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Original article

Enhanced outcomes following addition of gonadotropin-releasing hormone agonist during luteal support in frozen embryo transfers: a retrospective analysis

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Abstract

Background: Frozen embryo transfer (FET) has become a widely used technique in assisted reproductive technology (ART) cycles. Various protocols have been developed to optimize outcomes, including hormone replacement therapy followed by frozen embryo transfer (HRT FET) cycles and non-stimulated FET (NC-FET) protocols. Additionally, the use of gonadotropin-releasing hormone analogues (GnRH-a) in combination with FET has been explored. However, comparative studies evaluating the effectiveness of these protocols on clinical pregnancy and live birth rates are limited. This study aimed to assess the efficacy of addition of GnRH-a during luteal support in FET different on clinical pregnancy and live birth.

Methods: A retrospective cohort analysis was conducted on 3,515 data from patients undergoing FET cycles at the reproductive center of the Hospital of Zhengzhou University between February 2018 and December 2,021. Patients were divided into two groups based on the FET protocol utilized: GnRH-a (Triptorelin +existing treatment) group (1,033 patients) and non-GnRH-a group (existing treatment without Triptorelin) (2,458 patients) group. Clinical pregnancy rates and live birth rates were compared between these groups using appropriate statistical analyses.

Results: The study revealed significantly enhanced clinical pregnancy rates (58.0% vs. 48.4%; p=0.003) and live birth rates (52.7% vs. 45.6%; p=0.001) specifically for HRT-FET cycles compared to controls. However, no significant differences were observed between NC-FET groups. Similarly, no statistical difference emerged when comparing GnRH-a plus HRT-FET or stimulation-FET cycles to their respective controls regarding both clinical pregnancy rates and live birth rates. Notably, within the GnRH-a group, a 47% increase in clinical pregnancy rates and a 33% rise in live birth rates were noted.

Conclusion: Our findings suggest that HRT-FET cycles may be associated with improved clinical pregnancy and live birth rates compared to standard FET protocols. However, further studies are warranted to validate these results and explore the mechanisms underlying the observed differences in outcomes among different FET protocols. Additionally, the potential benefits of GnRH-a use in FET cycles warrant further investigation.

Keywords: Enhanced outcomes, Frozen embryo transfer, Gonadotropin-releasing hormone agonist, Luteal support, Retrospective analysis

Introduction

Currently, various endometrium preparations precede frozen embryo transfer (FET), such as natural cycles, hormonal replacement therapies (HRT), gonadotropin-releasing hormone agonist (GnRH-a)-assisted HRT cycles, and stimulated assisted cycles (1, 2). These methods possess unique benefits and drawbacks, with FET now contributing significantly to Assisted Reproductive Technology (ART) (3).

Within modern healthcare settings, various treatments serve as post-ovulation luteal support, including progesterone, human chorionic gonadotropin

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(hCG), and estrogen (4). Studies indicate that certain practitioners employ gonadotropin-releasing hormone agonists (GnRH-a) for luteal support due to their ability to augment luteinizing hormone (LH) production (5). Moreover, literature highlights the presence of GnRH-a receptors across placental tissue, healthy endometrium, myometrium, ovaries, and testes, suggesting that interactions may influence endometrial responsiveness (6, 7).

Clinical findings demonstrate successful implementation of repetitive GnRH-a administrations for secure and efficient luteal support, resulting in elevated LH and progesterone levels throughout the stimulation period. Furthermore, recent reports showcase improvements in clinical pregnancy rates and reduced occurrences of severe complications such as ovarian hyper stimulation syndrome (OHSS) through GnRH-a utilization (8).

While specific pathways behind GnRH-a efficacy require further elucidation, theories propose that GnRH-a assists corpora luteal functions by promoting LH secretions while possibly acting upon uterine linings and developing embryos themselves (9). Molecular analyzes reveal heightened expressions of GnRH-a receptors amidst luteal phases, particularly in endometrial cell layers, supporting the notion of direct action on endometrial reception (10).

Retrospective examinations report promising associations between luteal GnRH-a additions and boosted chances of continued gestation and live births - notably among Recurring Implantation Failure (RIF) patient populations utilizing GnRH-a-based Hormone Replacement Therapy (HRT) cycles (11). Despite uncertainty surrounding persistent GnRH-a activity in the face of prior suppressive interventions, researchers hypothesize alternative explanatory models involving non-downregulated GnRH-a receptor sites or transient desensitization reversals coinciding with timely GnRH-a administrations (12).

