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# Measuring the Level of the Hormone Progesterone on the Day of Transfer of Fresh and Frozen Embryos and its Impact on Pregnancy Outcomes

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**Abstract**
**Objective:** Prediction of the effect of the hormone progesterone on pregnancy results that measured on the day of transfer of fresh and frozen embryos.
**Methods:** This research at reproductive centres in Baghdad from May 2022 to April 2023 examined how serum progesterone levels affect embryo transfers in ICSI procedures in 100 fresh or frozen cycle women. The study examined clinical data and endometrial preparation techniques to determine if progesterone levels affect pregnancy rates. Data were analysed using SPSS to determine how pre-transfer hormonal levels affected implantation and pregnancy outcomes. Participants were selected according on inclusion and exclusion criteria. The study shows that progesterone is essential for IVF embryo transfers.
**Results:** Compared to frozen cycles in females, fresh cycles had greater mean age and progesterone levels but lower COC number and 2PN levels, with no changes in BMI, MII, or injectable MII. Frozen cycles produced more good-grade embryos, whereas fresh cycles produced more fair-grade embryos. There were no differences in the number of "C" embryos. Fresh cycle groups had greater pregnancy rates (59.1%) than frozen cycle groups (38.9%), but progesterone levels did not change between cycle types.
**Conclusion:** The study found that fresh cycles include older participants, greater progesterone but lower COC and 2PN levels, and higher pregnancy rates than frozen cycles. BMI, MII levels, embryo count, poor grade "C" embryos, and progesterone levels associated to pregnancy outcomes did not differ across groups.
**Keywords:** Progesterone, transfer, fresh, frozen, embryos, impact, pregnancy outcomes

## Introduction

When exploring the complex landscape of assisted reproductive technologies (ART), the role of hormonal environments cannot be overstated, particularly the impact of progesterone levels on the outcome of embryo transfers, whether fresh or frozen. This introduction delves into the significance of progesterone measurement on the day of embryo transfer and its consequential influence on pregnancy outcomes, providing a comprehensive overview that bridges hormonal physiology with reproductive biotechnology.[1] Progesterone is a steroid hormone pivotal in the regulation of the menstrual cycle, pregnancy, and embryogenesis. It is secreted by the corpus luteum in the ovary post-ovulation and plays a critical role in preparing the endometrium for implantation and maintaining a pregnancy. In the context of ART, supplemental progesterone is often administered to support the endometrial lining and enhance the chances of successful implantation following embryo transfer. The precise timing, dosage, and route of administration of progesterone are subjects of ongoing research and optimization in the field of reproductive medicine.[2] The distinction between fresh and frozen embryo transfers (FET) introduces an additional layer of complexity in understanding the role of progesterone. Fresh transfers occur within the same menstrual cycle as egg retrieval, often requiring careful hormonal manipulation to mimic the natural cycle and prepare the uterus for implantation. In contrast, FET involves the thawing and transfer of previously frozen embryos into a uterus that has been prepared hormonally, allowing for more flexibility in timing and potentially a more controlled hormonal environment.[3] Recent studies have indicated that the level of progesterone on the day of embryo transfer can significantly affect the outcome of both fresh and frozen transfers. Optimal progesterone levels are crucial for endometrial receptivity, a state in which the endometrial lining is most conducive to embryo implantation. Levels that are either too low or excessively high have been associated with decreased implantation rates and lower pregnancy outcomes, highlighting the need for precise hormonal management.[4] The impact of progesterone levels is particularly pronounced in the case of FET. The endometrial preparation for FET typically involves estrogen supplementation followed by progesterone to mimic the natural preparatory phase of the endometrium for implantation. The flexibility in timing allows for the adjustment of progesterone levels to ideal ranges before transfer, potentially improving outcomes compared to fresh transfers where the window for hormonal correction is narrower.[5]

