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Outcomes of three endometrial preparation protocols for frozen-thawed embryo transfer cycles in regularly ovulating women: a prospective randomized clinical trial

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Abstract

Background: In recent years, with the continuous technical advancement in cryopreservation techniques, frozen embryo transfer (FET) has become mainstream and a viable alternative to fresh embryo transfer. Despite the upward trend in FET, the most optimal priming strategy of the endometrium before FET remains controversial. For ages, the hormone replacement treatment (HRT) cycle protocol has been favored as the first-line regimen for endometrial priming in FET; however, nowadays some patients and physicians may opt for other protocols rather than using high-dose hormones with their potential adverse risks. ***Objective***: The aim of this work is to compare three endometrial preparation protocols for FET; either by HRT cycles, ovulation induction (OI) cycles with aromatase inhibitors (AI), or modified natural cycles (mNC). ***Methods*:** This prospective randomized clinical trial included 120 patients preparing for FET. Participants were randomized into three equal groups according to the protocol of endometrial preparation. Group A underwent HRT protocol by daily 6 mg oral Estradiol valerate, followed by daily 800 mg vaginal progesterone (P4). Group B underwent OI protocol, using 2.5 mg Letrozole, twice daily, for 5 days. Group C underwent mNC protocol, using 5000 IU human chorionic gonadotropin (hCG) as a trigger for ovulation. ***Results***: there were no significant differences between the three groups regarding number of days before FET, mean endometrial thickness, or reproductive outcomes. In normo-ovulatory women, there was a trend toward improved reproductive outcomes in the mNC group regarding implantation, clinical pregnancy, and ongoing pregnancy rates; nevertheless neither of these outcomes reached a statistical significance. ***Conclusion***: In normo-ovulatory women, there was a trend toward improved reproductive outcomes in the mNC group, so we recommend the mNC protocol for endometrial preparation before FET as the protocol of choice in these women.

Keywords: Frozen embryo transfer, hormone replacement treatment, ovulation induction, modified natural cycles.

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Introduction

In recent years, with the continuous technical advancement in cryopreservation, frozen embryo transfer (FET) has become mainstream and a viable alternative to fresh embryo transfer.(1) Despite the upward trend in FET, the most optimal priming strategy of the endometrium before FET remains controversial. To date, different endometrial priming protocols have been proposed, including natural cycle (NC), hormone replacement therapy (HRT) cycle, and ovulation induction (OI) cycle (2, 3).

A. Hormone replacement therapy (HRT) cycle FET

In HRT cycle FET, sequential exogenous estradiol (E2) and progesterone (P4) are administered to prepare the endometrium and inhibit follicular growth. Compared to other endometrial priming protocols, the HRT cycle protocol is more flexible and convenient. However, the main disadvantages of this regimen are the potentially detrimental effects caused by exogenous E2 and the absence of corpus luteum (CL) (4).

E2 can be given orally, transdermally, or vaginally; however, the oral route is the most popular one due to its convenience (5-8). The duration of E2 administration is very flexible (9-11). An ultrasound assessment is typically scheduled after an initial phase of E2 administration to determine endometrial thickness and rule out the presence of a pre-ovulatory follicle, CL, or luteinized endometrium before beginning P4 supplementation (12).

P4 supplementation is initiated when the endometrium is triple-line and exceeding 7 mm in thickness (13). The preferred P4 routes are the vaginal and the intramuscular (IM); however, owing to its convenience, ease of usage, and reduced pain, most patients choose vaginal over IM P4 (14). Nowadays, the majority of cleavage-stage embryos are transferred on the fourth day of P4 therapy, while blastocysts are often transferred on the sixth day of P4 therapy (15, 16). E2 and P4 are usually continued until the 12th week of gestation, at which point placental autonomy is developed to replace the absent CL (17-19).

B. Natural cycle (NC) - FET

In NC-FET, endometrial preparation is based on endogenous E2 and P4 produced by the dominant follicle. NC-FET includes true natural cycle (tNC), and modified natural cycle (mNC). Regularly ovulating females can benefit from NC-FET protocol as it eliminates the need for administering large doses of exogenous

hormones, making it cheaper and more physiological than other endometrial preparation protocols (4).

a) ***True natural cycle (tNC) - FET***

In tNC, FET scheduling is determined by tracking the physiologically occurring luteinizing hormone (LH) surge, occuring about 34 to 36 hours before follicular rupture,(20, 21) and monitoring sonographic evidence of impending ovulation. Serial transvaginal sonographic scans are conducted, beginning on the eighth day of the cycle (22). A follicular size of approximately 18 to 20 mm and a trilaminar endometrium of more than 7 mm thickness usually predict approaching ovulation and good endometrial receptivity (13).

