Pharmaceutical Sciences and Drug Design
ISSN: 3062-4428
2022, Volume 2, Page No: 170-180

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Galaxy Publication

GnRH Agonist Pretreatment Prior to Hormone Replacement Therapy Improves Live Birth Rates in Frozen-Thawed Embryo Transfer Among Overweight and Obese Women: A Large Retrospective Cohort Study

Eli Mitchell[1]*, Hazel Simmons[1]

[1]Department of Phytochemistry, Faculty of Pharmacy, University of British Columbia, Vancouver, Canada.

***E-mail** ✉ eli.mitch.ca@gmail.com

Received: 28 May 2022; **Revised:** 06 September 2022; **Accepted:** 08 September 2022

ABSTRACT

Excess body weight is frequently associated with suboptimal endometrial readiness and poorer reproductive success in frozen-thawed embryo transfer (FET) cycles. Administering a depot gonadotropin-releasing hormone agonist (GnRH-a) prior to starting hormone replacement therapy (HRT) has been suggested to enhance endometrial performance through several biological pathways; however, whether this strategy benefits overweight or obese individuals has not been clearly determined. This retrospective analysis included 1968 FET cycles carried out at a major fertility center in Jiangxi Province from January 2016 to December 2021. Overweight/obesity—defined according to Chinese criteria as body mass index ≥ 24.0 kg/m²—was used to identify eligible participants, who were then assigned to either the HRT group (n = 946) or the GnRH-a+HRT group (n = 1022). The study focused primarily on the live birth rate. To limit bias, propensity score matching (1:1) and multivariable logistic regression were applied. Additional subgroup analyses examined outcomes according to lipid-metabolism status. After matching, 539 cycles per group remained, with baseline variables well balanced. Live birth was more frequent in the GnRH-a+HRT cohort than in the HRT-only cohort (55.84% vs 49.35%, P = 0.033). This pretreatment strategy was also associated with higher rates of positive hCG testing (77.18% vs 68.65%, P = 0.002), clinical pregnancy (68.09% vs 60.48%, P = 0.009), and implantation (52.41% vs 47.47%, P = 0.039). Miscarriage rates did not differ significantly (17.71% vs 16.87%, P = 0.771). Among women with dyslipidemia, the advantage for live birth persisted (adjusted OR 1.75, 95% CI 1.08–2.85), whereas no clear improvement was detectable in those with normal lipid profiles (adjusted OR 1.18, 95% CI 0.87–1.58). These results provide new clinical evidence that GnRH-a pretreatment may enhance FET outcomes in overweight and obese patients, particularly when dyslipidemia is present. An individualized endometrial-preparation approach may therefore be warranted. Larger multicenter randomized trials are required for confirmation.

Keywords: Dyslipidemia, Frozen-thawed embryo transfer, Gonadotropin-releasing hormone agonist, Obesity, Pregnancy outcomes

How to Cite This Article: Mitchell E, Simmons H. GnRH Agonist Pretreatment Prior to Hormone Replacement Therapy Improves Live Birth Rates in Frozen-Thawed Embryo Transfer Among Overweight and Obese Women: A Large Retrospective Cohort Study. Pharm Sci Drug Des. 2022;2:170-80. https://doi.org/10.51847/FcJg30dMrJ

Introduction

Growing rates of overweight and obesity have turned these conditions into major global health concerns. According to the Global Burden of Disease 2021 assessment, approximately 2.11 billion adults aged 25 years or older—representing 45.1% of the worldwide adult population—fall within these categories [1]. Within China, 27.1% of women aged 18–49 years were classified as overweight/obese in 2019 [2].

The detrimental reproductive implications of excess adiposity are well established [3]. Increased fat mass can interfere with central reproductive-axis regulation, producing menstrual irregularities, anovulatory cycles, and diminished fertility [4]. In assisted conception, a high BMI has been linked to weaker ovarian stimulation, impaired fertilization, poorer embryo development, and reduced rates of both clinical pregnancy and live birth [5–7]. Even after transferring embryos deemed normal in appearance and chromosomal status, obese patients

continue to show lower implantation potential and higher risk of pregnancy loss [8–10], strongly implicating impaired endometrial readiness.

During frozen-embryo cycles, creating a receptive endometrium is a fundamental requirement. One of the most widely adopted approaches is hormone replacement therapy (HRT), which uses exogenous estrogen followed by progesterone to regulate endometrial maturation [11]. This method is user-friendly, flexible, and applicable to women with or without ovulation. Nonetheless, spontaneous follicle development and ovulation may still occur in 1.9%–7.4% of cycles [12, 13]. To prevent endogenous hormonal surges, some clinicians employ a depot GnRH-a before HRT, providing pituitary suppression and more predictable endometrial timing.

Although studies involving patients with otherwise favorable prognoses show comparable outcomes between HRT and GnRH-a+HRT [14, 15], accumulating research suggests that pretreatment may be advantageous in conditions associated with compromised endometrial function—such as intrauterine adhesions [16], inadequate endometrial thickness [17, 18], recurrent implantation failure [19, 20], and polycystic ovary syndrome [14]. However, the impact of this strategy specifically on overweight or obese women has not been rigorously evaluated.

The present investigation aimed to compare reproductive outcomes between standard HRT and GnRH-a-supplemented HRT in overweight/obese individuals undergoing FET cycles.

