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Proportion and Pregnancy Outcomes of Rescued Frozen-Thawed Cycles with Low Serum Progesterone Levels: A Cross-Sectional Study

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Abstract

Background: Low serum progesterone concentration on the day of frozen embryo transfer (FET) has been associated with adverse pregnancy outcomes. Progesterone supplementation has been shown to improve the outcomes in these cycles. The purpose of the current study was to investigate the prevalence, pregnancy outcomes, and factors associated with rescued FET cycles involving low serum progesterone concentrations.

Methods: A cross-sectional study was conducted on 367 FET cycles with hormonal endometrium preparation (oral estradiol+vaginal progesterone) at Hung Vuong Hospital, Vietnam, from October 2022 to February 2023. Serum progesterone concentrations were measured on the day of FET. All cycles with serum progesterone <10 *ng/ml* were supplemented with intramuscular progesterone according to the hospital protocol, and outcomes were subsequently observed.

Results: The prevalence of cycles with low serum progesterone concentration was 71.66% (263/367). Factors associated with low serum progesterone were female body weight (ORadj=1.04; 95%CI: 1.0006–1.07) and duration from the last progesterone dose to blood sampling (ORadj=1.11; 95%CI: 1.03–1.19). Despite being rescued with progesterone supplementation, cycles with serum progesterone <10 *ng/ml* had significantly lower chemical (ORadj=0.52; 95%CI: 0.31–0.89), clinical (ORadj=0.54; 95%CI: 0.31–0.93) and ongoing (ORadj=0.54; 95%CI: 0.31–0.94) pregnancy rates.

Conclusion: A high prevalence of frozen-thawed cycles with low serum progesterone concentrations was observed in this study, which was associated with female body weight and duration from the last progesterone dose to blood sampling. Despite progesterone rescue, lower pregnancy rates were detected in cycles with serum progesterone <10 *ng/ml*.

Keywords: Artificial cycle, Frozen embryo transfer, Rescue, Serum progesterone.
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Introduction

The production and secretion of progesterone by the corpus luteum are crucial for proper transformation of endometrium, which is necessary to achieve and maintain pregnancy. In an artificial cycle, exogenous progesterone is administered in combination with estradiol to prepare the endometrium for frozen embryo transfer.

A previous study reported that serum progesterone levels on the day of frozen embryo transfer in artificial cycles were significantly lower than those observed in spontaneous or ovarian stimulation cycles (1). In such cycles, low serum progesterone levels are associated with decreased pregnancy and live birth rates, and increased miscar-

riage rate (2, 3). Approximately 37% of cycles have been reported to exhibit serum progesterone levels below 10 *ng/ml* on the day of embryo transfer (2). Several factors have been linked to serum progesterone levels on the day of frozen embryo transfer, including patient age, height, and the duration of hormone replacement therapy (3). Ideally, detecting low serum progesterone levels could help identify high-risk cases for individualized treatment, such as administering an additional "rescue" dose of progesterone. Various routes of progesterone supplementation including vaginal, subcutaneous, intramuscular, and oral administration have been investigated. However, research has shown controversial results on the effectiveness of progesterone rescue in cases of low serum progesterone levels (4).

At Hung Vuong Hospital, cycles with low progesterone levels prior to embryo transfer are routinely managed with daily intramuscular progesterone supplementation as part of standard clinical practice. Although such practice is relatively common in Vietnam, the paucity of supporting scientific evidence underscored the necessity for conducting this research. In this article, an attempt was made to identify the proportion of frozen-thawed cycles with low serum progesterone levels on the day of embryo transfer, as well as the associated factors. Moreover, the pregnancy outcomes of cycles with low serum progesterone levels that were rescued with intramuscular progesterone supplementation, in accordance with our hospital protocol, were also evaluated. To the best of our knowledge, no studies have been conducted in Vietnam to investigate cycles with low serum progesterone levels rescued with progesterone supplementation.