The typical practice to schedule FET at the blastocyst stage is on LH surge + 6 day, and at the cleavage stage is on LH surge + 4 day, considering the day of the LH surge as Day 0 (23). Little definitive evidence exists on the actual benefits of luteal phase support (LPS) in tNC-FET. Some authors favored LPS use in tNC-FET (24, 25), while others found nearly similar reproductive outcomes in tNC-FET with or without LPS (26-29).

Modified natural cycle (mNC) -FET

This protocol is more flexible and necessitates less endocrine and sonographic surveillance than tNC-FET. When the dominant follicle reaches a diameter of 16–20 mm and the endometrial thickness exceeds 7 mm, ovulation is induced by injecting human chorionic gonadotropin (hCG) (3).

Regarding FET timing, the typical practice to schedule mNC-FET at the blastocyst stage is on hCG administration + 7 day (22), and at the cleavage stage is on hCG + 5 day (3). Since hCG has a half-life of at least seven days, it may provide sufficient luteotropic effects throughout the early luteal phase, so LPS might not be needed in mNC-FET (30).

C. Ovulation induction (OI) cycle FET

In this protocol, either oral ovulatory agents, such as clomiphene citrate (CC) and letrozole, exogenous gonadotropins, or combinations of them can be utilized (22). The rationale behind OI in women who cycle regularly is alleviating minor abnormalities

in folliculogenesis and the ensuing luteal phase, aiming for a more favorable endocrine environment for embryo implantation (31, 32). Serial transvaginal ultrasonographic surveillance are done to assess the follicular response. HCG is given once the leading follicle's diameter measures 16-20 mm, and the endometrial thickness exceeds 7 mm (22).

The typical practice to schedule OI cycle FET at the blastocyst stage is on hCG administration + 7 day, and at the cleavage stage is on hCG + 5 day (3). Despite utilizing LPS for OI- FET in most in vitro fertilization (IVF) centers (31, 33), further well-designed randomized controlled trials (RCTs) are required to determine the role of LPS in this protocol.

Objective

The aim of this work was to compare three endometrial preparation protocols for FET; either by HRT cycles, OI cycles with AI, or mNC.

Patients & Methods

This study is a prospective randomized clinical trial performed at multiple private ART centers.

Sample size:

Sample size was calculated at Medical Research Institute, Department of Biomedical Informatics and Medical Statistics, Alexandria University. A total hypothesized sample size of 120 eligible infertile couples undergoing frozen-thawed embryo transfer (40 per group) was needed to compare three different endometrial preparation protocols for FET; either by HRT cycles, OI cycles with AI, or mNCs in regularly ovulating women; taking into consideration 95% confidence level, effect size of 85% and 80% power using Chi Square-test.

Participants:.

This study included 120 infertile women undergoing FET. Participants were randomly divided into three equal groups according to the protocol of endometrial preparation (Figure 1)

- **Group A:** 40 cases underwent HRT endometrial preparation protocol.
- **Group B:** 40 cases underwent OI protocol for endometrial preparation, using letrozole.

- **Group C:** 40 cases underwent mNC protocol for endometrial preparation, using 5000 IU hCG as a trigger for ovulation.

Inclusion & exclusion criteria:

- We included women aged 20-38 years with spontaneous regular ovulatory cycles and body mass index (BMI) <35, and having good quality cryopreserved embryos.
- Women with comorbidities including hypertension, diabetes mellitus, or other endocrinopathies, also women with endometriomas, untreated hydrosalpinges, müllerian anomalies (even if corrected), and previous uterine surgeries were excluded from this study.

Before enrollment in the study, all patients were subjected to routine medical evaluation to make sure of presence of inclusion criteria and absence of exclusion criteria. Baseline ultrasound scan was performed on day 2-3 of the cycle to measure the endometrial thickness and exclude the presence of functional ovarian cysts.

Randomization:

A total of 120 eligible patients who met all inclusion criteria and none of the exclusion criteria were randomized into three groups according to a computer-generated simple randomization list with allocation assignment 1:1:1. Therefore, patients were randomly divided into three equal groups according to the endometrial preparation protocol.

Endometrial preparation:

- ***Group A:*** 40 cases underwent HRT endometrial preparation protocol, starting on cycle day-3, by 6 mg/day oral E2 valerate (Cycloprogynova 2mg-Bayer®). Endometrial thickness was followed up by transvaginal ultrasound after 7 days of E2 valerate use. Another transvaginal ultrasound scan was performed after 10-12 days of E2 use to confirm that no dominant follicle emerged and to measure the endometrial thickness. When the endometrial thickness was ≥ 8 mm, P4 was started, in the form of vaginal suppositories, 800mg/day (Prontogest®400mg, twice daily), to initiate secretory changes.
- ***Group B:*** 40 cases underwent OI protocol for endometrial preparation, starting on cycle day-3, by 2.5 mg oral letrozole twice daily for five consecutive days. Transvaginal sonographic follow-up of follicular growth and endometrial thickness was done after 7 days of letrozole use and then every other day. When the dominant follicle reached 16-20 mm in diameter and the endometrial thickness was ≥ 8 mm, 5000 IU hCG were given to induce final follicular

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maturation and ovulation. LPS with P4 (prontogest ®400 mg daily) was started 48 hours after hCG triggering.

- ***Group C:*** 40 Cases underwent mNC protocol of endometrial preparation. Following a baseline ultrasound scan on day 2-3 of cycle, a follow-up scan was done on cycle day 8-10 and continued on alternate days or daily until the dominant follicle reached 16-20 mm in diameter and endometrial thickness was ≥ 8 mm, during which 5000 IU hCG were employed. LPS with P4 (prontogest ®400 mg daily) was started 48 hours after hCG triggering.

In group A, day-3 and day-5 embryos were transferred on the 4th and 6th day of P4 respectively, whereas in Group B and C, day-3 and day-5 embryos were transferred 5 and 7 days after hCG administration respectively. Pregnancy test was done 14 days after embryo transfer. On positive pregnancy test, LPS was continued till approximately 10-12 weeks of gestation.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using number and percent. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

The used tests were:

1- **Chi-square test:** For categorical variables, to compare between different groups.

2- **Monte Carlo correction:** Correction for chi-square when more than 20% of the cells have expected count less than 5.

3- **F-test (ANOVA):** For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey).

4– **Kruskal Wallis test**: For abnormally distributed quantitative variables, to compare between more than two studied groups. Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons.

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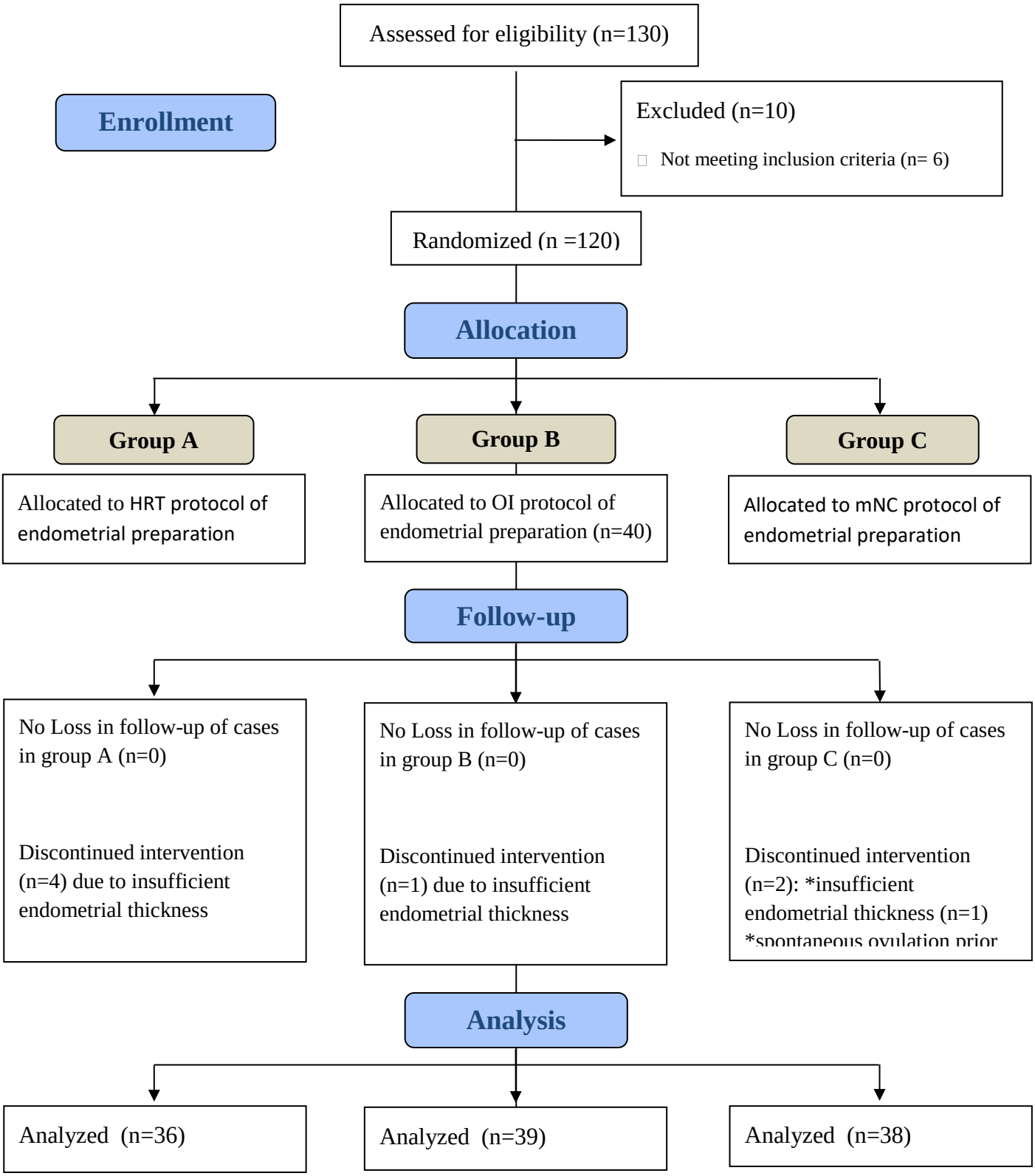

Figure 1 - CONSORT statement flow-chart

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Results

The three groups were compared according to the basic patient characteristics, and showed no statistically significant differences in relation to the patients' age, BMI, and type of infertility. Regarding the duration of infertility, the mean duration was (4.80 ± 3.30) years in group A, (4.30 ± 1.59) years in group B, and (5.34 ± 2.18) years in group C. The duration of infertility in group C was significantly higher than in group A (p_2=0.022*), and group B (p_3=0.041*). However, no statistically significant difference was found between group A and B (p_1=0.802). (Table 1)

Regarding baseline investigations, there was no statistically significant difference in anti-müllerian hormone (AMH) level among the groups, with mean AMH level 2.34 ± 0.60, 2.24 ± 0.53, and 2.17 ± 0.55 ng/ml in group A, B & C respectively. Also, there were no statistically significant differences among the three groups regarding serum thyroid stimulating hormone (TSH) and mid-luteal serum P4 in a previous cycle. Regarding serum prolactin (PRL) level, significantly higher serum PRL levels were observed in group "C" than in group "A". However, all patients in the three groups had their serum PRL levels within the normal range (Table 1).

Four cases in group "A" and one case in group "B" were cancelled due to insufficient endometrial thickness. In group "C" two cases were cancelled; one was due to insufficient endometrial thickness and the other was due to spontaneous ovulation prior to hCG injection (Figure 1).

Regarding the endometrial thickness before adding P4 in group A & at hCG administration in group B & C; there was no statistically significant difference among the groups, with mean endometrial thickness 8.93 ± 1.58, 9.20 ± 1.08, and 9.07 ± 1.0 in group A, B & C respectively. Regarding the number of days before FET; there was no statistically significant difference among the groups, with mean number of days 17.53 ± 1.42, 17.31 ± 1.47, and 17.97 ± 1.88 in group A, B & C respectively. There was no statistically significant difference between the three groups regarding the number and the developmental stage of the transferred embryos (Table 2).

Regarding pregnancy outcomes, there was no statistically significant difference in the rate of positive BHCG, 14 days after FET between the three studied groups, P value 0.810. The overall implantation rate was not significantly different among the studied groups, P value 0.902. The clinical pregnancy rate (CPR) was highest in group "C" (50 %) and lowest in group "A" (47.2%), but the difference between the three groups was not statistically significant, P value 0.972. Regarding ongoing pregnancy rate (OPR), there was no statistically significant difference among the three studied groups, P value 0.994 (Table 2).

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Table 1 - Comparison between the three studied groups according to basic patient characteristics and baseline investigations

Parameter	Group A (n = 40)	Group B (n = 40)	Group C (n = 40)	p
Age (mean) years	31.80 ± 5.18	31.90 ± 4.49	32.55 ± 4.65	0.749
BMI (mean) kg/m²	26.97 ± 2.14	26.46 ± 2.03	26.41 ± 1.86	0.933
Type of infertility				
Primary	28 (70%)	28 (70%)	27 (67.5%)	0.962
Secondary	12 (30%)	12 (30%)	13 (32.5%)	
Duration of infertility (mean) years	4.80 ± 3.30	4.30 ± 1.59	5.34 ± 2.18	0.042*
Significance bet. groups		p_1=0.802,p_2=0.022*,p_3=0.041*		
AMH (mean) ng/ml	2.34 ± 0.60	2.24 ± 0.53	2.17 ± 0.55	0.261
PRL (mean) ng/ml	17.62 ± 2.69	18.48 ± 2.19	19.20 ± 2.07	0.012*
Significance bet. groups		p_1= 0.228,p_2= 0.009*,p_3=0.361		
TSH (mean) mU/L	1.85 ± 0.92	1.92 ± 0.86	1.79 ± 0.76	0.774
Mid-luteal P4 in previous cycle (mean) ng/ml	16.39 ± 3.25	16.16 ± 2.72	16.61 ± 2.67	0.778

p: p value for comparing between the three studied groups; P_1: p value for comparing **Group A** and **B;** P_2: p value for comparing **Group A** and **C;** P_3: p value for comparing **Group B** and **C;** *: Statistically significant at $p \leq 0.05$

In our study, there was a trend toward improved pregnancy outcomes in group "C" regarding implantation rate, CPR, and OPR; nevertheless neither of these outcomes reached a statistical significance in relation to the other 2 groups (Table 2).

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Table 2 - Comparison between the three studied groups according to cycle parameters and pregnancy outcomes

Parameter	Group A (n = 36)#		Group B (n = 39)#		Group C (n = 38)#		p
	No.	%	No.	%	No.	%	
Mean endometrial thickness before adding P4 in group A & at hCG administration in group B & C (mm)	8.93 ± 1.58		9.20 ± 1.08		9.07 ± 1.0		0.519
Days before FET (mean)	17.53 ± 1.42		17.31 ± 1.47		17.97 ± 1.88		0.402
Number of embryos transferred (Mean)	2.86 ± 0.68		2.87 ± 0.66		3.11 ± 0.56		0.162
Developmental stage of transferred embryos							
Day 3 (cleavage stage)	21	(58.3%)	20	(51.3%)	22	(57.9%)	0.785
Day 5 (blastocyst stage)	15	(41.7&)	19	(48.7%)	16	(42.1%)	
Positive BHCG 14 days after FET	18 / 36 (50%)		22 / 39 (56.4%)		19 / 38 (50%)		0.810
Number of gestational sacs (mean)	0.58 ± 0.69		0.62 ± 0.71		0.71 ± 0.80		0.831
Implantation rate (mean)	22.67 ± 27.60%		24.54 ± 29.57%		23.89 ± 26.79%		0.954
Total number of gestational sacs	**21**		**24**		**27**		
Total number of transferred embryos	**103**		**112**		**118**		
Overall implantation rate	**21 / 103 (20.4%)**		**24 / 112 (21.4%)**		**27 / 118 (22.9%)**		0.902
CPR	17 / 36 (47.2%)		19 / 39 (48.7%)		19 / 38 (50%)		0.972
OPR	16 / 36 (44.4%)		17 / 39(43.6%)		17 /38 (44.7%)		0.994

p: p value for comparing between the three studied groups; *: Statistically significant at p ≤ 0.05

#: Cancelled cases were excluded

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Discussion

Endometrial preparation in FET cycles can be accomplished through three commonly used protocols: NC with or without hCG-triggered ovulation; HRT cycles, with or without gonadotropin releasing hormone agonist (GnRHa) down-regulation; and OI cycles.(34) Nevertheless, the optimal FET strategy for endometrial preparation remains controversial (34, 35).