Materials and Methods

Study design and participants

This work used a retrospective cohort framework and took place at the Center for Reproductive Medicine of Jiangxi Maternal and Child Health Hospital, affiliated with Nanchang Medical College. It followed the ethical standards outlined in the Declaration of Helsinki. Authorization was granted by the hospital's Ethics Committee (No. 2024–03-040). Because the project relied solely on pre-existing anonymized records, with no patient interaction or intervention, written informed consent was not required. All datasets were handled in a fully de-identified format to maintain privacy.

A total of 17192 HRT-FET cycles from January 2016 to December 2021 were evaluated. Eligible women were 20–40 years old, experienced infertility linked to tubal and/or male factors, and had a BMI ≥24.0 kg/m² under the Chinese classification of overweight/obesity [21]. Individuals were excluded if they had: (1) congenital uterine abnormalities such as unicornuate, bicornuate, septate, or duplicated uterus; (2) acquired uterine or endometrial issues (endometriosis, adenomyosis, submucosal fibroid, intrauterine adhesions, or lining <7 mm); (3) polycystic ovary syndrome or endocrine-related obesity (e.g., Cushing's syndrome); (4) recurrent miscarriage or repeated implantation failure; (5) donor gametes or chromosomal anomalies in either partner; (6) cycles using PGT; or (7) incomplete follow-up or missing essential information. Cycles showing spontaneous follicular rise during HRT were also removed. If a patient underwent several FET cycles during the study window, only her first was included.

Endometrial preparation protocols

For the HRT approach, endometrial preparation began on cycle day 2 or 3 with 6 mg/day of oral estradiol valerate (Progynova, Bayer, Germany). Weekly ultrasound and serum progesterone evaluation were performed to follow lining progression and rule out unexpected follicular development. Progesterone injections (60 mg, Xianju Pharma, China) were initiated once the endometrium reached ≥7 mm and progesterone stayed <1.5 ng/mL, marking the shift to the secretory phase.

In the GnRH-a+HRT protocol, 3.75 mg of leuprorelin (Beiyi, Lizhu Pharma, China) was administered intramuscularly on day 2 or 3 of the cycle prior to FET. After 28 days, pituitary suppression was verified with ultrasound and hormone levels: endometrial thickness <5 mm, FSH <5 mIU/mL, LH <5 mIU/mL, and estradiol <50 pg/mL. Hormonal preparation then followed the identical regimen used for standard HRT. Use of GnRH-a pretreatment was not medically mandated; instead, it was jointly chosen by clinicians and patients to enhance scheduling convenience and minimize cancellation.

Embryo transfer and luteal-phase support

Embryos were vitrified and thawed using commercial kits from Kitazato Biopharma (Japan), following previously detailed methods [22]. High-quality embryos included cleavage-stage embryos of grade I–II with 7–10

blastomeres, and day 5–6 blastocysts with scores ≥4BB according to Gardner and Schoolcraft criteria [23, 24]. A maximum of two embryos was transferred in each cycle. Cleavage-stage embryos were placed on the fourth day after progesterone initiation, while blastocysts were transferred on the sixth day, maintaining synchronization between embryonic development (P+0 = Day 0) and the expected implantation window [11]. All transfers were conducted under abdominal ultrasound guidance.

Luteal-phase treatment started on the day of transfer and consisted of 90 mg/day vaginal progesterone gel (Crinone, Merck Serono, Germany) together with 20 mg/day oral progesterone (Duphaston, Abbott Biologicals, Netherlands). If pregnancy was confirmed, estradiol was tapered and progesterone was continued until 10 weeks of gestation.

Outcome measures

The main endpoint was the live birth rate per transfer. Secondary endpoints were the positive hCG rate, clinical pregnancy rate, implantation rate, and miscarriage rate. A live birth is referred to as the delivery of a viable infant at ≥28 weeks of gestation. A positive biochemical test required β-hCG ≥5 IU/L at 14 days after transfer. Clinical pregnancy was determined by ultrasound identification of a gestational sac roughly one month post-transfer, irrespective of fetal cardiac activity. Implantation rate represented the proportion of gestational sacs relative to embryos transferred. Miscarriage was defined as any spontaneous pregnancy loss occurring before 24 weeks of gestation.

Statistical analysis

Continuous measures were reported as the mean together with the standard deviation (SD). The Shapiro–Wilk procedure was used to determine whether these variables followed a normal distribution. Based on the outcome of this test, comparisons between groups employed either Student's t-test (for normally distributed data) or the Mann–Whitney U-test (for skewed data). Categorical parameters were expressed as frequencies with percentages, and differences between groups were evaluated via chi-square testing or Fisher's exact method when indicated. To reduce imbalances between the HRT and GnRH-a+HRT cohorts and limit selection bias, a 1:1 propensity score matching (PSM) strategy was applied. Nearest-neighbor matching without replacement was implemented, using a caliper equal to 0.1 SD of the logit-transformed propensity score. Variables incorporated into the matching model included maternal age, BMI, antral follicle count, infertility duration, infertility type and etiology, the COS protocol, peak endometrial thickness during the COS cycle, oocyte yield, fertilization technique, number of earlier transfer failures, length of estradiol treatment, number and developmental stage of transferred embryos, the use of high-quality embryos, and the duration of cryostorage. Histograms of the estimated scores were used to assess covariate distribution before and after matching.

To examine whether treatment effects varied across subgroups, analyses were stratified by BMI according to Chinese standards: overweight (24.0–27.9 kg/m²) and obesity (≥28.0 kg/m²) [21]. Because lipid abnormalities may substantially influence reproductive outcomes [25–28], overweight and obese participants were further classified by dyslipidemia status. Dyslipidemia complied with Chinese guidelines [29] and required meeting at least one of the following thresholds: triglycerides ≥1.70 mmol/L, total cholesterol ≥5.20 mmol/L, LDL-C ≥3.40 mmol/L, or HDL-C <1.00 mmol/L. Additionally, multivariable logistic regression models were constructed to estimate adjusted odds ratios (aORs) with 95% confidence intervals (CIs) comparing the two preparation strategies, using the same covariates applied within the PSM.