Methods

Design and setting: This cross-sectional study was approved by the Institutional Review Board of Hung Vuong Hospital, Vietnam (Approval Letter No 5313/HĐĐĐ-BVHV). The study was conducted at Hung Vuong Hospital between October 2022 and February 2023.

Study population: Our target population included patients with infertility undergoing IVF and frozen embryo transfer who underwent endometrial preparation using exogenous estradiol and progesterone at Hung Vuong Hospital, Vietnam.

Sample size calculation: The sample size was calculated using the formula for estimating proporti-

ons in a population, with the proportion of frozen embryo transfer cycles exhibiting low serum progesterone levels (<10 *ng/ml*) on the day of transfer estimated to be 37% (2). A cutoff value of 10 *ng/ml* for progesterone levels was selected based on the evidence from multiple studies indicating that serum progesterone levels below 8.8–10 *ng/ml* are associated with adverse treatment outcomes (2, 3, 5). This threshold is also widely used for diagnosing luteal phase deficiency in natural conception cycles, given its practicality and ease of application in clinical settings. Hung Vuong Hospital has incorporated this cutoff into its clinical practice protocols. The minimum sample size required was 360 frozen embryo transfer cycles.

Sampling method: All Vietnamese women aged 18 years or older, undergoing IVF with frozen embryo transfer and artificial endometrial preparation using exogenous hormones at Hung Vuong Hospital, were enrolled from the start of the study in October 2022 until the minimum required sample size (N=360) was reached. Exclusion criteria included women who were unable to undergo embryo transfer due to acute conditions requiring treatment (*e.g*., abnormal uterine bleeding or genital tract infections), non-compliance with treatment protocols (*e.g*., missed medication doses or incorrect administration), or uterine abnormalities (*e.g*., uterine malformations, endometrial polyps, fibroids, or adenomyosis). From October 2022 to February 2023, a total of 367 frozen embryo transfer cycles were included in the study.

Outcomes: The primary outcome was the proportion of frozen embryo transfer cycles with low progesterone levels on the day of transfer, defined as <10 *ng/ml*. Patient characteristics such as age, female body weight (*kg*), height (*cm*), history of infertility treatment, indications for treatment (tubal obstruction, polycystic ovary syndrome, endometriosis, oligomenorrhea), anti-Müllerian hormone (AMH) levels (*ng/ml*), oocyte donation, and the hormonal regimen used in the FET cycles were recorded to investigate their relationship with low progesterone levels.

Early pregnancy outcomes were assessed, including chemical pregnancy (defined as serum ß-hCG ≥5 *mIU/ml*), clinical pregnancy (presence of at least one gestational sac with a detectable heartbeat on ultrasound at 7 weeks), and ongoing pregnancy (characterized by the continuation of a

viable pregnancy beyond 12 weeks of gestation). The relationship between various factors including serum progesterone levels, age, serum AMH levels, oocyte donation, endometrial thickness, progesterone dosage and timing, embryo developmental stage at transfer, as well as embryo quality and number was analyzed in relation to pregnancy outcomes. Embryo morphology was assessed using the "Cummins criteria" for Day 3 embryos and the "Gardner and Schoolcraft grading system" for blastocysts.

Study protocol

Endometrial preparation: On the second day of the menstrual cycle, the patient was prescribed estradiol valerate (Valiera) 2 *mg* orally once daily. Every 5 days, the patient underwent ultrasound evaluation of the endometrial lining. If the endometrial lining was <7 *mm* thick, the dose of estradiol valerate was increased to 2 *mg* orally twice daily or two tablets once daily. When the endometrial lining was ≥ 8 *mm* thick with a triple-line pattern, the patient was administered vaginal progesterone (Utrogestan 200 *mg*, Cyclogest 200 *mg* or 400 *mg*, or Crinone 8%) for 3 days prior to Day 3 embryo transfer or for 5 days prior to Day 5 embryo transfer. The type of medication, dosage, and duration of progesterone use were recorded as variables to investigate their association with low progesterone levels in the logistic regression model.

