibitor therapy. Letrozole is relatively safe during pregnancy, and no congenital anomalies were reported for pregnant women who received letrozole. Considering that the clinical trial studies are limited in PCOS patients and given the controversial nature of this subject, the present study was designed as a randomized clinical trial to compare the reproductive outcomes following stimulated cycles with letrozole plus human menopausal gonadotropin (HMG) for endometrial preparation with routine AC-FET.

Methods
Study design and population
This randomized controlled trial was carried out on infertile PCOS patients who underwent IVF/ICSI and frozen embryo transfer in Arash Women’s Hospital affiliated to Tehran University of Medical Sciences between September 2018 and January 2020. The study was approved by the Ethics Committee, Tehran University of
Regardless of the dominant follicle size. Thick ness was below 7 mm, the cycle was cancelled re- final oocyte triggering. On day 20, if the endometrial mon, 5000 IU, i.m., IBSA company) were injected for the cycle stimulated with letrozole plus human menopausal gonadotropin (HMG) (intervention group) and routine artificial hormonal endometrial preparation (control group). Permuted block randomization was conducted by the methodological advisor according to a computer-generated list. The patients’ enrolment and assignment to intervention and control groups were carried out by a researcher midwife in the clinic. Each patient participated in the study only once and on the condition of written consent.

Endometrial preparation
The eligible patients were randomly allocated into two groups: cycle stimulated with letrozole plus human menopausal gonadotropin (HMG) (intervention group) and routine artificial hormonal endometrial preparation (control group). The ongoing pregnancy rate was defined as pregnancies observed gestational sacs divided by the number of embryos transferred.

Outcome measures
The primary outcomes were implantation and chemical and clinical pregnancy rates. The secondary outcomes consist of early miscarriage and ongoing pregnancy rates. Implantation rate was calculated as the number of observed gestational sacs divided by the number of embryos transferred for each patient. Clinical pregnancy was detected by the presence of a gestational sac with fetal heartbeat on the vaginal ultrasound. The spontaneous loss of a clinical pregnancy between 14 and 20 weeks of gestation was considered a late miscarriage. The ongoing pregnancy rate was defined as pregnancies.
continued for at least 21 weeks after ET and confirmed by the ultrasound scan.

**Calculation of sample size**
The sample size was calculated by using the following statistical formula assuming $z_{\alpha/2} = 1.96$ and $z_\beta = 0.85$. A sample size of 60 patients was required in each group at a significance level (alpha level) of 0.05 and a power of 80.

\[
\frac{z^2 \times p (1-p)}{e^2} + (\frac{z^2 \times p (1-p)}{e^2} N) = \frac{z^2 \times p (1-p)}{e^2}
\]

**Statistical analysis**
Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 23.0. The continuous variables were compared between groups by student's t-test and presented as mean ± standard deviation (SD). The chi-square test was applied for comparing the categorical variables between groups, and the results were reported as numbers/percentages. The statistically significant level was considered at $p$-value < 0.05.

**Results**
The flow chart of the study subjects' sampling according to the Consolidated Standards of Reporting Trials (CONSORT) guideline was as shown in Fig. 1. During the study period, 177 infertile patients were evaluated for participation in the study. Of these, 57 women were excluded due to non-eligibility for entering the study, and a total of 120 women were randomly assigned to either the artificial cycle group ($n = 60$) or stimulated cycle with letrozole + HMG ($n = 60$). After follow up, the cycle outcomes of 57 patients in the intervention group and 59 patients in the control group were compared (Fig. 1). The baseline characteristics of patients are presented in Table 1, and according to the results, the two groups did not show significant differences in terms of demographic and baseline characteristics. The cycle characteristics and pregnancy outcomes were compared between groups in Table 2. There were no significant differences in implantation rate, chemical,
ectopic and clinical pregnancy rates between groups. Moreover, the rates of miscarriage and ongoing pregnancy were similar between groups. The rate of cycle cancellation due to inappropriate endometrium in the stimulated cycle was higher than that of the artificial cycle groups; however, this difference was not significant between groups ($P = 0.29$). Two cases of cycle cancellation in the stimulated cycle group occurred due to unexpected LH rise midcycle or failure to reach an optimum endometrial thickness or both. Despite the recommendation for contraception use, a case of heterotopic pregnancy occurred in the letrozole + HMG group. Laparoscopic surgery was done to remove ectopic pregnancy, and the subject eventually had a successful live birth (Table 2).