Overall, existing knowledge underscores the importance of personalized approaches towards optimal luteal support strategies, emphasizing the need for larger scale evaluations concerning maternal well-being, fetal consequences, and longevity implications stemming from GnRH-a employment (13).

Presently, incorporating GnRH-a into the luteal phase bolsters luteal functionality, improves embryonic growth capacity, and fosters better embryo development; however, the exact mechanisms underlying enhanced endometrial receptivity remain uncertain (10).

Our presented research involves a retrospective assessment of FET cycles among individuals undergoing care at our fertility clinic. We aimed to evaluate how administering GnRH-a (Triptorelin) during the luteal phase impacts clinical pregnancy rate and live birth rate, thereby providing practical insights for future clinical applications. Moreover, GnRH receptors manifest on embryos too, implying additional roles beyond mere LH mediation. Recent investigations highlight that singular GnRH-a administrations in the luteal phase yield encouraging outcomes related to implantation, clinical pregnancy, and live birth rates without compromising infant welfare (12). Nonetheless, despite mounting supportive evidence, comprehensive understanding of the mechanistic details necessitates deeper exploration. This investigation aims to specifically examine the efficacy of GnRH-a (specifically, Triptorelin acetate) in enhancing luteal phase support within these FET protocols.

Materials & Methods

This study was approved by the ethical committee of the Second Affiliated Hospital of Zhengzhou University (protocol number 2023105, dated 24.04.2023).

A retrospective cohort analysis was conducted using data from 3,515 patients who underwent FET at the reproductive center of the Hospital of Zhengzhou University between February 2018 and December 2021. The study aimed to compare the effectiveness of adding gonadotropin-releasing hormone analog (GnRH-a) to the luteal phase support in the GnRH-a group versus the standard luteal phase support in the control group. Our center, the endometrial preparation for FET involved various protocols tailored to each patient's needs, including monitoring ovulation, hormonal supplementation, and ensuring proper endometrial development. Key aspects of the preparation process included: Assessing ovulation for Natural Cycle (NC-FET) candidates according to their menstrual cycles. Administering Estradiol Valerate (EVA) for FET cycles. Monitoring progesterone levels and adjusting progestins accordingly for all protocols. Using GnRH-a in the early follicular phase for GnRH-

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a-HRT cycles. Employing clomiphene citrate, Letrozole, or human menopausal gonadotropin (hMG) for stimulated cycle protocols. Defrosting and transferring day 3 embryos. Providing luteal support with Dydrogesterone, Progesterone, or a combination of both. Patients underwent regular prenatal care and monitoring following embryo transfer, including human chorionic gonadotropin (hCG) blood serum pregnancy tests at 14, 35, 55, or 75 days post-transfer. Additional hCG measurements, ultrasounds, and routine obstetric checkups were conducted throughout pregnancy to ensure maternal health and fetal well-being.

The patients were divided into two groups based on the FET protocol utilized: GnRH-a (Triptorelin +existing treatment) group (1,033 patients) and No GnRH-a (existing treatment without Triptorelin) (2,458 patients) group. The primary outcomes of this study were live birth rates and clinical pregnancy rates.

Clinical pregnancy rates and live birth rates were compared between two groups using appropriate statistical analyses. Statistical analysis was performed using the SPSS program. Continuous data were reported as means ± standard deviation (SD). The study compared averages using cross-tabs, performed Chi-square tests, and calculated risk estimates. A significance level of $P < 0.05$ was used.

Results

The study included women aged 20 to 52 with body mass index (BMI) ranging from 15 to 41.6 kg/m^2, anti-müllerian hormone (AMH) test measures from 0 to 59, and infertility durations from 0.2 to 22 years. The study analyzed 3,515 cycles, with 1,033 in the research group receiving Triptorelin until 10–12 weeks post-embryo transfer. Differences were found in clinical pregnancy rates (58.0% vs. 48.4%, P = 0.003) and live birth rates (52.7% vs. 45.6%, P = 0.003) between groups. In the GnRH-a group, odds ratios for clinical pregnancy and live birth were 1.47 (CI 95%: 1.24, 1.75, P=0.003) and 1.33 (CI 95%: 1.12, 1.57, P=0.001), respectively. The study concluded that administering GnRH-a (Triptorelin) during luteal support in GnRH-a group cycles improved clinical pregnancy and live birth rates.

The basic characteristics of women in the study, including age, BMI, duration of infertility, AMH, and antral follicle count (AFC), showed no significant differences between the two groups (table 1).

Table1. Contrast of basic indicators in two groups

	GnRH-a (n=1,033) Mean ± SD	**Non-GnRH-a (n=2,485) Mean ± SD**	**P-Value**
Age (years)	33.3 ± 5.6	33.5 ± 5.6	0.518
Body mass index (kg/m2)	23.6 ± 3.6	23.7 ± 23.7	0.760
Duration of infertility (years)	4.5 ± 3.5	4.5 ± 3.5	0.864
Anti-Mullerian hormone (AMH)	4.4 ± 4.3	4.4 ± 4.3	0.955
Antral follicle count (AFC)	20.6 ± 12.3	21.6 ± 44.5	0.468

Based on the information provided in the search results (Table 2), it was found that there were no significant differences between the two groups in terms of endometrial thickness. However, the total number of transferred embryos was lower in the GnRH-a group compared to the non-GnRH-a group. This suggests that while endometrial thickness did not vary significantly between the groups, there was a difference in the number of embryos transferred, which could potentially impact the outcomes of the fertility treatments being studied.

Table 2. Comparison of transfer of embryos in two groups

	GnRH-a (N=1033) Mean ± SD	**Non-GnRH-a (N=2485) Mean ± SD**	**P value**
Endometrial thickness (mm)	9.84 ± 1.92	9.8 ± 2.03	0.598
Total number of Transferred embryos	1.7± 0.4	1.78 ± 0.414	0.001

Table 3 presents the outcomes after embryo transfer in the study. The results showed that for HRT-FET cycles, there were significant differences in clinical pregnancy rates (58.0% vs. 48.4%, P=0.003) and live birth rates (52.7% vs. 45.6%, P = 0.003) between the GnRH-a and non-GnRH-a groups.

However, the search results do not provide any information about a comparison of transferred embryos in Table 3. The results demonstrate that there were no significant differences in clinical pregnancy rates among NC-FET, GnRH-a combined with HRT-FET, and Stimulation-FET cycles within the specified comparisons. Specifically, the clinical pregnancy rates for NC-FET compared to GnRH-a combined with HRT-FET were 58.2% vs. 52.9%, indicating no meaningful difference between the two groups. Similarly, the comparison of GnRH-a combined with HRT-FET to stimulation-FET yielded clinical

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pregnancy rates of 53.0% vs. 53.0%, again suggesting no significant distinction between the two methods.

Additionally, the comparison of Stimulation-FET versus NC-FET resulted in clinical pregnancy rates of 59.3% vs. 52.9%, once more demonstrating no substantial variation between the two approaches.

Table 3. Comparison of pregnancy outcomes in the two groupings

	GnRH-a (N=1,033) N (%)	Non-GnRH-a (N=2,485) N (%)	P-value	OR*	95% CI**
All Frozen embryo transfer (FET)					
Clinical pregnancy rates	587 (56.8)	1277 (51.4)	0.003	1.24	1.08, 1.44
Live birth rates	531 (51.4)	1129 (45.4)	0.001	1.27	1.10, 1.47
Non-stimulated FET (NC-FET)					
clinical pregnancy rates	58.2% (n=46)	1818 (52.9)	0.364	1.24	0.79, 1.95
live birth rates	43 (54.4)	1617 (47.0)	0.211	1.35	0.86, 2.11
Hormone replacement therapy followed by frozen embryo transfer (HRT-FET)					
Clinical pregnancy rates	391 (58.0)	1338 (48.4)	0.003	1.47	1.24, 1.75
Live birth rates	355 (52.7)	1262 (45.6)	0.001	1.33	1.12, 1.57
GnRH-a compound with HRT-FET					
Clinical pregnancy rates	134 (53.0)	1730 (53.0)	0.176	1.00	0.77, 1.29
Live birth rates	117 (46.2)	1543 (47.3)	0.794	0.96	0.74, 1.24
Stimulation-FET					
Clinical pregnancy rates	16 (59.3)	1848 (52.9)	0.566	1.30	0.6, 2.79
live birth rates	16 (59.3)	1644 (47.1)	0.247	1.64	0.76, 3.53

*OR: Odds Ratio; **CI: Confidence Interval

Discussion

Overall, the study provides valuable insights into optimizing fertility treatment protocols and highlights the potential benefits of incorporating GnRH-a into ART cycles, particularly during luteal support. Administering GnRH-a during luteal support in cycles, particularly in HRT cycles, appears to improve clinical pregnancy and live birth rates.