It is well-established that several factors, including the patient's baseline characteristics, the cause and history of infertility, and the efficiency of the process of ovulation, IVF, implantation, and maintenance of pregnancy, all influence the outcomes of IVF treatment at different stages of the intervention. In view of the available literature on different endometrial preparation protocols, a certain degree of heterogeneity exists among studies complicating comparisons. Inclusion criteria concerning patient age, ovulatory state, as well as quality, developmental stage, and number of transferred embryos differ among different studies. Furthermore, protocols differ in utilizing LPS and pituitary down-regulation. Consequently, a variety of confounding factors could be the cause of the controversial outcomes among the available studies.

In our study, we targeted patients with regular menstrual cycles, as this group is probably linked to better ovarian and endometrial functions. We compared 3 different endometrial preparation protocols for FET, mNC, OI cycles, and HRT cycle. Patients in the HRT group relied entirely on exogenous E2 and P4, without pituitary down-regulation, patients in the mNC group received exogenous hCG (as an ovulatory trigger), and those in the mild-OS group received letrozole to support follicular growth and exogenous hCG (as an ovulatory trigger). Vaginal P4 was prescribed as a LPS to those in the mNC and OI groups to offset any possibility of a defective endogenous luteal phase. Most patients' basic characteristics were comparable between the three groups. Also, baseline laboratory investigations were not significantly different among the groups except for the mean PRL level which was significantly higher in the mNC group than the HRT group; however, it was within the normal range. We found that there were no significant differences in reproductive outcomes between the three endometrial preparation protocols in patients with regular menstrual cycles.

Our results are comparable to those of the retrospective study conducted by Yong Jin Kim et al. (36) comparing the clinical outcomes of mNC, OI (CC or letrozole), and HRT endometrial preparation FET protocols. In line with our study, the authors found no significant differences between the three studied groups regarding the mean endometrial thickness before administering P4 or hCG, implantation rate, or CPR.

Another study by Song-jun Li et al. (37) analyzed 517 NC (tNC and mNC), 359 letrozole-OI, and 354 HRT FET cycles. In line with our study, there were no significant differences between letrozole-OI and NC groups in endometrial thickness, implantation rate, or CPR. On the other hand, they found significantly higher endometrial thickness, implantation rate, and CPR in the letrozole-OI and the NC groups than in the HRT group. In our study, we only found insignificantly higher endometrial thickness, implantation rate, and CPR in the letrozole and the NC groups than in the HRT group, but this might be due to the smaller sample size in our study.

Our findings are consistent with those of the study conducted by Toshihiro Kawamura et al. (38), comparing mNC and HRT cycle endometrial preparation protocols for FET. In their study, reproductive outcomes were comparable in both protocols. No statistically significant difference was found between both groups regarding positive bHCG rate, 14 days after FET. Authors also reported non-significantly higher endometrial thickness, CPR, implantation rate, and OPR in the mNC group than in the HRT group.

A retrospective study by Eun Mi Chang et al., (34) reported significantly superior outcomes in mNC than in HRT cycle endometrial preparation protocol. They found significantly higher endometrial thickness, implantation rate, CPR, and OPR in the mNC group than in the HRT group. In our study, we only found a non-significant increase in these reproductive outcomes. This might be due to the smaller number of cycles in our study.

On the other hand, Carolyn R Givens et al. (39) reported better outcomes in HRT than mNC. Authors found a significantly higher CPR in HRT-FET cycles than in mNC-FET in patients using their own oocytes. In this study, the number of studied cases was larger (602 mNC cases and 205 HRT cases), the mean age was higher (35.1 ± 4.1 and 34.8 ± 5.0 years for mNC and HRT groups respectively), and the mean number of transferred embryos was far higher (2496 in mNC and 808 in HRT cycles) than our study. Moreover, in contrast to our study, patients undergoing mNC protocol were monitored for LH surge using urinary kits, and 10,000 IU HCG were given to all patients, whether to trigger ovulation in those without a detected LH surge or to supplement the detected LH surge. FET was done 5 or 6 days (for day 3 and day 5 embryos respectively) following the detected LH surge or the administered HCG trigger.

Kai N. Holder et al. (40) conducted a retrospective cohort study on 183 FET cycles. Patients were assigned either to letrozole OI or NC (tNC or mNC) endometrial preparation protocol depending on clinician preference or previous ovulation history. Letrozole (2.5-7.5 mg daily for 7 days) was used in 79 cycles and the remaining cycles

were NC-FET. In line with our study, there were no significant differences in implantation rate, CPR, and OPR between NC and OI cycle FET.