Hormone measurement: A 2 *ml* venous blood sample was collected from the patients at 8:00 AM on the same day, prior to embryo transfer, to quantify serum progesterone levels using the electrochemiluminescence immunoassay (ECLIA) method (Cobas® E 801; Roche Diagnostics, Germany). The assay has a measurable range of 0.05-60 *ng/ml*, with an intra-assay variability of 2-6%, and an inter-assay variability of 2-4%.

Embryo transfer and follow-up: Embryo transfer was performed on day 4 (for Day 3 embryo transfer) or day 6 (for Day 5 embryo transfer) after the initiation of progesterone. After thawing, a maximum of two embryos were transferred into the uterus under abdominal ultrasound guidance. Estradiol and progesterone for luteal phase support were continued until the day of β-hCG testing (after 2 weeks). According to our hospital protocol, all cycles with low serum progesterone levels (<10 *ng/ml*) were supplemented with intramuscular administration of progesterone at a dose of 25 *mg/per* day, initiated after embryo

transfer and continued until β-hCG testing. If the pregnancy test was positive, the luteal phase support regimen was maintained until 12 weeks of gestation.

Statistical analysis: The data was collected using Microsoft Excel software and analyzed with R 4.3.0 software. Variables with a p-value <0.05 in the multivariate logistic regression model were identified as significantly correlated with low serum progesterone levels. Similarly, multivariate logistic regression models were applied to assess the association between pregnancy outcomes and multiple predictors, including serum progesterone levels.

Results

Between October 2022 and February 2023, 367 frozen embryo transfer cycles with artificial endometrial preparation were included in the study. Low serum progesterone levels (<10 *ng/ml*) were observed in 71.66% of cycles. The mean serum progesterone level on the day of transfer was 8.2± 0.45 *ng/ml*. Baseline characteristics and treatment details are presented in table 1.

Most participants were aged 31–40 years (58.86%). Mean weight and height were 54.66± 0.79 *kg* and 156±0.52 *cm*, respectively. A history of previous embryo transfer was observed in 54.22% of cycles (199/367), with a mean of 0.86± 0.06 prior transfers. The most common infertility causes were ovulation disorders (23.98%), tubal obstruction (22.34%), polycystic ovary syndrome (13.62%), and endometriosis (7.9%). Mean serum AMH was 3.88±0.31 *ng/ml*. The mean estradiol treatment duration before embryo transfer was 19.95±0.4 days. The average endometrial thickness at the initiation of implantation window was 10.53±0.13 *mm*. Crinone 8% gel was the most frequently used progesterone preparation (49.04%), followed by Utrogestan 200 *mg* (46.87%) and Cyclogest 200/400 *mg* (4.09%). The mean daily progesterone dose was 365.31±26.15 *mg*, administered 2.09±0.05 times per day. The mean interval between the last progesterone dose and serum sampling was 11.39±0.5 *hr*.

In table 2 multivariate logistic regression identified two in-dependent variables correlated with low serum progesterone levels (<10 *ng/ml*) on the day of embryo transfer. These variables included each 1 *kg* increase in weight (OR 1.04; 95%CI: 1.0006–1.07; p=0.05) and each 1-*hr* increase in the interval between the last progesterone dose

Table 1. The demographic characteristics and treatment details

Variables	p-value
Age (years)	32.84±0.52
Female body weight (*kg*)	54.66±0.79
Polycystic ovary syndrome	13.62%
Tubal obstruction	22.34%
Oligomenorrhea	23.98%
Serum AMH levels (*ng/ml*)	3.88±0.31
Oocyte donation	5.18%
Endometrial thickness at the onset of progesterone administration (*mm*)	10.53±0.13
Types of vaginal progesterone	
Utrogestan® 200 *mg*	46.87%
Cyclogest® 200 or 400 *mg*	4.09%
Crinone® 8%	49.04%
Progesterone dosage (total mg per day)	365.31±26.15
Progesterone dosage (times per day)	2.09±0.05
Embryo developmental stage	
Day 3 embryo	21.8%
Day 5 embryo	78.2%
Number of embryo transfers	
1	72.48%
2	27.52%
Number of high-quality embryo transfers	
0	35.15%
1	62.94%
2	9.1%
Duration from the last progesterone dose to blood sampling (*hr*)	11.39± 0.5