**Discussion**

The results of the present study showed similar pregnancy outcomes and cancellation rates after vitrified-warmed ET in both endometrial preparation methods in PCOS patients. Therefore, since minimally stimulated cycles have fewer side effects, it can be suggested as an alternative method. Especially considering recent studies have shown a plausible association between the absence of corpus luteum in FET cycles and adverse obstetrical outcomes [18], the application of endometrial preparation with minimally stimulated cycles is preferable.

To the best of our knowledge, five retrospective [2, 11, 12, 19, 20] and two clinical trial studies [9, 14] have evaluated the effects of letrozole utilization for endometrial preparation before FET; however, only one of the previously published clinical trials [14] possessed a registration number in the specific clinical trial cites. The recent retrospective study, by Zhang et al. in 2019 evaluated a total of 2664 patients with PCOS undergoing FET cycles, reporting that letrozole stimulation during FET cycles significantly improved live birth rates with a decrease in the pregnancy loss rate.

Similarly, three retrospective studies in China and one in Egypt found higher implantation, clinical, ongoing and live birth rates in letrozole stimulated cycles compared with AC [11, 12, 19] and natural cycles [20]. Elsewhere, Peigné et al. (2019) in a retrospective study concluded that live birth rate was significantly higher with mild OS than with the AC preparation, even after adjusting for potential confounders; suggesting that the first-line endometrial preparation could be OS instead of an AC and a potential defect of the luteal phase may exist in AC preparation [21]. However, Aleyasin et al. in a randomized clinical trial found no significant

### Table 1: Comparison of demographic and clinical characteristics of study participants between groups

| Variables                         | Artificial cycle ($n = 59$) | Simulated cycle by letrozole + HMG ($n = 57$) | $P$-value |
|----------------------------------|----------------------------|-----------------------------------------------|-----------|
| Age (years)                      | 29.45 ± 0.42               | 30.12 ± 0.33                                  | 0.21      |
| BMI (kg/m²)                      | 26.10 ± 0.48               | 25.70 ± 0.46                                  | 0.55      |
| Duration of infertility (Years)  | 2.80 ± 0.21                | 3.10 ± 0.26                                   | 0.38      |
| Basal serum FSH level (IU/l)     | 5.86 ± 0.11                | 5.52 ± 0.13                                   | 0.06      |
| Basal serum LH level (IU/l)      | 7.23 ± 0.37                | 7.43 ± 0.28                                   | 0.65      |
| Serum AMH level (ng/ml)          | 6.87 ± 0.48                | 7.52 ± 0.49                                   | 0.35      |
| No. of retrieved oocytes of previous COH | 21.70 ± 0.96            | 22.40 ± 0.96                                  | 0.61      |

Descriptive data were presented as Mean ± SD. $P$-value $\leq 0.05$ was considered statistically significant. BMI Body mass index, No. number, FSH Follicle-stimulating hormone, LH Luteinizing hormone, AMH Anti-Müllerian hormone, COH Controlled ovarian stimulation, HMG Human menopausal gonadotropin

### Table 2: Comparison of cycles and pregnancy outcomes between groups

| Outcomes                              | Artificial cycle ($n = 59$) | Simulated cycle by letrozole + HMG ($n = 57$) | $P$-value |
|---------------------------------------|----------------------------|-----------------------------------------------|-----------|
| Endometrial thickness at ET day (mm)  | 9.92 ± 0.19                | 9.45 ± 0.16                                   | 0.065     |
| Cycle cancellation rate (%)           | 1 (1.6)                    | 3 (5.2)                                       | 0.29      |
| Implantation rate (%)                 | 20%                        | 22%                                           | 0.67      |
| Chemical pregnancy rate (%)           | 25 (42.3)                  | 28 (49.1)                                     | 0.46      |
| Clinical pregnancy rate (%)           | 22 (37.2)                  | 24 (42.1)                                     | 0.59      |
| Ectopic pregnancy                     | 1 (1.6)                    | 2 (3.5)                                       | 0.53      |
| Heterotopic pregnancy                 | 0 (0)                      | 1 (1.75)                                      | 0.30      |
| Miscarriage rate (%)                  | 2 (3.3)                    | 2 (3.5)                                       | 0.97      |
| Ongoing pregnancy rate (%)            | 19 (32.2)                  | 20 (35)                                       | 0.74      |

