Does Inclusion of GnRH Agonist in Luteal Phase Support Improve Pregnancy Rates in Frozen Embryo Transfer Cycles?

Original Article

Mohamed Zakaria Sayer Dayer and Abdelbar Mohamed Sharaf

Department of Obstetrics &Gynecology, Faculty of Medicine, Menoufia University, Menoufia, Egypt

ABSTRACT

Background and Objective: The endometrium and embryos have been reported to be directly affected by GnRH agonists (GnRHa). Additionally, as demonstrated by numerous meta-analyses, the utilization of GnRHa during the luteal phase of fresh IVF cycles has been correlated with an increase in the rates of live birth and pregnancy. This study aimed to ascertain whether GnRHa administration during the luteal phase could enhance the results of in vitro fertilization (IVF) in patients who are undergoing frozen embryo transfer (FET) cycles.

Materials and Methods: In order to prepare the endometrium, hormone replacement therapy (HRT) was implemented in a total of 166 frozen embryo transfer cycles during this randomized controlled trial. The cycles were divided into two equal groups, each consisting of 83 cycles. Two hours after the embryo transfer, a single subcutaneous dose of 0.2 mg triptorelin (Decapeptyl) was administered to the GnRHa group. Devoid of the administration of luteal GnRHa, the control group underwent embryo transfer. In this study, the clinical pregnancy rate was the primary outcome, while the ongoing pregnancy rate was the secondary outcome.

Results: Both groups exhibited remarkable similarities in their baseline and cycle characteristics. The luteal GnRHa group has a clinical pregnancy rate that was significantly higher than those of the control group (57.8% vs. 41.0%, $P = 0.030$). Rates of ongoing pregnancy were comparable in both groups (41.0% vs. 25.3%; P = 0.476). Furthermore, the use of a luteal GnRH agonist was identified as a significant independent predictor of clinical pregnancy in FET-HRT cycles, as indicated by the multivariate analysis (OR 1.512, 95% CI 1.020-2.241, $P = 0.039$).

Conclusions: The clinical pregnancy rates of patients who are undergoing the HRT-FET protocol may be enhanced by addition of a single luteal dose of GnRHa.

Key Words: Frozen embryo transfer, hormone replacement therapy cycles, luteal GnRHa, pregnancy rates.

Received: 24 June 2024, **Accepted:** 29 October 2024

Corresponding Author: Mohamed Zakaria Sayer Dayer, Department of Obstetrics &Gynecology, Faculty of Medicine, Menoufia University, Menoufia, Egypt, **Tel.:** +20 10 0477 8794, **E-mail:** Mimo_zsd@yahoo.com

ISSN: 2090-7265, November 2024, Vol. 14, No. 4

INTRODUCTION

The utilization of frozen-thawed embryo transfer (FET) in IVF programs has been increased because it is associated with a decrease in the prevalence of ovarian hyperstimulation syndrome[1-4]. Embryo-endometrial communication, a prepared endometrium and high-quality embryos are the factors that result in implantation. The embryos and the endometrium both contain GnRH and GnRH receptors (GnRH-R)[5–10]. In fact, prior research has demonstrated that GnRH enhances endometrial receptivity and embryonic development[11–14].

It has been demonstrated in specific meta-analyses and comprehensive reviews that the ongoing pregnancy rate and live birth rate were significantly increased by the addition of GnRHa to progesterone in cycles that involve fresh embryo transfer[15–18]. Furthermore, the administration of GnRHa during the luteal phase resulted in an increased clinical pregnancy rate in both fresh and frozen embryo transfer cycles. This was shown by a systematic review and meta-analysis that encompassed 20 investigations and included 5497 women[19].

Supplementing with luteal GnRHa may have an impact through two possible pathways: stimulation of the release of endogenous pituitary gonadotropins and the activation of the GnRH-Receptors, which is expressed locally in the endometrium and embryos[7,8]. Thus the endometrial receptivity and implantation are both improved by the GnRH-GnRH-R network.

In humans, GnRHa may induce the expression of factors essential for implantation including endometrial integrin αvβ3 expression , HOXA10 and LIF[11,12]. Research on human decidual stromal cells has also shown that

DOI:10.21608/EBWHJ.2024.331727.1374

GnRH has the ability to control matrix metalloproteinases (MMPs) and the endogenous inhibitors of these enzymes, namely TIMPs[21], both are linked to decidualization and cyclic remodeling of the endometrium[22]. Additionally, an essential step in embryo implantation preparation, GnRHa has directly induced endometrial stromal cells invasion and migration[23]. The findings suggested that GnRHa could enhance endometrial receptivity and the implantation process.

In addition, GnRH- R and GnRH have been identified in preimplantation embryos in both inner cell mass of the blastocyst stage and trophectoderm cells. These findings provide more evidence that, the GnRH-GnRH-R pathway may be crucial for controlling the early phases of embryonic development. The development of embryos and the inhibition of apoptosis in animal models can be facilitated by the administration of GnRHa in culture medium[9,10,13,14].

Evidence suggests that GnRHa may modulate urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor (PAI-1) expression in human extravillous cytotrophoblasts, potentially facilitating trophoblast invasion[24-26]. Additionally, it is possible that GnRHa affects hCG production and secretion in the placenta and preimplantation embryo[27,28].

Consequently, we asked if boosting the luteal phase with GnRHa at the FET-HRT protocol is beneficial. Nevertheless, this topic has been the subject of only a few empirical investigations. Therefore, a single dose of GnRHa was administered during the luteal phase to patients who were undergoing HRT FET cycles in this randomized controlled trial to investigate its effects.

MATERIALS AND METHODS

From September 2021 to September 2023, a randomized controlled trial was implemented at a specialized fertility and gynecology center. Menoufia University's Institutional Review Board authorized the investigation, and each participant signed a written informed consent after being instructed of the study's objectives, procedures, and nature.

Women aged 23-40 years, underwent hormone replacement FET, endometrial thickness more than eight mm at the day of progesterone initiation and ≥ one top-quality embryo transferred were invited for inclusion in our investigation.

The following criteria were listed for exclusion: 1) Patients who are over the age of 40 years, 2) Patients with uterine factor infertility, 3) Fresh ET cycles, 4) Frozen ET cycles other than hormonal replacement therapy, 5) After estradiol priming, patients with a refractory thin endometrium (< 8 mm), 6) Patients who had preimplantation genetic testing, and 7) Patients with testicular sperm extraction (TESE).

A total of 166 patients were randomly assigned to either the control group (n = 83) or the luteal GnRHa group (n = 83). Collective allocation was concealed by sealed opaque identical serially numbered envelopes using computer generated randomization sheet by MedCalc © version 13. Each envelope contained a letter that corresponded to the designated group. A subcutaneous injection of 0.2 mg triptorelin (Decapeptyl ®; Ferring, Malmo, Sweden) was administered to the luteal GnRHa group two hours after ET.

Endometrial preparation and frozen–thawed embryo transfer

The HRT protocol was employed in all FET cycles in this study. Endometrial preparation was commenced on the 1st menstrual cycle day with oral estradiol valerate (Cyclo-Progynova®; Bayer, Germany) at a daily dose of 6-8 mg. Transvaginal ultrasound was done and endometrial thickness was assessed 14 days after giving the drugs consecutively. When the thickness of endometrium was less than eight mm, the dosage of estrogen was increased, and the duration of medication was extended. After 20 days of estrogen therapy, the cycle was cancelled if the thickness of endometrium was less than or equal to eight millimeters. IM progesterone (100 mg/day) and progesterone vaginal pessaries (400 mg twice daily) were administered in conjunction with E2 administration to sustain the luteal phase when the thickness of endometrial was 8 mm or greater.

Transfer of embryos either in the cleavage stage or blastocyst stage were implemented on the fourth or sixth day of progesterone administration respectively. The transabdominal ultrasound was used to guide the embryo transfer. Within the luteal GnRHa group, at 2 hours post-ET, 0.2 mg of triptorelin (Decapeptyl ®; Ferring, Malmo, Sweden) was administered once subcutaneously to all patients.

Outcome measures

In this study, the clinical pregnancy rate was the primary outcome, while the ongoing pregnancy rate was the secondary outcome. At around 6-7 weeks of gestation, transvaginal ultrasonography was utilized to detect clinical pregnancy by visualizing the embryonic heart activity. The term "ongoing pregnancy" was used to describe a healthy pregnancy that continued beyond the 12th week.

Statistical analysis

Employing the Statistical Package for the Social Sciences (IBM Corp., 2017) and Version 25 of SPSS Statistics for Windows, the data was analyzed, classified,

and tabulated. As a normality measure, the test of Kolmogorov-Smirnov was implemented. In order to determine the statistical significance of a non-parametric variable that encompassed two study groups, Mann-Whitney test was implemented. To compare two qualitative variables, Chi-Square test was employed. P values that are less than 0.05 are considered significant. Risk factor prediction was conducted within a 95% confidence interval using ordinal regression and logistic analyses. P values less than 0.05 are considered significant.