Table 2. Multivariate logistic regression analysis of multiple factors correlated with low serum progesterone levels on the day of frozen embryo transfer

Variables	Low serum progesterone levels	
	OR$_{adj}$ (95%CI)	p-value
Female body weight (*kg*)	1.04 (1.0006-1.07)	0.05
Polycystic ovary syndrome	1.25 (0.4-4.03)	0.7
Tubal obstruction	1.5 (0.8-2.93)	0.22
Oligomenorrhea	1.39 (0.61-3.31)	0.44
Serum AMH levels (*ng/ml*)	1.06 (0.95-1.19)	0.33
Oocyte donation	3.18 (0.76-22.09)	0.16
Types of vaginal progesterone		
Utrogestan® 200 *mg*	1	-
Cyclogest® 200/400 *mg*	0.26 (0.02-2.39)	0.24
Crinone® 8%	1.83 (0.13-46.92)	0.66
Progesterone dosage (total mg per day)	1 (0.99-1.009)	0.95
Progesterone dosage (times per day)	0.72 (0.19-2.51)	0.6
Duration from the last progesterone dose to blood sampling	1.11 (1.03-1.19)	0.004

and blood sampling (OR 1.11; 95%CI: 1.03–1.19; p=0.004). Table 3 shows that chemical (ORadj= 0.52; 95%CI: 0.31–0.89), clinical (ORadj=0.54; 95%CI: 0.31–0.93), and ongoing (ORadj=0.54; 95%CI: 0.31–0.94) pregnancy rates were all correlated with low serum progesterone levels. These

Table 3. Logistic regression analysis of multiple factors correlated with pregnancy outcomes in frozen-thawed cycles with hormonal endometrium preparation

	Chemical pregnancy		Clinical pregnancy		Ongoing pregnancy	
	OR_{adj} (95% CI)	p	OR_{adj} (95%CI)	p	OR_{adj} (95%CI)	p
Low serum progesterone levels	0.52 (0.31–0.89)	0.02	0.54 (0.31–0.93)	0.03	0.54 (0.31–0.94)	0.03
Age (year)	0.95 (0.9–1)	0.08	0.97 (0.91–1.02)	0.23	0.95 (0.9–1)	0.1
Serum AMH levels (*ng/ml*)	1.04 (0.96–1.13)	0.34	1.05 (0.97–1.14)	0.26	1.04 (0.96–1.12)	0.36
Oocyte donation	1.03 (0.3–3.24)	0.97	0.79 (0.19–2.71)	0.72	0.9 (0.22–3.12)	0.88
Types of vaginal progesterone						
Utrogestan® 200 *mg*	1	-	1	-	1	-
Cyclogest® 200/400 *mg*	2.83×10^{-6} (0–6 x 10^{39})	0.99	1.13×10^{-5} ($0^{-\infty}$)	0.99	1.1×10^{-5} ($0^{-\infty}$)	0.99
Crinone® 8%	2.19×10^{6} ($0^{-\infty}$)	0.99	2.41×10^{6} ($0^{-\infty}$)	0.99	2.3×10^{6} ($0^{-\infty}$)	0.99
Progesterone dosage (total *mg per day*)	1.04 ($0.72^{-\infty}$)	0.99	1.04 ($0.72^{-\infty}$)	0.99	1.04 ($0.72^{-\infty}$)	0.99
Progesterone dosage (times per day)	0.03 ($0–2\times10^{11}$)	0.99	0.05 ($0^{-\infty}$)	0.99	0.05 ($0^{-\infty}$)	0.99
Embryo developmental stage						
Day 3 embryo	1	-	1	-	1	-
Day 5 embryo	2.5 (1.28–5.1)	0.01	2.85 (1.35–6.43)	0.01	3 (1.39–6.97)	0.01
Number of embryo transfers						
1	1	-	1	-	1	-
2	0.65 (0.35–1.2)	0.17	0.57 (0.28–1.1)	0.1	0.6 (0.29–1.17)	0.14
Number of high-quality embryo transfers						
0	1	-	1	-	1	-
1	1.76 (1–3.1)	0.05	1.41 (0.78–2.59)	0.26	1.43 (0.79–2.65)	0.24
2	4.35 (0.82–25.39)	0.08	6.2 (1.14–37.14)	0.03	6.5 (1.18 – 39.5)	0.03
Endometrium thickness (*mm*)	1.17 (0.97–1.4)	0.1	1.2 (0.99–1.45)	0.06	1.16 (0.96–1.4)	0.13
Duration from the last progesterone dose to blood sampling	1 (0.95–1.07)	0.88	1 (0.94–1.07)	0.95	1 (0.94–1.07)	0.89

findings demonstrate that low serum progesterone levels are significantly associated with poorer pregnancy outcomes, despite progesterone rescue. In the multivariate logistic regression model, other factors correlated with poor pregnancy outcomes included embryo developmental stage and the number of high-quality embryos transferred.

Discussion

In our study of 367 frozen embryo transfer cycles, the mean serum progesterone level on the day of transfer was 8.2±0.45 *ng/ml*, with 71.66% of cycles exhibiting low serum progesterone levels (<10 *ng/ml*). This prevalence was notably higher than that reported in previous international studies. Cédrin-Durnerin et al. reported an average serum progesterone level of 11.4±4 *ng/ml* on the day of embryo transfer, with 37% of cycles exhibiting low serum progesterone levels (<10 *ng/ml*) in a study of 227 frozen embryo transfer cycles (2). Labarta et al. surveyed 1105 embryo transfer cycles and found that serum progesterone levels on the day of embryo transfer was ≤9.9 *ng/ml* in 40% of cases (3). In Vietnam, a study by Vuong et al. showed that in artificial endometrial preparation cycles, 25% of cycles had serum progesterone levels <10.9 *ng/ml* on the day of embryo transfer (5). Thus, the observed rate of

71.66% is markedly higher than that reported in the literature. At Hung Vuong Hospital, ECLIA method was used to quantify progesterone, with a measurable range of 0.05-60 *ng/ml*, assay variability of 2-6%, and inter-assay variability of 2-4%, similar to the study by Vuong et al (5). Other researchers also quantified progesterone using ECLIA method, with the only difference being the version used (2, 3, 5). In terms of population characteristics, our study is similar to the study conducted by Vuong et al. regarding age, weight, height, as well as factors related to the causes and treatment history of infertility. Thus, the main reason for the difference in the rate of cycles with low serum progesterone levels may be related to the process of endometrial preparation. At Infertility Department of Hung Vuong Hospital, and similarly across many healthcare facilities in Vietnam, standardized protocols for progesterone administration to establish the implantation window are not yet universally implemented. In the absence of widely adopted evidence-based guidelines, progesterone regimens are frequently individualized based on clinicians' judgment and prior experience. In our study, three types of progesterone preparations (Crinone 8%, Utrogestan 200 *mg*, and Cyclogest 200 *mg*/400 *mg*) were utilized with varying frequencies and doses.

The majority of patients received Crinone 8% vaginal gel (90 *mg* natural progesterone) administered once daily, which may have contributed to prolonged intervals—often exceeding 12 *hr*—between the last progesterone dose and blood sampling. This extended interval could partially explain the high proportion of cycles with low serum progesterone levels observed. In cycles preparing the endometrium with vaginal progesterone, studies have shown rapid progesterone absorption, reaching peak serum levels within 3–6 *hr*, with a half-life of approximately 13 *hr* after administration 6-8. These findings align with our observation that a longer interval between the last progesterone dose and blood sampling increases the risk of low serum progesterone levels before embryo transfer. Therefore, the use of endometrial preparation protocols with excessively long intervals between progesterone administrations (such as with Crinone 8%) may have contributed to the significantly higher rate of cycles with low serum progesterone levels observed in our study.