All statistical work was performed using SAS 9.4 (SAS Institute Inc., USA). A two-sided $P < 0.05$ signified statistical significance.

Results and Discussion

Baseline characteristics

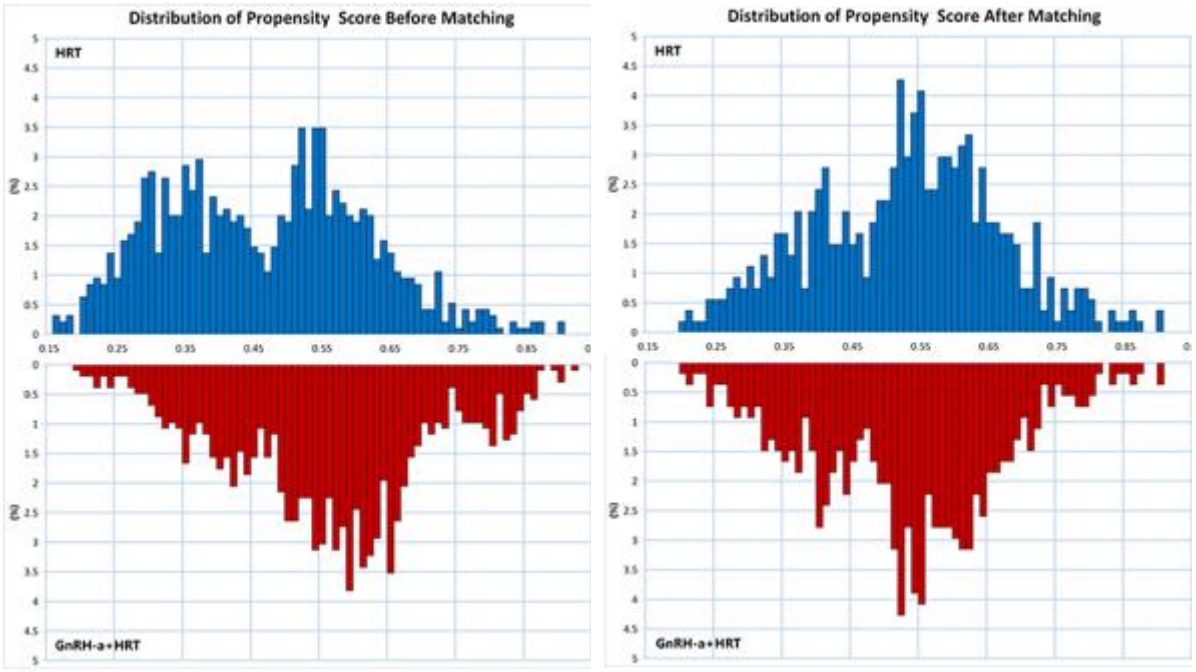
Table 1 shows the baseline characteristics according to the endometrial preparation method. Before matching, 1,968 patients met the study criteria, with 946 managed using HRT and 1,022 receiving the GnRH-a+HRT regimen. Relative to the HRT group, those in the GnRH-a+HRT cohort tended to be older, exhibited a smaller antral follicle count, showed a higher frequency of secondary infertility, and had experienced more earlier transfer failures. During the COS cycle, they less often underwent a GnRH-a protocol, had fewer retrieved oocytes, and demonstrated a thinner measured peak endometrial lining. In FET cycles, single-embryo and blastocyst transfers

occurred more commonly in the GnRH-a+HRT group, and their embryos had been stored for a longer period prior to use.

Following PSM, 539 individuals remained in each group, and no variable displayed meaningful imbalance, as shown in **Table 1** and **Figure 1**.

Table 1. Baseline Characteristics Grouped by the Endometrial Preparation Regimen

Characteristic	Before Propensity Score Matching	After Propensity Score Matching
	HRT (n=946)	GnRH-a+HRT (n=1022)
Age (years)	31.07 ± 4.41	32.26 ± 4.23
Body mass index (kg/m²)	26.13 ± 2.19	26.15 ± 2.10
Antral follicle count	13.40 ± 5.47	12.04 ± 5.73
Duration of infertility (years)	4.79 ± 3.33	4.79 ± 3.17
Type of infertility, n (%)		
Primary	269 (28.44%)	246 (24.07%)
Secondary	677 (71.56%)	776 (75.93%)
Etiology of infertility, n (%)		
Tubal factor	662 (69.98%)	710 (69.47%)
Male factor	106 (11.21%)	127 (12.43%)
Tubal + male factors	178 (18.82%)	185 (18.10%)
Stimulation protocol in fresh cycle, n (%)		
GnRH agonist	799 (84.46%)	752 (73.58%)
GnRH antagonist	81 (8.56%)	153 (14.97%)
Others	66 (6.98%)	117 (11.45%)
Peak endometrial thickness in fresh cycle (mm)	10.85 ± 2.44	10.55 ± 2.72
Number of oocytes retrieved	15.03 ± 7.58	12.93 ± 7.47
Fertilization method, n (%)		
Conventional IVF	730 (77.17%)	765 (74.85%)
ICSI	179 (18.92%)	223 (21.82%)
IVF + ICSI	37 (3.91%)	34 (3.33%)
Number of previous failed embryo transfers, n (%)		
0	577 (60.99%)	502 (49.12%)
1	340 (35.94%)	412 (40.31%)
2	29 (3.07%)	108 (10.57%)
Duration of estradiol administration (days)	14.08 ± 2.20	13.87 ± 2.12
Number of embryos transferred, n (%)		
Single	326 (34.46%)	413 (40.41%)
Double	620 (65.54%)	609 (59.59%)
Embryo developmental stage at transfer, n (%)		
Cleavage stage	549 (58.03%)	437 (42.76%)
Blastocyst	397 (41.97%)	585 (57.24%)
Transfer of at least one top-quality embryo, n (%)	588 (62.16%)	611 (59.78%)
Duration of embryo cryopreservation (days)	30.08 ± 4.49	31.13 ± 4.43



a) b)

Figure 1. Distribution of propensity scores before and after matching.
Abbreviations: HRT, hormone replacement therapy; GnRH-a, gonadotropin-releasing hormone agonist.

Cycle outcomes

Table 2 summarizes the clinical outcomes. Endometrial thickness during the FET cycle was significantly greater in the GnRH-a+HRT cohort (9.82±2.07 mm) relative to the HRT cohort (9.48±1.76 mm, P = 0.011). The live birth rate favored the GnRH-a+HRT regimen (55.84% vs 49.35%, P = 0.033). Higher values were also recorded for positive hCG (77.18% vs 68.65%, P = 0.002), clinical pregnancy (68.09% vs 60.48%, P = 0.009), and implantation (52.41% vs 47.47%, P = 0.039) in the GnRH-a pretreated group. Miscarriage frequencies remained statistically indistinguishable (17.71% vs 16.87%, P = 0.771).

Table 2. Cycle Outcomes Grouped by the Endometrial Preparation Regimen

Outcome	HRT (n=539)	GnRH-a+HRT (n=539)	P value
Endometrial thickness on transfer day (mm)	9.48 ± 1.76	9.82 ± 2.07	0.011
Positive hCG rate, n (%)	370 (68.65%)	416 (77.18%)	0.002
Clinical pregnancy rate, n (%)	326 (60.48%)	367 (68.09%)	0.009
Implantation rate, n/N (%)	413/870 (47.47%)	456/870 (52.41%)	0.039
Miscarriage rate, n (%)	55 (16.87%)	65 (17.71%)	0.771
Live birth rate, n (%)	266 (49.35%)	301 (55.84%)	0.033

Abbreviations: HRT, hormone replacement therapy; GnRH-a, gonadotropin-releasing hormone agonist; hCG, human chorionic gonadotropin.

Further analyses, adjusted for potential confounders, are presented in **Table 3**. After controlling for these variables, the GnRH-a+HRT protocol continued to show elevated probabilities of a positive hCG result, clinical gestation, and live birth, with adjusted odds ratios of 1.63 (95% CI: 1.23–2.17), 1.45 (95% CI: 1.12–1.88), and 1.29 (95% CI: 1.01–1.65), respectively. The addition of GnRH-a prior to HRT demonstrated no meaningful influence on miscarriage risk (aOR: 1.18, 95% CI: 0.78–1.77).

Table 3. Crude and Adjusted Analyses of Pregnancy Outcomes in the Matched Cohort

Outcome	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Positive hCG	1.55 (1.18–2.03)	0.002	1.63 (1.23–2.17)	0.001

Clinical pregnancy	1.39 (1.09–1.79)	0.009	1.45 (1.12–1.88)	0.005
Miscarriage	1.06 (0.72–1.57)	0.771	1.18 (0.78–1.77)	0.439
Live birth	1.30 (1.02–1.65)	0.033	1.29 (1.01–1.65)	0.043

Abbreviations: OR, odds ratio; CI, confidence interval; hCG, human chorionic gonadotropin.

Subgroup analysis

Findings for the stratified evaluation based on dyslipidemia status are provided in **Tables 4 and 5**. In participants with dyslipidemia, the GnRH-a+HRT protocol yielded a higher live birth proportion compared with HRT alone (57.47% vs 45.91%; aOR: 1.75, 95% CI: 1.08–2.85). This contrast was not statistically confirmed in the normolipidemic cohort (55.07% vs 50.79%; aOR: 1.18, 95% CI: 0.87–1.58). Within the dyslipidemic subgroup, advantages for the GnRH-a+HRT regimen also appeared for positive hCG test rate, clinical pregnancy, and implantation, whereas in normolipidemic patients, only the hCG outcome differed.

Table 4. Subgroup Analysis According to the Status of Dyslipidemia

Subgroup: Dyslipidemia	**HRT (n=159)**	**GnRH-a+HRT (n=174)**	**P value**
Lipid profile			
Triglycerides (TG, mmol/L)	2.06 ± 1.20	2.03 ± 1.15	0.606
Total cholesterol (TC, mmol/L)	4.80 ± 0.97	4.85 ± 0.96	0.605
LDL-cholesterol (LDL-C, mmol/L)	2.73 ± 1.01	2.82 ± 1.00	0.397
HDL-cholesterol (HDL-C, mmol/L)	1.25 ± 0.32	1.32 ± 0.36	0.066
Positive hCG rate, n (%)	104 (65.41%)	137 (78.74%)	0.007
Clinical pregnancy rate, n (%)	90 (56.60%)	120 (68.97%)	0.020
Implantation rate, n/N (%)	112/258 (43.41%)	154/295 (52.20%)	0.039
Miscarriage rate, n (%)	16 (17.78%)	19 (15.83%)	0.708
Live birth rate, n (%)	73 (45.91%)	100 (57.47%)	0.035
Subgroup: Normolipidemia	**HRT (n=380)**	**GnRH-a+HRT (n=365)**	**P value**
Lipid profile			
Triglycerides (TG, mmol/L)	0.96 ± 0.36	0.94 ± 0.31	0.703
Total cholesterol (TC, mmol/L)	4.14 ± 0.55	4.11 ± 0.53	0.575
LDL-cholesterol (LDL-C, mmol/L)	2.41 ± 0.48	2.43 ± 0.48	0.621
HDL-cholesterol (HDL-C, mmol/L)	1.39 ± 0.23	1.43 ± 0.30	0.115
Positive hCG rate, n (%)	266 (70.00%)	279 (76.44%)	0.047
Clinical pregnancy rate, n (%)	236 (62.11%)	247 (67.67%)	0.112
Implantation rate, n/N (%)	301/612 (49.18%)	302/575 (52.52%)	0.250
Miscarriage rate, n (%)	39 (16.53%)	46 (18.62%)	0.545
Live birth rate, n (%)	193 (50.79%)	201 (55.07%)	0.242

Abbreviations: HRT, hormone replacement therapy; GnRH-a, gonadotropin-releasing hormone agonist; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; hCG, human chorionic gonadotropin.

Table 5. Crude and Adjusted Analyses of Pregnancy Outcomes in the Subgroups

Outcome	**Dyslipidemia Subgroup**	**Normolipidemia Subgroup**
	Crude OR (95% CI)	Adjusted OR (95% CI)
Positive hCG test	1.96 (1.20–3.19)	2.49 (1.41–4.40)
Clinical pregnancy	1.70 (1.09–2.67)	1.87 (1.14–3.08)
Miscarriage	0.87 (0.42–1.81)	0.86 (0.38–1.94)

Live birth	1.59 (1.03–2.46)	1.75 (1.08–2.85)

Abbreviations: OR, odds ratio; CI, confidence interval; hCG, human chorionic gonadotropin.

When stratifying by BMI group, 913 individuals were classified as overweight (BMI <28 kg/m²), and 165 were categorized as obese (BMI ≥28 kg/m²). Among overweight participants, cycles using GnRH-a+HRT consistently surpassed HRT-only cycles in positive hCG rate, clinical pregnancy, and live birth. In contrast, the obese subgroup showed no significant between-protocol differences, either before or after statistical adjustment.

Frozen embryo transfer (FET) is increasingly favored in assisted reproduction to reduce detrimental effects linked to controlled ovarian stimulation, including impaired endometrial receptivity and the risk of ovarian hyperstimulation syndrome [30]. Because endometrial status directly influences embryo implantation, preparation strategies are pivotal. In this cohort analysis, we compared outcomes from HRT and GnRH-a+HRT cycles among overweight and obese individuals. The findings demonstrate that the long-acting downregulation approach leads to notable improvements in clinical pregnancy and live birth, particularly for patients presenting with dyslipidemia.

Previous research has examined in depth whether GnRH-a administration before HRT-FET offers measurable advantages, particularly because this strategy adds financial burden, lengthens treatment timelines, and may cause adverse effects. In unselected patient groups, a recent meta-analysis of 14 randomized trials involving 1,244 cycles with pretreatment and 1,208 controls found no significant changes in clinical pregnancy (OR 1.09, 95% CI 0.87–1.36), implantation (OR 1.01, 95% CI 0.85–1.20), miscarriage (OR 0.75, 95% CI 0.31–1.82), or live birth (OR 1.14, 95% CI 0.68–1.41), indicating that routine use is not justified [14]. Nevertheless, evidence from specific populations paints a different picture. In a retrospective analysis of patients with recurrent implantation failure, the GnRH-a+HRT protocol yielded a markedly higher live birth proportion compared with HRT alone and natural cycles (36.55% vs 22.16% vs 16.92%, P<0.001) [20]. Meta-analytic findings in polycystic ovary syndrome further reported that pituitary downregulation increased live birth odds by 22% and lowered miscarriage by 25% [14]. Additional studies have noted amplified benefits in women younger than 40 years, those with primary infertility, PCOS, menstrual irregularities, or undergoing blastocyst-stage transfer or hysteroscopic removal of multiple polyps [31–33]. Conversely, pretreatment has not demonstrated benefit in older patients with intramural fibroids [34]. These observations underscore that responsiveness to the protocol is highly dependent on the underlying clinical profile. In the present study, we add new clinical support showing that overweight and obese patients also experience improved outcomes with the GnRH-a+HRT approach, thereby broadening the clinical scenarios in which this method may be advantageous. This may help refine treatment selection and increase reproductive success among FET candidates with excess body weight.