HMG Human menopausal gonadotropin. $P$-value $\leq 0.05$ was considered statistically significant
difference in terms of live birth rate between the letrozole plus HMG method and the artificial FET protocol [14]. In another clinical trial by Tahoon et al. it was reported that using letrozole stimulated cycles for endometrial preparation in cryopreserved ET yields a significantly higher ongoing pregnancy rate than artificial cycles [9]. An overview of past studies reveals that the use of letrozole for endometrial preparation can be advantageous. Two possible reasons for the observed qualities of the letrozole approach were proposed which is as follows: i) letrozole decreases intraovarian and serum estrogen levels by blocking the conversion of androgens to estrogens in the ovarian granulosa cells [20], subsequently low estrogen levels reduce ubiquitination of estrogen receptors, this process leads to faster endometrial proliferation and increased blood level in the uterus and endometrium, with positive effects on pregnancy outcomes [19] and ii) there is some evidence that letrozole may potentially improve endometrial receptivity [2]. In this way, Miller et al. concluded that the lack of endometrial avß3 integrin expression is associated with a poor prognosis outcome in IVF cycles that might be improved with letrozole co-treatment [22]. Elsewhere, Ganesh et al., in a preliminary study reported that OS with the use of letrozole was associated with a significant increase in the epithelial and stromal expression of uterine receptivity markers, including integrin, leukaemia inhibitory factor, and L-selectin, in women with unexplained infertility compared with natural cycles [23]; all of which may have positive effects on the pregnancy rate after ET [2].

Meanwhile, we observed a slight increase in the endometrial thickness on the day of ET in the letrozole-treated group. Similarly, Zhang et al. found the endometrial thickness was significantly greater in the letrozole group than in the AC group, both on the day of starting progesterone and on the day of ET [2]. However, some studies found no significant difference [14, 21] or even higher endometrial thickness after the artificial preparation method [9]. Therefore, more randomized trials with higher sample size are still required to conclude and comment on this regards.

In terms of the long-term safety of fertility treatment for future offspring, Tatsumi et al. in a recent population-based study suggested that the administration of letrozole during IVF cycles neither increased the risk of major congenital anomalies nor compromised neonatal outcomes of the newborns compared with natural cycles [24].

Noting that this study is the largest randomized clinical trial design at the time of writing; yet, this trial could not find a significant effect to prioritize the use of letrozole for endometrial preparation over the hormonal method, which may be due to sample size limitation. Therefore, we suggest that a multi-centre clinical trial with a larger sample size be conducted in this field. Another limitation is that we did not assay the endometrial receptivity markers on both groups of patients, so further studies on this subject are warranted to explore the mechanism of the effect of letrozole on the endometrium.

In conclusion, we found similar pregnancy outcomes after evaluating two endometrial preparation methods. Noting that each treatment centre should select the most beneficial and cost-effective method with the least adverse effects for patients, letrozole preparations for FET could be incorporated into possible options; however, establishing this approach as first-line treatment is premature in light of current evidence, and future randomized clinical trials with larger sample sizes are required for widespread application.

Abbreviations
FET: Frozen embryo transfer; PCOS: Polycystic ovary syndrome; OHSS: Ovarian hyperstimulation syndrome; rFSH: Recombinant follicle stimulating hormone; AC: Artificial-cycle; E2: Estradiol; HMG: Human menopausal gonadotrophins; hCG: Human chorionic gonadotropin; SD: Standard deviation

Acknowledgements
We would like to thank all of the participants and co-workers in Arash Women’s Hospital for their assistance in this study.

Authors’ contributions
AM and AH designed the research. AH, AM, LK and MFM contributed in patient selection, data collection, interpretation of data and manuscript writing/editing. AH, AM and LK wrote the manuscript. EH and ES assisted in the analysis of the data. All authors read and approved the final manuscript.

Authors’ information
1- Infertility fellowship at Department of Gynecology and Obstetrics, Arash women’s Hospital, Tehran University of Medical Sciences, Tehran, Iran.
2- Professor at Department of Gynecology and Obstetrics, Arash women’s Hospital, Tehran University of Medical Sciences, Tehran, Iran.
3- Associate Professor at Department of Gynecology and Obstetrics, Arash women’s Hospital, Tehran University of Medical Sciences, Tehran, Iran.
4- Assistant professor at Laparoscopic Research Centre, Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran.
5- Embryologist at Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Funding
This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding authors on reasonable request.