Sample size calculation

Based on review of past literature[20] who found that clinical pregnancy rate was 54.2 and 30.6% in luteal GnRHa group and control group respectively. The least sample size calculated using statistics and sample size pro is 76 participants per each group and increase up to 83 participants per each group to avoid 10% dropout rate. The calculated total sample size was 166 participants (83 participants per each group), power of study= 80%, and α error=0.05.

RESULTS

According to the flowchart of the CONSORT scheme (Figure 1), the present investigation assessed the eligibility of 175 HRT-FET cycles that took place between September 2021 and September 2023. Only three patients declined to participate, while six patients failed to satisfy the inclusion criteria. Therefore, nine cycles were excluded from the investigation. The remaining 166 cycles were randomly assigned to the control group (n = 83) and the luteal GnRHa group (n = 83). The trial has been completed by all participants.

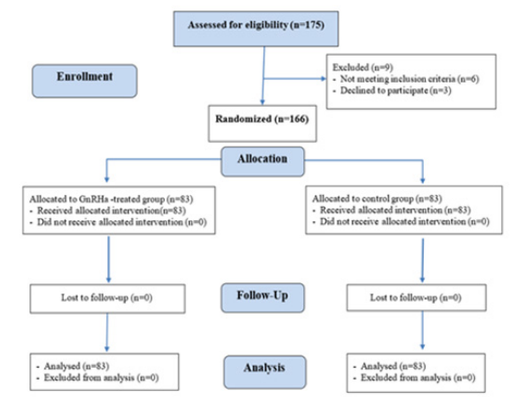

Fig. 1: The CONSORT flow chart

The study population's baseline and ICSI cycle characteristics are summarized in (Table 1). In terms of age (p = 0.964), body mass index (p = 0.416), and duration of subfertility (p = 0.498), there were no significant differences among the two groups. Furthermore, in relation to the characteristics of the ICSI cycle, the two groups did not exhibit any significant differences in the number of Mii oocytes (p = 0.617), endometrial thickness (p = 0.542), number of embryos transferred (p = 0.739), and grade of transferred embryos (p = 0.231).

Table 1: Baseline and ICSI cycle characteristics of the study population (N=166):

Studied variables	Control group (N=83)	GnRH group (N=83)	Test of sig	*P value*
Age (years) Mean ±SD Median(range)	 28.9±5.6 29.0(19.0 – 42.0)	 29±5.8 29.0(19.0 – 40.0)	U= 3430.5	0.964
BMI (kg/m²) Mean ±SD Median(range)	 25.0±2.60 25.0(19.0 – 30.0)	 25.2±3.2 26.0(19.0 – 31.0)	U= 3194.0	0.416
Duration of subfertility (years) Mean ±SD Median(range)	 4.40±1.50 4.00(3.00 – 9.00)	 4.30±1.50 4.00(3.00 – 9.00)	U= 3242.5	0.498
Cause of subfertility Unexplained Anovulation Diminished ovarian reserve Endometriosis Tubal Mixed Male factor	N (%) 35(42.2) 6(7.20) 13(15.70) 5(6.00) 2(2.40) 8(9.60) 14(16.9)	N (%) 32(38.6) 17(20.5) 7(8.44) 5(6.00) 6(7.23) 6(7.23) 10(12.00)	X²= 10.148	0.114
Number of Mii oocytes Mean ±SD Median (range)	 12.9±8.70 11.0(2.00 – 45.0)	 13.8±9.50 12.0(1.00 – 45.0)	U= 3209.5	0.617
Endometrial thickness (mm) Mean ±SD Median (range)	 9.80±1.30 10.0(7.00 – 12.0)	 9.70±1.00 10.0(7.00 – 12.0)	U= 3263.0	0.542
Number of embryos transferred Mean ±SD Median (range)	 2.30±0.50 2.00(2.00 – 3.00)	 2.30±0.50 2.00(2.00 – 3.00)	U= 3361.5	0.739
Quality of embryos transferred Grade A Grade B	N (%) 75(90.4) 8(9.60)	N (%) 79(95.2) 4(4.80)	X²= 1.437	0.231

GnRH: gonadotropin-releasing hormone agonist; BMI: body mass index N: number %: percentage SD: Standard deviation U: Mann Whitney test χ^2: Chi square test

(Table 2) presented are the outcomes of the frozen-thawed ET cycles of the study population. The incidence of clinical pregnancy in the luteal GnRHa group was substantially higher than that of the control group (57.8% vs. 41.0%, $p = 0.030$). Rates of ongoing pregnancy were comparable in both groups (41.0% vs. 25.3%; $p = 0.476$).