The biological explanation for the enhanced benefit in women with elevated BMI likely relates to the more compromised endometrial environment frequently observed in this group, given the influence of hormonal dysregulation, metabolic impairment, and persistent low-grade inflammation [35]. GnRH-a pretreatment may mitigate these factors through several pathways. It enhances the endometrial presence of molecules essential for receptivity, such as leukemia inhibitory factor, integrin β3, HOXA10, prolactin, and IGFBP-1, all of which participate in cell adhesion and stromal decidualization [36, 37]. In murine adenomyosis models, GnRH-a has also been shown to increase the density and maturation of pinopodes, structures necessary for successful embryo contact [37]. Additionally, the treatment may reduce estrogen receptor abundance and improve progesterone responsiveness, helping re-establish appropriate steroid hormone signaling [38]. Parallel reductions in inflammatory mediators—such as tumor necrosis factor-α and monocyte chemotactic protein-1—also contribute to a more permissive endometrial immune milieu [39]. Another mechanism involves modulation of uterine immunity: GnRH-a enhances natural killer (NK) cell activity [40], which is crucial for healthy placentation and extravillous trophoblast invasion [41]. It also shifts T-cell balance by lowering the Th17/Treg ratio, promoting immune tolerance [42]. Altogether, these effects lead to a more receptive endometrial phenotype. In alignment with these mechanisms, our study recorded increased endometrial thickness—a commonly used ultrasound marker of receptivity—in overweight/obese individuals receiving GnRH-a+HRT for their FET cycles. Despite these findings, no animal research has specifically evaluated GnRH-a pretreatment in overweight or obese models, and such studies would provide valuable mechanistic confirmation of our clinical observations.

Stratified analysis

In the stratified evaluation, patients with dyslipidemia showed a notably stronger response to the GnRH-a+HRT regimen than individuals with normal lipid profiles. This trend is consistent with recent reports demonstrating that abnormal lipid metabolism negatively influences live birth rates following embryo transfer and is linked to greater miscarriage risk [25, 26, 28]. At the mechanistic level, Zhang *et al.* [43] found that women with dyslipidemia displayed higher levels of CD56dim NK cells, an elevated M1/M2 macrophage ratio during the mid-secretory phase, and disrupted cytokine/chemokine expression, creating an immune environment associated with a shifted implantation window and increased colonization by harmful bacteria [43]. Both human and animal studies also indicate that dyslipidemia alters the expression of tight-junction proteins Claudin-3 and Claudin-4, weakening epithelial barrier function [44]. Collectively, these abnormalities suggest that dyslipidemia undermines endometrial receptivity by inducing immune disturbances and compromising epithelial integrity. The more substantial improvement seen with GnRH-a+HRT in this subgroup may reflect its capacity to counteract portions of these detrimental processes, consistent with the broader mechanisms previously outlined. Still, this interpretation is provisional and should be tested in future research.

Limitations

To date, work addressing this clinical question remains sparse, and our results add supporting evidence on the effect of GnRH-a downregulated HRT cycles for overweight and obese women undergoing FET. Several constraints, however, must be considered. First, although propensity score matching was implemented to reduce imbalance and selection bias, the observational framework cannot fully eliminate unmeasured confounding variables—including lifestyle habits such as physical activity or diet. Additionally, the absence of PGT data means that chromosomal abnormalities could not be excluded as contributors to unsuccessful pregnancies. Second, the study was conducted at a single institution, which may limit generalizability and reduce statistical power, particularly for subgroup comparisons. Notably, even though we classified women into overweight and obese groups, no significant effects were detected in the obese cohort, likely because of the smaller sample size. Larger studies involving more obese participants are needed to determine whether BMI modifies the impact of GnRH-a pretreatment. Third, overweight and obese individuals may also have coexisting metabolic disturbances (e.g., insulin resistance), and treatments such as metformin might influence reproductive outcomes. These data were not available in our dataset, preventing adjustment for these factors. Future work incorporating these variables will be critical for a more precise interpretation.

Conclusion

Overall, this retrospective cohort analysis shows that GnRH-a pretreatment enhances FET outcomes compared with HRT alone in overweight and obese patients, with the greatest improvement observed in those with dyslipidemia. These findings offer practical guidance for clinical decision-making and highlight the need for large, multicenter randomized trials to validate the results.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. M Ng, E Gakidou, J Lo, YH Abate, C Abbafat. Global, regional, and national prevalence of adult overweight and obesity, 1990-2021, with forecasts to 2050: a forecasting study for the Global Burden of Disease Study 2021. Lancet. 2025;405(10481):813–38. doi:10.1016/s0140-6736(25)00355-1
2. Wu S, Dong J, Ma X, He Y. Prevalence and risk factors of underweight, overweight and obesity in women of reproductive age in China. Chin J Clin Nutr. 2022;30(2):79–86. doi:10.3760/cma.j.cn115822-20220206-00021