Jie Zhang et al. (41) conducted a retrospective cohort study comparing letrozole OI and HRT endometrial preparation protocols before FET in women with PCOS. They found a significantly thicker endometrium in the letrozole group than in the HRT group. We also found a thicker endometrium in the letrozole group than in the HRT group; however, our result did not reach a statistical significance. This discrepancy could be due to the larger sample size, or due to supplementation with human menopausal gonadotropin (HMG) if the dominant follicle was less than 14 mm on cycle day 10, in their study. On the other hand, in line with our study, there were no significant differences between both groups regarding implantation rate, CPR, and OPR.

A trend towards improved reproductive outcomes in OI FET protocol than the NC and HRT protocols was reported by some studies (42, 43). Tatsumi et al. (42) compared tNC, Letrozole-OI, and HRT FET endometrial preparation protocols. They included 110722 FET cycles utilizing blastocysts or cleavage-stage embryos. Comparable to our study, CPR was insignificantly higher in the NC group than in the HRT group. However, in contrast to our study, they found a significantly higher CPR in the Letrozole-OI group than in the other two groups. This might be due to the significantly younger age of patients in the letrozole group than in the tNC group, or due to the significantly higher percentage of blastocyst-stage embryos in the Letrozole group than in the other 2 groups. Furthermore, although this study had the privilege of including a significant number of cycles, it was also hampered by the absence of data on key patients' variables, such as the history of ART failures. Also, no information was provided about the dosage or duration of letrozole consumption. Moreover, LPS was significantly variable among the 3 groups.

Also, Hui-Juan Guan et al. (43) compared the reproductive outcomes of mNC, OI, and HRT endometrial preparation protocol in 1071 FET cycles in normo-ovulatory females. In line with our study, there were no statistically significant differences in implantation rate or CPR between mNC and HRT groups. However, implantation rate was significantly higher in the OI group than in the mNC and HRT groups. The CPR was non-statistically higher in the OI group than in the other 2 groups. This study differs from ours in that the OI group was supplemented by 75 IU HMG after letrozole. HMG might improve the luteal phase and endometrial receptivity (44). In addition, females in the OI group had a significantly lower BMI than the other 2 groups. Moreover, significantly more blastocyst-stage and top-quality embryos were transferred in the OI group than in the other 2 groups.

In fact, while evaluating the benefits of an endometrial preparation protocol, reproductive outcomes are not the sole concern; one must also account for its convenience, affordability, and risk of complications. The HRT protocol might still be the first choice for women in FET cycles as it can be more easily scheduled, and might decrease cancellation rates relative to other FET protocols (45). Nonetheless, these benefits are partially mitigated by its potential negative consequences, including exposure to high doses of exogenous hormones with the risk of thromboembolic accidents in obese and high-risk patients, and high financial load on the patients.(17-19) So, in regularly menstruating women, it is preferred to find other options.

Although NC-FET protocol requires frequent clinic's visits for monitoring of follicular development and assessment of hormonal levels, it reduces financial burdens, drug utilization and complications. So, NC is easier, cheaper, and more physiological than other endometrial preparation protocols. In normo-ovulatory women, our study confirmed that, the mNC protocol could yield comparable reproductive outcomes to other endometrial preparation protocols. Considering these, we recommend the mNC protocol for endometrial preparation before FET as the protocol of choice in normo-ovulatory women.

The limitations of the current study are the small sample size and the brief duration of follow-up, which precludes the assessment of the live birth rate. Large-scale randomized clinical trials with longer follow-up periods are needed to confirm our findings and assess the live birth rate in different endometrial preparation protocols.

Conclusion

In normo-ovulatory women, The HRT, OI, and mNC endometrial preparation FET protocols result in comparable reproductive outcomes. In this study, although there were no statistically significant differences between the studied groups in terms of implantation, clinical pregnancy, and ongoing pregnancy rates, there was a marginal trend toward improved reproductive outcomes in the mNC group. It is worth conduction of further studies with larger sample sizes to confirm the conclusions of this study and verify the advantages of the mNC-FET protocol. Also, further studies with longer periods of follow-up are needed to compare the live birth rate among different endometrial preparation protocols.

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Conflict of Interest: The authors declare that they have no competing interests.

Ethics Approval: Approval was obtained from the ethics committee of Faculty of medicine, Alexandria University in 2022. Approval number was 0201668.

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Consent to Participate: Informed consent was obtained from all participants included in the study.

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