Through multivariate analysis, two variables were identified as being associated with low serum progesterone levels on the day of embryo transfer: body weight and the time from the last progesterone dose to blood sampling. According to a study conducted by González-Foruria et al., factors related to low serum progesterone levels include age, weight, low serum progesterone levels in the previous cycle, and timing of blood tests during the day (9). Similar to González-Foruria et al.'s study, an inverse relationship between weight and serum progesterone levels was observed in our study. Specifically, an increase in weight was associated with an increased risk of low serum progesterone levels. Lower serum progesterone levels observed in overweight and obese patients may be explained by increased distribution relative to body weight, leading to reduced circulating concentrations. Moreover, as a lipophilic steroid hormone, progesterone is prone to sequestration in adipose tissue, which is typically elevated in individuals with higher BMI. Brady et al. also found similar results in cycles with thin endometrial lining (10).

No correlation was observed between age and the risk of low serum progesterone levels. According to Levy et al., a higher rate of vaginal progesterone absorption was observed in women over 40 years of age compared to younger individuals, possibly due to thinning and atrophy of the vaginal mucosa (8). In our study population, individuals over the age of 40 accounted for only 6.58% of cases, and therefore were considered to have minimal impact on the overall results. Additionally, a decline in progesterone levels was reported by González-Foruria et al. when blood sampling was performed later in the day, suggesting the presence of significant diurnal fluctuations. This aligns with our findings, in which a longer interval between the last progesterone dose and blood sampling or later embryo transfer timing was associated with an increased risk of low serum progesterone levels on the day of transfer. In hormonally prepared cycles, serum progesterone concentrations were entirely dependent on exogenous administration. Thus, it can be hypothesized that delayed embryo transfer or prolonged intervals from progesterone administration may increase the likelihood of suboptimal serum progesterone levels. A positive correlation between the number of progesterone doses per day and live birth rates was demonstrated in a previous study (11). This is also consistent with our findings, as a higher dosing frequency was associated with a shorter interval between the last progesterone administration and the time of

blood sampling.

Despite rescue with intramuscular progesterone, low serum progesterone levels are significantly associated with poorer pregnancy outcomes. A meta-analysis by Stavridis et al. reported that, overall, progesterone rescue was associated with clinical, ongoing, and live birth rates comparable to those observed in cycles with sufficient progesterone levels (4). However, subgroup analysis revealed that outcomes were not improved with intramuscular or vaginal progesterone rescue, whereas subcutaneous and oral supplementation were associated with results comparable to those of cycles with adequate progesterone levels. These findings are consistent with those observed in our study. This study has several limitations. Its cross-sectional design prevents causal inference and limits evaluation of different progesterone supplementation strategies. As data were collected at a single center, the findings may not be generalizable. Estradiol levels during endometrial preparation and the potential impact of supra-physiologic progesterone levels on pregnancy outcomes were not assessed, as they were beyond the study's scope. Future prospective, multicenter studies are needed to address these limitations and further clarify optimal progesterone management.

Conclusion

In this study, a high proportion of FET cycles with low serum progesterone levels was observed, and these levels were found to be correlated with female body weight and the interval between the last progesterone dose to blood sampling. Despite progesterone rescue, serum progesterone <10 *ng/ml* before FET correlated with poorer pregnancy outcomes. These findings underscore the importance of serum progesterone monitoring and individualized dose adjustment, particularly in women with elevated body weight, to minimize the risk of suboptimal progesterone levels on the day of embryo transfer.

Acknowledgement

Conflict of Interest

The authors declare no conflict of interest.

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