3. Schon SB, Cabre HE, Redman LM. The impact of obesity on reproductive health and metabolism in reproductive-age females. Fertil Steril. 2024;122(2):194–203. doi:10.1016/j.fertnstert.2024.04.036
4. Broughton DE, Moley KH. Obesity and female infertility: potential mediators of obesity's impact. Fertil Steril. 2017;107(4):840–7. doi:10.1016/j.fertnstert.2017.01.017
5. Sermondade N, Huberlant S, Bourhis-Lefebvre V, et al. Female obesity is negatively associated with live birth rate following IVF: a systematic review and meta-analysis. Hum Reprod Update. 2019;25(4):439–51. doi:10.1093/humupd/dmz011
6. Ribeiro LM, Sasaki LM, Silva AA, Souza ES, Lyrio AO, Figueiredo AC, et al. Overweight, obesity and assisted reproduction: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2022;271:117–27. doi:10.1016/j.ejogrb.2022.01.019
7. Kasum M, Orešković S, Čehić E, Lila A, Ejubović E, Soldo D. The role of female obesity on in vitro fertilization outcomes. Gynecol Endocrinol. 2018;34(3):184–8. doi:10.1080/09513590.2017.1391209
8. Bakkensen JB, Strom D, Boots CE. Frozen embryo transfer outcomes decline with increasing female body mass index in female but not male factor infertility: analysis of 56,564 euploid blastocyst transfers. Fertil Steril. 2024;121(2):271–80. doi:10.1016/j.fertnstert.2023.07.027
9. Cozzolino M, García-Velasco JA, Meseguer M, Pellicer A, Bellver J. Female obesity increases the risk of miscarriage of euploid embryos. Fertil Steril. 2021;115(6):1495–502. doi:10.1016/j.fertnstert.2020.09.139
10. Tremellen K, Pearce K, Zander-Fox D. Increased miscarriage of euploid pregnancies in obese women undergoing cryopreserved embryo transfer. Reprod Biomed Online. 2017;34(1):90–7. doi:10.1016/j.rbmo.2016.09.011
11. Mackens S, Santos-Ribeiro S, Van De Vijver A, Racca A, Van Landuyt L, Tournaye H, et al. Frozen embryo transfer: a review on the optimal endometrial preparation and timing. Hum Reprod. 2017;32(11):2234–42. doi:10.1093/humrep/dex285
12. Van De Vijver A, Polyzos NP, Van Landuyt L, De Vos M, Camus M, Stoop D, et al. Cryopreserved embryo transfer in an artificial cycle: is GnRH agonist down-regulation necessary? Reprod Biomed Online. 2014;29(5):588–94. doi:10.1016/j.rbmo.2014.08.005
13. Dal Prato L, Borini A, Cattoli M, Bonu MA, Sciajno R, Flamigni C. Endometrial preparation for frozen-thawed embryo transfer with or without pretreatment with gonadotropin-releasing hormone agonist. Fertil Steril. 2002;77(5):956–60. doi:10.1016/s0015-0282(02)02960-6
14. Ho NT, Ho DK, Tomai XH, Nguyen NN, Nguyen HS, Hu YM, et al. Pituitary Suppression with Gonadotropin-Releasing Hormone Agonist Prior to Artificial Endometrial Preparation in Frozen-Thawed Embryo Transfer Cycles: a Systematic Review and Meta-Analysis of Different Protocols and Infertile Populations. Biomedicines. 2024;12(4):760. doi:10.3390/biomedicines12040760
15. Wu H, Wei F, Tan W, Dong M, Tan Y, Zhang X, et al. Administration of depot GnRH agonist prior to programmed frozen-thawed embryo transfer does not improve the live birth rate in ovulatory women: a large, multi-center retrospective study. Medicine (Baltimore). 2022;101(40):e30991. doi:10.1097/md.0000000000030991
16. Mo M, Zheng Q, Zhang H, Xu S, Xu F, Wang Y, et al. Hormone replacement therapy with GnRH agonist pretreatment improves pregnancy outcomes in patients with previous intrauterine adhesions. J Gynecol Obstet Hum Reprod. 2022;51(8):102439. doi:10.1016/j.jogoh.2022.102439
17. Liu Y, Ma L, Zhu M, Yin H, Yan H, Shi M. STROBE-GnRHa pretreatment in frozen-embryo transfer cycles improves clinical outcomes for patients with persistent thin endometrium: a case-control study. Medicine (Baltimore). 2022;101(31):e29928. doi:10.1097/md.0000000000029928
18. Wei L, Tian B, Wang S, Xu S, Zhang C. Gonadotropin Releasing Hormone Agonists Combined with Hormone Replacement Therapy Significantly Improves Reproductive Outcomes for Patients with Thin Endometrium and Intramural Fibroids in Frozen Embryo Transfer Cycles. Drug Des Devel Ther. 2025;19:173–83. doi:10.2147/dddt.S501809
19. Xia L, Tian L, Zhang S, Huang J, Wu Q. Hormonal Replacement Treatment for Frozen-Thawed Embryo Transfer With or Without GnRH Agonist Pretreatment: a Retrospective Cohort Study Stratified by Times of Embryo Implantation Failures. Front Endocrinol (Lausanne). 2022;13:803471. doi:10.3389/fendo.2022.803471
20. Pan D, Yang J, Zhang N, Wang L, Li N, Shi J, et al. Gonadotropin-releasing hormone agonist downregulation combined with hormone replacement therapy improves the reproductive outcome in frozen-thawed embryo

transfer cycles for patients of advanced reproductive age with idiopathic recurrent implantation failure. Reprod Biol Endocrinol. 2022;20(1):26. doi:10.1186/s12958-022-00897-3