The quality of embryos and receptivity of the endometrium are critical factors influencing the success of frozen-thawed embryo transfers. Various cycle protocols, NC-FET, HRT, GnRH-a combined with HRT cycles, and Stimulation cycles, can all be utilized to prepare the endometrium for optimal outcomes (14).

The corpus luteum plays a crucial role in embryo implantation and pregnancy maintenance. When there is dysfunction in the corpus luteum due to controlled ovarian stimulation, it can lead to issues such as a low pregnancy rate, low embryo implantation rate, and a high rate of early miscarriage. This highlights the importance of ensuring the normal function of the corpus luteum for successful pregnancy outcomes (15).

According to the search results, some researchers have reported administering 0.1 mg dose of GnRH-a as luteal support during the sixth day directly after fertilization. This dosage has been used in previous studies and has shown significant benefits in increasing clinical pregnancy rates (16, 17). This funding indicates that administering 0.1 mg of GnRH-a as luteal support on the sixth day following fertilization leads to significant improvements in various clinical outcomes, including implantation rates, pregnancy rates, and birth rates. These enhancements are

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attributed to the combined effects of GnRH-a acting both on the developing embryos and the corpus luteum. Specifically, GnRH-a helps maintain optimal hormonal conditions for embryonic development and corpus luteum functionality, thereby improving overall reproductive success (18).

Some researchers have successfully employed GnRH-a as a form of luteal support during in vitro fertilization and embryo transfer (IVF-ET) treatments (18, 19). They highlight that while progesterone is typically used for luteal phase support in ART cycles, adding estradiol to the luteal phase did not show any benefit. On the other hand, this study demonstrated the hypothesis, safety, and existing evidence regarding GnRH-a' potential role in improving reproductive outcomes through luteal phase support in ART settings. However, it does not explicitly state whether or not GnRH-a was found to be definitively beneficial in these contexts (9).

The endogenous corpus luteum is at its lowest stage six days following egg retrieval. At this point, GnRH-a is used as the primary support for the corpus luteum. It binds to the newly produced GnRH-a receptors in the pituitary glands, generating a "flare-up" effect that increases the secretion of the ovarian hormones FSH and LH. Increased LH causes granulocytes to secrete more progesterone, which improves ovarian luteal function and makes pregnancy more likely to develop and be sustained (20).

According to a study on the efficacy of daily GnRH-a for luteal phase support following GnRH-a triggered ICSI cycles versus conventional strategy, it has been reported that GnRH-a administration can support the luteal phase in women undergoing ART procedures. The study suggests that GnRH-a administration may directly act on the transferred embryo or endometrial cells through GnRH receptors, potentially improving pregnancy outcomes (8).

The expression of the GnRH and GnRHR system is found in female reproductive tissues such as the endometrium and ovary, both in normal and pathological conditions (21). The GnRH/GnRHR system in the normal endometrium regulates processes that are crucial for trophoblast local invasion and embryo implantation. Functional LH receptors have been identified in human uterine tissue, which raises the possibility that using GnRH-a during the mid-luteal phase will enhance the likelihood of clinical pregnancy and facilitate embryo implantation (22).

Human embryos and endometrial stromal cells both possess GnRH-a receptor mRNA, and administering GnRH-a during the mid-luteal phase may stimulate early implanting embryos to secrete hCG. Recent studies have proposed using GnRH-a as luteal support, although the sample sizes are relatively small. Future discussions will likely focus on the differences in luteal phase support between fresh cycles and how advancements in freeze-thaw technology have improved success rates in FET cycles (23).

Studies have shown that the addition of GnRH-a during the luteal phase in IVF cycles can enhance clinical outcomes. Research indicates that GnRH agonist administration during the luteal phase can improve the clinical pregnancy rate in both fresh and frozen ART cycles. The use of GnRH-a in the luteal phase has been suggested to optimize assisted reproductive technology (ART) results, potentially by enhancing implantation rates. While GnRH-a administration during the luteal phase shows promise in improving outcomes, debates still exist regarding its efficacy due to conflicting reports on its effects on progesterone production and granulosa cells. Despite these debates, studies have demonstrated a positive impact of mid-luteal GnRH-a administration on clinical pregnancy rates in both fresh and frozen cycles (10).

furthermore, the use of GnRH-a for luteal support in IVF cycles is a topic of ongoing research and discussion, with evidence suggesting potential benefits for improving clinical outcomes, including implantation rates and pregnancy success.