Ethics approval and consent to participate
The Institutional Review Boards and the Ethics Committees of Tehran University of Medical Sciences approved this study (ethics code: IR.TUMS.MEDICINE.REC.1398.834). All procedures performed in studies involving human participants were in accordance with the ethical standards of Tehran University of Medical Sciences and the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The eligible patients signed written informed consent forms prior to participation in the study.
Consent for publication
Not applicable.

Competing interests
All authors have nothing to disclose.

Author details
1Department of Gynecology and Obstetrics, Arash women’s Hospital, Tehran University of Medical Sciences, Tehran, Iran. 2Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran. 3Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran. 4Department of Obstetrics and Gynecology, Shiraz University of Medical Sciences, Shiraz, Iran. 5Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Received: 1 July 2020 Accepted: 13 November 2020
Published online: 20 November 2020

References
1. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. Clinics. 2015;70(11): 765–9.
2. Zhang J, Liu H, Wang Y, Mao X, Chen Q, Fan Y, et al. Letrozole use during frozen embryo transfer cycles in women with polycystic ovary syndrome. Fertil Steril. 2019.
3. Roque M, Valles M, Guimaraes F, Sampaio M, Geber S. Cost-effectiveness of the freeze-all policy. JBRA assisted reproduction. 2015;19(3):125–30.
4. Groenewoud E, Cohen B, Al-Oraibi A, Brinkhuis E, Broekmans F, De Bruin J, et al. A randomized controlled, non-inferiority trial of modified natural versus artificial cycle for cryo-thawed embryo transfer. Hum Reprod. 2016; 31(7):1483–92.
5. Ghobara T, Gelbaya TA, Ayeleke RO. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev. 2017;7.
6. Behnam-Rasouli M, Nikravesh MR. The Abortional and the Teratogenetical effects of 17-beta estradiol Valerate administration during embryonic development in rats. Iran Biomed J. 1997;14(4):53–7.
7. Wright KP, Guibert J, Wetzen S, Davy C, Fauche P, Olivennes F. Artificial versus stimulated cycles for endometrial preparation prior to frozen-thawed embryo transfer. Reprod BioMed Online. 2006;13(3):321–5.
8. Jouan C, Emonard V, Ruggeri P, Debelle L, Hincourt N, Lorquet S, et al. Pregnancy outcome following frozen embryo transfer after artificial cycle or treatment by clomiphene citrate. Gynecol Endocrinol. 2016;32(10):907–10.
9. Tahoon AS, Abdrabo HA, Mustafa MK. Randomized controlled trial comparing pregnancy outcome using artificial versus letrozole stimulated cycle in cryo preserved embryo transfer. Egypt J Hosp Med. 2018;72(6): 4644–9.
10. Hatoum I, Bellon L, Swierkowski N, Ouazana M, Boubal S, Fatallah H, et al. Disparities in reproductive outcomes according to the endometrial preparation protocol in frozen embryo transfer. J Assist Reprod Genet. 2018; 35(3):425–9.
11. Sj L, Yj Z, Xs C, Mf N, Y-y Z, Ji C, et al. Letrozole ovulation induction: an effective option in endometrial preparation for frozen-thawed embryo transfer. Arch Gynecol Obstet. 2014;289(3):687–93.
12. Sibai H, El Housseini A, Elgindy E. Letrozole versus artificial hormonal endometrial preparation for vitrified-warmed embryos transfer cycles. Middle East Fertility Soc. J. 2016;21(2):96–100.
13. Yu J, Ma Y, Wu Z, Li Y, Yang T, Li Y, et al. Endometrial preparation protocol of the frozen-thawed embryo transfer in patients with polycystic ovary syndrome. Arch Gynecol Obstet. 2015;291(1):201–11.
14. Aleyasin A, Aghahosseini M, Safarian L, Noorzadeh M, Falahi F, Rezaeean Z, et al. Can letrozole plus HMG protocol improve pregnancy outcomes in frozen-thawed embryo transfer? An RCT. Int J Reprod BioMed. 2017;15(2):83.
15. Garcia-Velasco JA. The use of aromatase inhibitors in in vitro fertilization. Fertil Steril. 2012;98(6):1356–8.
16. Wallace KL, Johnson V, Sopelak V, Hines R. Clomiphene citrate versus letrozole: molecular analysis of the endometrium in women with polycystic ovary syndrome. Fertil Steril. 2011;96(4):1051–6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
• fast, convenient online submission
• thorough peer review by experienced researchers in your field
• rapid publication on acceptance
• support for research data, including large and complex data types
• gold Open Access which fosters wider collaboration and increased citations
• maximum visibility for your research: over 100M views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions