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Role of intramuscular progesterone supplementation on the day of embryo transfer in artificial frozen cycles

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Abstract

Background Sufficient endometrial preparation with or without progesterone supplementation is crucial in artificial cycles with frozen embryo transfer (FET). We aimed to study the effect of intramuscular progesterone supplementation on the day of embryo transfer (ET) in artificial frozen cycles.

Methods A clinical cohort study involved women undergoing FET with artificially prepared endometrium. Serum progesterone levels were assessed on the day of ET. Accordingly, we recruited 177 women with progesterone levels more than 9.2 ng/ml in group 1, and 177 other women with progesterone levels less than 9.2 ng/ml in group 2. Women in group 1 received only 400 mg vaginal progesterone twice-daily after ET, while women in group 2 received additional intramuscular progesterone supplementation. The chemical, clinical, and ongoing pregnancy rates, as well as the pregnancy loss rate, were assessed in both groups.

Results Expectantly, both groups showed a significant difference regarding the serum progesterone level on the day of ET (13.43 ± 4.65 vs 4.62 ± 2.77, $P = 574$). However, with additional intramuscular progesterone supplementation in group 2, both groups showed no significant difference regarding the chemical pregnancy rate (68.93% in group 1 vs 63.84% in group 2, $P = 0.311$), the clinical pregnancy rate (61.02% in group 1 vs 58.76% in group 2, $P = 0.664$), ongoing pregnancy rate (56.50% in group 1 vs 53.11% in group 2, $P = 0.522$), and pregnancy loss rate (7.41% in group 1 vs 9.62% in group 2, $P = 0.564$).

Conclusions Intramuscular progesterone supplementation in women with decreased serum progesterone levels could improve pregnancy outcomes in artificial frozen cycles.

Trial registration It was first registered at ClinicalTrials.gov on 8/4/2021 with registration number NCT04837768.

Keywords Progesterone support, Luteal phase support, Artificial frozen cycle

Introduction

Cryopreservation of frozen-thawed embryos has become a cardinal procedure in assisted reproductive therapies. Therefore, it can be attributed to the development of the vitrification process and the improvement of embryo survival rates after thawing, leading to a progressive spread in the use of frozen embryo transfer (FET) [1].

Elective embryo cryopreservation or "freeze all" technique was designed primarily for women at high risk of ovarian hyperstimulation syndrome (OHSS) [2]. Its use

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has now been extended to cover other indications, such as cycles involving pre-implantation genetic diagnosis, late-follicular increased progesterone levels [3], and embryo-endometrial asynchrony [4]. Moreover, the need for FET has increased greatly due to the implementation of the single embryo transfer (ET) policy in many areas worldwide to reduce multiple pregnancies. This strategy has led to an increase in surplus embryos derived from ovarian stimulation cycles [5].

Despite this increased need for FET, there is a continuous debate on the best method to prepare the endometrium for ET to improve pregnancy outcomes in the artificial frozen cycle, whether through the natural cycle (NC) or the hormone replacement therapy (HRT) cycle with oestrogen and progesterone supplementation [6]. Exogenous administration of oestrogens and progesterone is used in an artificial cycle to mimic a natural cycle and, therefore, endometrial development.

In the HRT cycle, also known as the artificial cycle, progesterone supplementation begins once the endometrium has grown sufficiently with oestrogen medication. Progesterone is given to enhance the final stage of endometrial preparation before ET; however, there is no consensus on the ideal route or dose of progesterone supplementation [7].

The advantage of the HRT cycle is the easy scheduling and minimal cycle monitoring. There are, however, potential disadvantages associated with its widespread usage, including the increased expense and risks of oestrogen supplementation, such as thromboembolic events [8].

This study investigates whether intramuscular progesterone supplementation will improve the chemical, clinical, and ongoing pregnancy rates in cases with decreased serum progesterone levels on the day of ET in artificial frozen cycles.