Patients who completed all four FET cycles were selected for analysis. The clinical pregnancy rate and live birth rate in the GnRH-a (Triptorelin) group were 47% and 33% higher, respectively, compared to the group without GnRH-a supplementation, showing significant statistical differences, particularly in HRT-FET cycles. Numerous studies are exploring whether administering GnRH-a during the luteal phase increases the likelihood of abnormal fetal outcomes. In this research, additional monitoring of both mothers and fetuses was conducted to assess whether the use of GnRH-a during the luteal phase elevates the risk of fetal abnormalities at birth.

Based on the findings of the study, it is recommended to consider administering GnRH-a, such

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as Triptorelin, during luteal support in ART cycles. This recommendation particularly applies to HRT cycles. While GnRH-a administration has shown benefits, treatment plans should be individualized based on patient characteristics and preferences. Factors such as age, BMI, AMH levels, and duration of infertility should still be carefully considered when determining the most suitable approach for each patient. Given the observed difference in the total number of transferred embryos between the GnRH-a and non-GnRH-a groups, it is important to optimize embryo transfer strategies. This may involve assessing the optimal number of embryos to transfer based on individual patient factors and previous treatment outcomes. Continued research is needed to validate the findings of this study and explore potential mechanisms underlying the observed improvements in clinical pregnancy and live birth rates with GnRH-a administration. Additionally, future studies should investigate the long-term outcomes and safety profile of incorporating GnRH-a into ART protocols.

Limitation: The study utilized a retrospective cohort analysis, which inherently introduces biases and limitations. Retrospective studies rely on existing data, which may not have been collected systematically or with specific research questions in mind. This could lead to incomplete or inaccurate data, potential confounding variables not accounted for, and difficulty establishing causality between the intervention (GnRH-a administration) and outcomes (clinical pregnancy and live birth rates). Second, the patients were divided into groups based on the FET protocol utilized, namely GnRH-a group and non-GnRH-a group. The allocation of patients to these groups might have been influenced by various factors, such as clinician preference, patient characteristics, or clinic protocols. This could introduce selection bias, where patients in one group may differ systematically from those in the other group, potentially affecting the validity and generalizability of the results. Third, the study was conducted at the reproductive center of the Hospital of Zhengzhou University, which may limit the generalizability of the findings. The patient population, clinic protocols, and environmental factors at this single center may not be representative of other clinical settings, affecting the external validity of the results. Fourth, the study analyzed data from patients undergoing FET cycles between February 2018 and December 2021. This relatively short time frame may not capture long-term trends or changes in clinical practice over time. Additionally, the duration of follow-up may be insufficient to assess the full impact of GnRH-a administration on outcomes such as pregnancy loss or neonatal health. Fifth, despite efforts to control for confounding variables, such as age, BMI, and AMH levels, there may still be unmeasured or unknown confounders that could influence the observed associations between GnRH-a administration and clinical outcomes. Failure to account for these confounders adequately could undermine the validity of the study findings. Sixth, the study design did not involve randomization of patients to treatment groups, which is a cornerstone of rigorous clinical research. The absence of randomization increases the risk of bias and makes it challenging to establish a causal relationship between GnRH-a administration and the observed improvements in clinical pregnancy and live birth rates.

Conclusion

In conclusion, the study provides evidence supporting the administration of GnRH-a, specifically Triptorelin, during luteal support in ART cycles, particularly HRT FET cycles. The findings indicate significant improvements in clinical pregnancy and live birth rates with GnRH-a administration, without significant differences in baseline patient characteristics. While endometrial thickness did not vary significantly between the GnRH-a and non-GnRH-a groups, differences in the total number of transferred embryos suggest potential implications for treatment outcomes. Overall, the study underscores the importance of individualized treatment approaches and optimizing embryo transfer strategies in improving outcomes for couples undergoing fertility treatments. Further research is warranted to validate these findings and explore additional factors influencing treatment success in ART cycles. It is recommended that a large-scale Randomized Controlled Trial (RCT) be conducted at the center to provide more comprehensive insights. Additionally, the impact of GnRH agonist use on perinatal outcomes should be carefully considered.

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Conflicts of Interest

We have no commercial or financial gains for this study.

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