## Methods

This comprehensive study, conducted from May 2022 to April 2023 at the Fertility Center for Infertility Treatment and IVF in Najaf Al-Ashraf and Kamal Al-Samarrai Teaching Hospital in Baghdad, aimed to investigate the influence of serum progesterone levels on the outcomes of embryo transfers in Intracytoplasmic Sperm Injection (ICSI) procedures. A total of 100 women were meticulously selected and evenly divided into

two groups: those undergoing fresh embryo transfer cycles and those undergoing frozen embryo transfer (FET) cycles. This division allowed for a detailed comparative analysis to assess the impact of progesterone levels measured on the day of embryo transfer on the success rates of implantation and pregnancy. The study paid close attention to a variety of clinical data, including both maternal and paternal demographics (age, Body Mass Index (BMI)), reproductive history (duration and causes of infertility), and specific details of the fertility treatment undertaken (ovarian stimulation protocols, reasons for embryo cryopreservation, embryo quality, and stage at transfer). Furthermore, the research team focused on the protocols for endometrial preparation and the measurement of endometrial thickness at the time of transfer, which are crucial for successful embryo implantation. The primary outcome of interest was the pregnancy rate within the two groups, aiming to establish a correlation between serum progesterone levels and clinical outcomes.

### *Inclusion and Exclusion Criteria*

Participants included were adults aged 18 and above, couples experiencing infertility for over two years, and those undergoing autologous, vitrified-warmed cleaved embryo transfer scheduled on the fourth day of progesterone supplementation. The exclusion criteria were participants under 18 or over 40 years of age, those lost to follow-up, having estradiol levels below 200 pg/ml or progesterone levels below 15 ng/ml, and refusal to participate in the study.

### *Data Collection*

Data was sourced from medical records at several fertility centers in Baghdad. The cohort was split into a standard group (underwent FET without hormonal measurements prior to transfer) and a surveillance subgroup (underwent FET with pre-transfer hormonal assessments). Those with insufficient hormonal levels in the surveillance group were excluded from further analysis. Hormonal assessments were performed using specific Cobas kits and analyzed with the Cobas e411 analyzer. All participants followed the same protocol for FET, which involved an initial oral administration of estrogen followed by a transvaginal scan to ensure appropriate endometrial thickness and absence of ovulation indicators, before proceeding to embryo transfer with subsequent progesterone supplementation. The statistical evaluation was carried out using SPSS version 22, employing a variety of tests to analyze the data. Frequency and percentage calculations were used for categorical data, while continuous data were described using mean, median, and standard deviation. The Chi-square test assessed the association between categorical variables, and the T-test analyzed differences in continuous variables. Significance was attributed to *P*-values of 0.05 or lower. This study is significant for its thorough examination of how serum progesterone levels affect the success of embryo transfers in ICSI procedures. By providing a detailed analysis of clinical outcomes in relation to progesterone levels, the research offers vital insights that could enhance fertility treatments and improve pregnancy rates among couples undergoing IVF procedures.

## Results

As shown in Table 1, there is significant difference in mean of females age fresh cycle have more mean age than frozen cycle, while no any differences in females BMI for both group.

As shown in Table 2, there is significant increase in progesterone level of females in fresh cycle while there is significant decrease in mean of COC number and 2PN level in

Table 1. **Differences in mean of variables in current study for both study groups**

| Variables | Group | Mean of age | SD | *P*-value |
|---|---|---|---|---|
| Female age (years) | Fresh cycle | 29.16 | 5.477 | **0.01** |
| | Frozen cycle | 26.59 | 4.346 | |
| **Variables** | **Group** | **Mean of BMI** | **SD** | ***P*-value** |
| BMI | Fresh cycle | 23.84 | 3.256 | 0.6 |
| | Frozen cycle | 23.57 | 2.689 | |

Table 2. **Differences in mean of variables in current study for both study groups**

| Variables | Group | Mean of Progesterone | SD | *P*-value |
|---|---|---|---|---|
| Progesterone | Fresh cycle | 64.52 | 40.751 | **0.0001** |
| | Frozen cycle | 34.23 | 19.544 | |
| **Variables** | **Group** | **Mean of COC no.** | **SD** | ***P*-value** |
| COC number | Fresh cycle | 16.73 | 6.293 | **0.036** |
| | Frozen cycle | 19.57 | 6.809 | |
| **Variables** | **Group** | **Mean of MII** | **SD** | ***P*-value** |
| MII | Fresh cycle | 14.66 | 5.383 | 0.4 |
| | Frozen cycle | 15.65 | 5.474 | |
| **Variables** | **Group** | **Mean of Injected MII** | **SD** | ***P*-value** |
| Injected MII | Fresh cycle | 14.11 | 6.766 | 0.07 |
| | Frozen cycle | 16.52 | 6.474 | |
| **Variables** | **Group** | **Mean of 2PN** | **SD** | ***P*-value** |
| 2PN | Fresh cycle | 11.14 | 4.032 | **0.008** |
| | Frozen cycle | 13.24 | 3.675 | |

Table 3. **Differences in mean of variables in current study for both study groups**

| Variables | Group | Mean of Embryo | SD | *P*-value |
|---|---|---|---|---|
| No. of embryos | Fresh cycle | 10.75 | 4.199 | 0.08 |
| | Frozen cycle | 12.06 | 3.212 | |
| **Variables** | **Group** | **Mean of Grade A** | **SD** | ***P*-value** |
| Good grade "A" | Fresh cycle | 4.07 | 2.444 | **0.008** |
| | Frozen cycle | 5.69 | 3.284 | |
| **Variables** | **Group** | **Mean of Grade B** | **SD** | ***P*-value** |
| Fair grade B | Fresh cycle | 3.91 | 2.476 | **0.01** |
| | Frozen cycle | 2.74 | 1.954 | |
| **Variables** | **Group** | **Mean of Grade C** | **SD** | ***P*-value** |
| Bad grade "C" | Fresh cycle | 2.45 | 1.302 | 0.2 |
| | Frozen cycle | 2.74 | .975 | |

Table 4. **Association between pregnancy outcome and both groups**

| | | Group | |
|---|---|---|---|
| | | **Fresh cycle** | **Frozen cycle** |
| Pregnancy outcomes | No | 18 | 33 |
| | | 40.9% | 61.1% |
| | Yes | 26 | 21 |
| | | 59.1% | 38.9% |
| Total | | 44 | 54 |
| | | 100.0% | 100.0% |

*P*-value = 0.05.

Table 5. **Differences in mean of variables in current study for both study groups**

| Variables | Outcome | Mean of Progesterone | SD | *P*-value |
|---|---|---|---|---|
| Progesterone | No pregnancy | 40.32 | 30.893 | **0.024** |
| | Pregnant | 55.97 | 36.171 | |

Table 6. **Differences in mean of Progesterone according to outcome in fresh cycle group**

| Variables | Outcome | Mean of Progesterone | SD | *P*-value |
|---|---|---|---|---|
| Progesterone | No pregnancy | 56.52 | 42.074 | 0.3 |
| | Pregnant | 70.05 | 39.682 | |

Table 7. **Differences in mean of Progesterone according to outcome in frozen cycle group**

| Variables | Outcome | Mean of BMI | SD | *P*-value |
|---|---|---|---|---|
| Progesterone | No pregnancy | 31.49 | 17.964 | 0.2 |
| | Pregnant | 38.54 | 21.541 | |

females in fresh cycle less than females in frozen cycle. No any significant differences in mean of MII and injectable MII for both fresh cycle and frozen cycle group.

As shown in Table 3, there is significant difference in mean no of good grade in females in frozen cycle more than fresh cycle. While mean of grade fair is more significant increase in females of fresh cycle. No any significant differences in mean of No. of embryos and Bad grade "C" for both fresh cycle and frozen cycle group.

As shown in Table 4, there is significant between outcome and both groups, 59.1% of females with fresh cycle have pregnancy while 38.9% of frozen cycle have pregnancy.

As shown in Table 5, there is significant in mean of progesterone level in pregnant females than in not pregnant females.