Methods

A clinical cohort study was conducted from July 2021 to March 2022 in the Assisted Reproduction Unit at the Obstetrics and Gynaecology Hospital at the Faculty of Medicine of Cairo University, after approval of the Research Ethics Committee with reference number (MD-124–2020), then the study was registered at Clinical trial.gov (NCT04837768). A total of 354 women undergoing FET with artificially prepared endometrium (HRT FET cycles) were recruited after obtaining informed consent to participate in the study. The primary outcome was the chemical and clinical pregnancy rates, while the secondary outcome was the ongoing pregnancy rate.

The inclusion criteria were restricted to infertile women aged 20–40 years, BMI of less than 40 kg/m^2, an endometrial thickness of more than 7 mm, and double ET (day 5) of grade 1 or 2. Women with autoimmune diseases, uncontrolled medical conditions, recurrent implantation failure, or anatomical uterine abnormalities (polyps, fibroids, or Müllerian anomalies) were excluded.

The recruited women received artificial endometrial preparation starting from day 2/3 of their cycle in the form of 2 mg Oestradiol Valerate pills three times daily (white pills of Cyclo-Progynova®, Bayer, Germany) and resumed for 7–10 days. After that, transvaginal 2D ultrasound was done using a Mindray DP-5 (50/60 Hz) model ultrasound to ensure the endometrial thickness greater than 7 mm. Progesterone supplementation was initiated via Prontogest® 400 mg vaginal pessaries twice daily (Prontogest®, Marcyrl, Egypt) for five complete days, ending with the day of ET.

On the morning of ET, a blood sample was drawn from the recruited women and sent to the laboratory for serum progesterone measurement. Women with more than 9.2 ng/ml serum progesterone levels were assigned to group 1, while those with serum progesterone levels less than 9.2 ng/ml were assigned to group 2. Patient recruitment in each group was stopped when the calculated sample size in each group was reached.

Women in group 1 (progesterone > 9.2 ng/ml) received only 400 mg vaginal progesterone twice-daily until quantitative β-HCG was done 14 days after the date of ET. On the other hand, women in group 2 (progesterone < 9.2 ng/ml) received intramuscular progesterone (Prontogest® 100 mg, IBSA, Egypt) twice-weekly in addition to the standard twice-daily vaginal progesterone, and both regimens continued until quantitative β-HCG was done 14 days after the date of ET.

For all patients in both groups, if confirmed β-HCG positive, an ultrasound was done to confirm/exclude clinical pregnancies after 4 weeks from the day of ET (primary outcome). If clinical pregnancy was confirmed, luteal phase support was continued until 12 weeks of gestation to detect the ongoing pregnancy rate (secondary outcome), after which luteal phase support was discontinued owing to the dominance of placental steroidogenesis by this time. Luteal phase support was discontinued if chemical or clinical pregnancy was not established.

Sample size

Sample size was calculated to detect a 16% difference (50–66%) in the clinical pregnancy rate between the two groups according to the serum progesterone level (< 9.2 ng/ml versus ≥ 9.2 ng/ml) [9] at a 95% level of confidence and an 80% power of the study. Calculation is performed using the following equation [10]: $n = \left[\frac{Z_{\alpha/2}+Z_{\beta}}{P_1-P_2}\right]^2 (p1q1 + p2q2)$, where n = sample size in each group, Zα/2 = critical value of the Z distribution

corresponding to the 95% level of confidence (=1.96), Zβ = critical value of the Z distribution corresponding to 80% power of the study (=0.84), p1 = clinical pregnancy rate in the group with serum progesterone levels < 9.2 ng/ml (50%) [9], p2 = clinical pregnancy rate in the group with serum progesterone levels ≥ 9.2 ng/ml (66%) [9], qi = 1-pi. Accordingly, the calculated sample size is 150 patients for each group. After considering 15% for drop-outs, a total of 177 patients in each group were recruited.

Statistical analysis

Data was analysed using the Statistical Package for Social Sciences (SPSS) version 25. Categorical variables were described as frequency and percentages, while quantitative variables were described as mean ± standard deviation with median and range. The differences between the two groups were tested for statistical significance through the chi-square test for qualitative variables and the unpaired Student's *t* test/Mann–Whitney test for quantitative variables. *P* values less than 0.05 were considered statistically significant at a 95% confidence level.

Results

During the time between July 2021 and March 2022, we recruited 177 women with serum progesterone more than 9.2 ng/ml on the day of ET to be assigned to group 1, as well as recruited 177 women with serum progesterone less than 9.2 ng/ml to be assigned to group 2 (Fig. 1).

The demographic data are shown in Table 1. There was no significant difference between both groups regarding age (*P* = 0.225), BMI (*P* = 0.826), gravidity (*P* = 0.824), parity (*P* = 0.891), infertility type (*P* = 0.749), and infertility duration (*P* = 0.723). The characteristics of IVF cycles are shown in Table 2, showing two good-quality embryos were transferred for each participant in both groups). There was no significant difference between both groups regarding the number of gestational sacs (*P* = 574). Expectantly, both groups showed a significant difference regarding the serum progesterone level on the day of ET (13.43 ± 4.65 vs 4.62 ± 2.77, *P* = 574).

Regarding the clinical outcomes in both groups, as shown in Table 3, There was no significant difference between both groups regarding the chemical pregnancy rate (68.93% in group 1 vs 63.84% in group 2, *P* = 0.311),

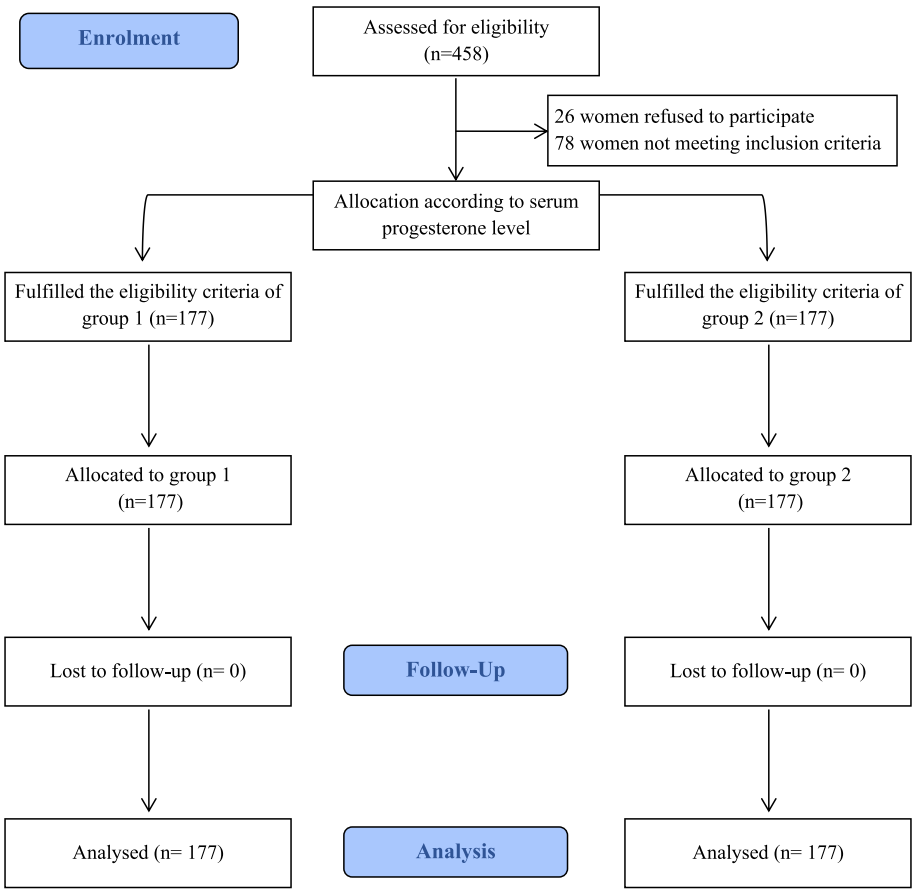

Fig. 1 Study enrolment and outcome

Table 1 The demographic characteristics of the two groups

	Group 1 ($n=177$)	Group 2 ($n=177$)	*P* value
Age (years)	30.06 ± 5.02 29 (19–39)	30.69 ± 4.68 31 (18–39)	0.225
BMI	28.01 ± 5.24 27.9 (17.3–40.4)	27.89 ± 4.79 27.9 (19–40.1)	0.826
Gravidity	0.98 ± 1.21 0 (0–5)	0.95 ± 1.18 0 (0–4)	0.824
Parity	0.52 ± 0.76 0 (0–3)	0.53 ± 0.78 0 (0–3)	0.891
Duration of infertility (years)	2.96 ± 1.29 3 (1–5)	3.01 ± 1.40 3 (1–7)	0.723
Type of infertility			
- Primary	93 (52.54%)	96 (54.24%)	0.749
- Secondary	84 (47.46%)	81 (45.76%)	

the clinical pregnancy rate (61.02% in group 1 vs 58.76% in group 2, $P=0.664$), ongoing pregnancy rate (56.50% in group 1 vs 53.11% in group 2, $P=0.522$), and pregnancy loss rate (7.41% in group 1 vs 9.62% in group 2, $P=0.564$).

Discussion

There still exists debate over the ideal route to prepare the endometrium for ET. There are limited data comparing IM versus vaginal progesterone administration, with some favouring the IM route [11] and others showing no significant differences in terms of outcome [7]. However, a more recent focus on intramuscular rather than transvaginal progesterone supplementation stems from newer mounting evidence that transvaginal supplementation not only increases vaginal discharge but also has a limited effect in increasing serum progesterone levels [12].

Labarta et al. (2017) conducted a prospective cohort study with similar parameters to ours but on oocyte donation cycles. They recruited 244 patients with an artificial endometrial preparation cycle via oestradiol valerate and vaginal progesterone (400 mg/12 h), similar to group 2 of our study. Serum progesterone was also withdrawn on the day of ET, and the mean level was found to be 12.7 ± 5.4 ng/ml. They investigated the relationship between serum progesterone levels on the day of ET and the ongoing pregnancy rate. Their study recommended a minimum serum progesterone threshold (9.2 ng/ml) to optimise the ongoing pregnancy rate in artificial cycles using transvaginal progesterone [9]. This value could theoretically be used to decide whether ET should be postponed or not.

Therefore, based on the study mentioned above that suggests that lower serum progesterone levels on the day of ET are associated with poorer pregnancy outcomes, we can deduct from our current study that the addition of intramuscular progesterone supplementation to the vaginal supplementation in those patients does in fact improve their pregnancy rates. This finding is illustrated by the lack of statistical significance between the two arms of our study. Despite group 1 having a mean serum progesterone level of 13.43 ng/ml, which is almost triple that of group 2 at 4.62 ng/ml, the pregnancy outcomes in group 2 with additional intramuscular progesterone supplementation showed no significant differences when compared to group 1, including the chemical pregnancy rate (68.93% in group 1 vs 63.84% in group 2, $P=0.311$), the clinical pregnancy rate (61.02% in group 1 vs 58.76% in group 2, $P=0.664$), ongoing pregnancy rate (56.50% in group 1 vs 53.11% in group 2, $P=0.522$), and pregnancy loss rate (7.41% in group 1 vs 9.62% in group 2, $P=0.564$).

Boynukalin et al. (2019) underwent a recent prospective cohort study involving day 5 single FET cycles. Their participants received HRT in the form of oestradiol valerate and 100 mg intramuscular progesterone till the 7th and 10th week of pregnancy, respectively. Serum progesterone was also analyzed immediately prior to ET, and their primary outcome was the presence or absence of an ongoing pregnancy at 16 weeks gestation. They found that the mean progesterone level on the day of ET was significantly higher in patients who later had an ongoing pregnancy (28 ng/ml) than those who did not have an

Table 3 Clinical outcomes in both groups

	Group 1 ($n=177$)	Group 2 ($n=177$)	*P* value
Chemical pregnancy	122 (68.93%)	113 (63.84%)	0.311
Clinical pregnancy	108 (61.02%)	104 (58.76%)	0.664
Ongoing pregnancy	100 (56.50%)	94 (53.11%)	0.522
Pregnancy loss	8/108 (7.41%)	10/104 (9.62%)	0.564

Table 2 Characteristics of IVF cycles

	Group 1 ($n=177$)	Group 2 ($n=177$)	*P* value
Serum progesterone (ng/ml)	13.43 ± 4.65 11.97 (9.25–40)	4.62 ± 2.77 3.56 (1.52–9.15)	< 0.001
Number of transferred embryos	2	2	N/A
Number of gestational sacs	0.66 ± 0.57 1 (0–2)	0.63 ± 0.56 1 (0–2)	0.574

ongoing pregnancy (16.4 ng/ml) with a p value of 0.039. They did, however, conclude that further investigation is needed for the individualisation of intramuscular progesterone doses to reach optimal pregnancy rates [13].

These findings support those of our study in that higher serum progesterone levels and supplementation are associated with superior pregnancy rates. It should be noted, however, that Boynukalin et al. (2019) provided all their progesterone supplementation in the form of intramuscular progesterone and did not use the vaginal dosing of progesterone twice-daily [13]. Furthermore, their cycles involved single ETs, whereas we used double ETs in our study. However, this was standardised in each study respectively and, therefore, should not affect any patterns deduced from our relative statistics.

Yovich et al. (2015) also investigated both serum progesterone and oestradiol levels in single FET HRT cycles, but hormonal levels in this study were sampled in the 'mid-luteal' phase, which they defined as being 8–9 days after progesterone pessary administration or 2–3 days after the ET. HRT was in the form of estradiol valerate tablets, in addition to vaginal pessaries containing both progesterone and estradiol. Clinical pregnancies and live birth rates were the main outcomes of the study, and the results showed that the pregnancy and birth rates in HRT FET cycles are highly dependent upon the circulating concentration of progesterone, with an optimal peak in progesterone concentration of 22–31 ng/ml. They also detected that mid-luteal serum progesterone levels > 31 ng/ml were associated after that with a significant decrease in pregnancy rates (p value 0.0047) and a non-significant reduction in live birth rates [14].

Contrary to the findings in our study, Kofinas et al. (2015) studied serum progesterone levels in single FET HRT cycles, where progesterone levels were analysed on the day of ET. They concluded that pregnancy and birth rates were inversely proportional to the serum progesterone level on the day of ET, with a downward trend in these rates as serum progesterone increased, particularly above the 40 ng/ml mark [15]. It is worth noting, however, that the progesterone supplementation in this study was exclusively intramuscular, as with Boynukalin et al. (2019), with the dosing varying between 50 and 75 mg daily, being continued till 9 weeks gestation [13]. Finally, another difference in methodology between Kofinas et al. (2015) and our study was the form of assisted reproductive technology undertaken. The majority of patients involved with Kofinas et al. (2015) underwent standard insemination technique as opposed to ICSI, as well as undergoing single, not double, embryo transfers [15].

Thus, more attention has been given to measuring serum progesterone during HRT FET cycles. Kofinas et al. (2015) suggested that progesterone levels > 20 ng/ml on the day of ET are associated with a reduction in the ongoing pregnancy and live birth rates [15]. This observation could be attributed to ovulation escape and subsequent embryo-endometrial asynchrony. On the other hand, Yovich et al. (2015) proposed that the optimal mid-luteal progesterone range from 22 to 31 ng/ml [14].

The main limitation of this study is that we could not design the study as a randomized controlled trial, with one group given intramuscular progesterone and the other without intramuscular progesterone. This could be explained by the fact that we had to provide the optimum management and the utmost care to all participants in the study. We had not to deprive these patients of the intramuscular progesterone, especially since they needed the luteal phase support. In addition, we did not study the side effects of progesterone injection, as we focused mainly on the great benefits of intramuscular progesterone for those patients with low progesterone levels.

Conclusion

Additional intramuscular progesterone supplementation is particularly important for patients with low progesterone levels undergoing artificial frozen cycles and gives promising pregnancy results similar to those patients with higher progesterone levels.

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None.

Authors' contributions
AB, FA, AHM, and RMF designed, conducted, and supervised the study. AE conducted the study and analyzed the data. MAR analyzed the data. All authors wrote, read, and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from Kasr El-Ainy Hospital, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are, however, available from the authors upon reasonable request and with permission of Kasr El-Ainy Hospital.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Research Ethics Committee with reference number (MD-124–2020). All methods were carried out in accordance with relevant guidelines and regulations. All participants gave their consent after being informed of the study's objective and design, and they were given the option to leave the study at any time.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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