21. Zhou BF. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults--study on optimal cut-off points of body mass index and waist circumference in Chinese adults. Biomed Environ Sci. 2002;15(1):83–96.
22. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 2007;67(1):73–80. doi:10.1016/j.theriogenology.2006.09.014
23. Cummins JM, Breen TM, Harrison KL, Shaw JM, Wilson LM, Hennessey JF. A formula for scoring human embryo growth rates in in vitro fertilization: its value in predicting pregnancy and in comparison with visual estimates of embryo quality. J in Vitro Fert Embryo Transf. 1986;3(5):284–95. doi:10.1007/BF01133388
24. Gardner DK, Schoolcraft WB. In vitro culture of human blastocyst. In: Jansen R, Mortimer D, editors. Towards Reproductive Certainty: Infertility and Genetics Beyond 1999. Carnforth: Parthenon Press; 1999:378–88.
25. Liu Z, Cong J, Liu X, Zhao H, Lai S, He S, et al. Dyslipidemia Is Negatively Associated With the Cumulative Live-Birth Rate in Patients Without PCOS Following IVF/ICSI. Front Physiol. 2021;12:713356. doi:10.3389/fphys.2021.713356
26. Yang F, Lu JC, Shen T, Jin YH, Liang YJ. Effect of hyperlipidemia on the outcome of in vitro fertilization in non-obese patients with polycystic ovary syndrome. Front Endocrinol (Lausanne). 2023;14:1281794. doi:10.3389/fendo.2023.1281794
27. Yang T, Zhao J, Liu F, Li Y. Lipid metabolism and endometrial receptivity. Hum Reprod Update. 2022;28(6):858–89. doi:10.1093/humupd/dmac026
28. Yang T, Zhao J, Zhang Q, Liu D, Liu N, Li Y, et al. Associations between dyslipidaemia and pregnancy outcomes in the first complete cycle of IVF/ICSI: a real-world analysis. Reprod Biomed Online. 2021;43(6):1095–105. doi:10.1016/j.rbmo.2021.08.030
29. Li JJ, Zhao SP, Zhao D, Lu GP, Peng DQ, Liu J, et al. 2023 Chinese guideline for lipid management. Front Pharmacol. 2023;14:1190934. doi:10.3389/fphar.2023.1190934
30. Devroey P, Polyzos NP, Blockeel C. An OHSS-Free Clinic by segmentation of IVF treatment. Hum Reprod. 2011;26(10):2593–7. doi:10.1093/humrep/der251
31. Xu B, Hou Z, Liu N, Zhao J, Li Y. Pretreatment with a long-acting GnRH agonist for frozen-thawed embryo transfer cycles: how to improve live birth? J Ovarian Res. 2023;16(1):197. doi:10.1186/s13048-023-01277-0
32. Hao H, Li M, Zhang C, Zhang S. The effect of gonadotropin-releasing hormone agonist downregulation in conjunction with hormone replacement therapy on endometrial preparation in patients for frozen-thawed embryo transfer. Front Med. 2024;11:1412126. doi:10.3389/fmed.2024.1412126
33. Wang J, Tong X, Zhu H, Wei M, Lin X, Wang X, et al. Pretreatment with long-acting gonadotropin-releasing hormone agonists improved pregnancy outcomes after hysteroscopic multiple polypectomies: a retrospective study of 660 frozen-thawed embryo transfer cycles. Taiwan J Obstet Gynecol. 2024;63(1):57–63. doi:10.1016/j.tjog.2023.09.020
34. Sun P, Dong Y, Yu Y, Xu H, Zhu L, Zhang P, et al. Gonadotropin-Releasing Hormone agonist (GnRH-a) Pretreatment before Hormone Replacement Therapy Does Not Improve Reproductive Outcomes of Frozen-Thawed Embryo Transfer Cycle in Older Patients with Intrauterine Fibroid: a Retrospective Cohort Study. J Clin Med. 2023;12(4):1401. doi:10.3390/jcm12041401
35. Gonnella F, Konstantinidou F, Donato M, Gatta DM, Peserico A, Barboni B, et al. The Molecular Link between Obesity and the Endometrial Environment: a Starting Point for Female Infertility. Int J Mol Sci. 2024;25(13):6855. doi:10.3390/ijms25136855
36. An J, Li L, Zhang X, Liu L, Wang L, Zhang X. A clinical and basic study of optimal endometrial preparation protocols for patients with infertility undergoing frozen-thawed embryo transfer. Exp Ther Med. 2020;20(3):2191–9. doi:10.3892/etm.2020.8914
37. Guo S, Li Z, Yan L, Sun Y, Feng Y. GnRH agonist improves pregnancy outcome in mice with induced adenomyosis by restoring endometrial receptivity. Drug Des Devel Ther. 2018;12:1621–31. doi:10.2147/dddt.S162541
38. MacLean II JA, Hayashi K. Progesterone Actions and Resistance in Gynecological Disorders. Cells. 2022;11(4):647. doi:10.3390/cells11040647

39. Mohammed NH, Al-Taie A, Albasry Z. Evaluation of goserelin effectiveness based on assessment of inflammatory cytokines and symptoms in uterine leiomyoma. Int J Clin Pharm. 2020;42(3):931–7. doi:10.1007/s11096-020-01030-3
40. Umesaki N, Tanaka T, Miyama M, Mizuno K, Kawamura N, Ogita S. Increased natural killer cell activities in patients treated with gonadotropin releasing hormone agonist. Gynecol Obstet Invest. 1999;48(1):66–8. doi:10.1159/000010137
41. Kanter J, Gordon SM, Mani S, Sokalska A, Park JY, Senapati S, et al. Hormonal stimulation reduces numbers and impairs function of human uterine natural killer cells during implantation. Hum Reprod. 2023;38(6):1047–59. doi:10.1093/humrep/dead069
42. Guo Z, Shi P, Hao C. Effect of GnRHa on Th17/Treg cells in peripheral blood of patients with unexplained repeated implantation failure. Arch Gynecol Obstet. 2018;298(6):1211–8. doi:10.1007/s00404-018-4874-6
43. Zhang Y, Sun X, Zhang Y, Zou Y, Zhang Y, Wang J, et al. Immune Dysregulation and Endometrial Receptivity Impairment in Women with Repeated Implantation Failure and Dyslipidemia. J Clin Endocrinol Metab. 2025;2025:dgaf100. doi:10.1210/clinem/dgaf100
44. Zhang Y, Zhang Y, Xia X, Gao L, Gao C, Zhou J, et al. Hyperlipidemia negatively impacts implantation by dysregulating tight junction and Claudin-3 and Claudin-4 expression in the endometrium. J Reprod Immunol. 2024;166:104326. doi:10.1016/j.jri.2024.104326