As shown in Tables 6 and 7, there is no significant differences in mean of Progesterone according to outcome in both fresh and frozen cycle group.

## Discussion

The study delineates significant distinctions in reproductive outcomes between fresh and frozen in vitro fertilization (IVF) cycles, emphasizing variances in patient age, hormone levels, egg and embryo quality, and pregnancy outcomes. It demonstrates that females undergoing fresh IVF cycles are notably older than those in frozen cycles, though Body Mass Index (BMI) showed no significant difference between the two groups. This finding aligns with existing literature suggesting age as a crucial determinant of IVF success, with a younger age favorably influencing outcomes, especially in fresh cycles. However, the impact of BMI remains ambiguous, with studies indicating both significant and negligible effects on IVF success rates, particularly concerning egg and embryo quality.[6,7] An interesting aspect of the study is the significant increase in progesterone levels in women undergoing fresh cycles compared to those in frozen cycles, potentially attributed to the ovarian stimulation in fresh cycles. Elevated progesterone is known to prepare the endometrium for implantation, although excessive levels might impair endometrial receptivity. The reduced number of cumulus-oocyte complexes (COCs) and two-pronuclei (2PN) stage embryos in fresh

cycles hint at possible differences in egg quality or the effects of ovarian stimulation, despite no significant variations in the maturity of eggs (MII stage) or use of injectable MII across the two cycle types.[8] The study further reveals that frozen cycles have a higher count of good grade embryos, whereas fresh cycles have more fair grade embryos, suggesting the freeze-thaw process may select more resilient embryos, thus impacting the overall embryo quality for transfer. Despite the higher pregnancy rates observed in fresh cycles, the choice between fresh and frozen cycles remains complex, influenced by a multitude of factors including clinical, physiological, and logistical considerations.[8,9] Moreover, the significant differentiation in progesterone levels between pregnant and non-pregnant females underscores the hormone's essential role in supporting early pregnancy and maintaining the endometrium for implantation and early embryonic development. This aligns with the body of evidence advocating for meticulous hormonal monitoring to enhance IVF outcomes, particularly in frozen embryo transfer (FET) cycles where hormonal replacement therapy (HRT) creates a synchronized environment for embryo transfer, improving pregnancy outcomes.[10,11] The ongoing debate around optimal hormone levels for FET outcomes is enriched by this study's findings, suggesting that individualized hormonal therapy may be crucial for maximizing FET success rates. It highlights the complex interplay of hormonal interactions and their consequential impact on FET success, emphasizing the need for tailored approaches in hormone administration.[12,13] Despite the significant findings, no differences were observed in outcomes based on age and BMI between hormonally monitored and unmonitored groups, suggesting these factors may have a less pronounced effect on FET cycle success than hormonal balance and timing.[14] Furthermore, the study aligns with recent research indicating that factors like endometrial thickness and infertility duration may have a greater impact on FET success than the number of embryos transferred,[15,16] advocating for a holistic approach to patient care in assisted reproductive technologies that prioritizes physiological readiness for embryo implantation over quantitative metrics. This study contributes to the intricate mosaic of reproductive medicine, underscoring the pivotal role of careful hormonal monitoring and the strategic selection of embryos in enhancing IVF and FET outcomes. It not only corroborates previous findings but also invites further exploration into the nuanced dynamics of reproductive technologies, aiming to optimize individual patient care and improve success rates in IVF treatments.

## Conclusion

The study indicates significant differences in the reproductive outcomes between fresh and frozen cycle groups among females, particularly in age, progesterone levels, COC number, 2PN level, and the quality of embryos. Fresh cycles are associated with older age, higher progesterone, but lower COC number and 2PN level than frozen cycles. Furthermore, pregnancy rates are notably higher in the fresh cycle group compared to the frozen cycle group. However, no significant differences were observed in BMI, MII and injectable MII levels, the number of embryos, or bad grade "C" embryos between the two groups, nor in progesterone levels based on pregnancy outcome within each group.

## Conflict of Interest

None.

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