In (Table 3), the effects of a supplementary luteal GnRH agonist on clinical pregnancy during FET-HRT cycles were assessed using logistic regression analysis. The following variables were analyzed: endometrial thickness, age, and body mass index, quality of transplanted embryos, number of Mii oocytes, number of transferred embryos, and duration of infertility. Furthermore, the use of a luteal GnRH agonist was identified as a significant independent predictor of clinical pregnancy in FET-HRT cycles, as indicated by the multivariate analysis (OR 1.512, 95% CI 1.020-2.241, $p= 0.039$). Age, BMI, thickness of endometrium, number of Mii oocytes, and number of transferred embryos did not manifest statistically significant associations with clinical pregnancy. This study's findings emphasize the importance of embryo quality and luteal GnRHa treatment in determining the clinical pregnancy rate in FET-HRT cycles.

Table 2: Outcomes of frozen-thawed ET cycles among the studied groups (N=166):

Studied variables	Control group (N=83)	GnRH group (N=83)	Test of sig.	*P value*
Clinical pregnancy	34(41.0%)	48(57.8%)	X^2= 4.724	0.030*
Ongoing pregnancy	21 (25.3%)	34(41.0%)	X^2= 0.741	0.476

*Significant χ^2: Chi square test

Table 3: Analysis of factors affecting the clinical pregnancy rate using logistic regression:

	Univariable		Multivariable	
	p	OR (95%CI)	*p*	OR (95%CI)
Luteal GnRHa administration	0.029*	1.540(1.046-2.268)	0.039*	1.512 (1.020-2.241)
Age	0.168	0.976(0.943-1.010)		
BMI	0.225	0.960(0.900-1.025)		
Duration of subfertility	0.296	1.072(0.941-1.221)		
Number of Mii oocytes	0.326	1.011(0.989-1.032)		
Endometrial thickness	0.861	1.015(0.86-1.198)		
Number of embryos transferred	0.080	0.688(0.453-1.046)		

BMI body mass index OR: Odds ratio CI: Confidence interval * significant

DISCUSSION

This randomized controlled trial assessed the effects of adding a single-dose of GnRHa during the luteal phase in patients who underwent FET-HRT cycles. According to the research, a higher incidence of clinical pregnancy was observed when a luteal GnRHa was administrated. Furthermore, the use of a luteal GnRH agonist was identified as a significant independent predictor of clinical pregnancy in FET-HRT cycles, as indicated by the multivariate analysis (OR 1.512, 95% CI 1.020-2.241, $P = 0.039$).

Supplementing with luteal GnRHa may have an impact through two possible pathways: stimulation of the release of endogenous pituitary gonadotropins and the activation of the GnRH-Receptors, which is expressed locally in the endometrium and embryos[7,8]. Thus the endometrial receptivity and implantation are both improved by the GnRH-GnRH-R network.

Cochrane meta-analysis of 10 RCTs involving 2861 women found that progesterone with GnRHa was linked with a greater incidence of live birth or continuing pregnancy compared to progesterone alone[15]. There are numerous additional systematic reviews and meta-analyses that have documented a comparable results[16–18].

To further assess the safety and effectiveness of various approaches of enhancing the luteal phase, a meta-analysis and systematic review of 89 RCTs covering 29,625 women was undertaken. Researchers found that compared to using progesterone alone, adding GnRHa to the progesterone significantly increased the live birth rate[29]. An additional comprehensive review and meta-analysis investigated the safety and efficacy of multiple-dose versus single-dose GnRHa strategies for luteal phase support in patients undergoing IVF/ICSI cycles. According to the results, the best way to increase the rates of clinical pregnancies and live births is to use the multiple-dose GnRHa strategy[30]. Nevertheless, these meta-analyses were limited to patients who were undergoing fresh ET cycles.

Later on, a meta-analysis and systematic review of frozen-thawed embryo transfer (FET) cycles found that luteal phase GnRHa therapy enhanced the clinical pregnancy rate in FET cycles, which was similar to the advantages of fresh cycles[19].

In accordance with the present findings, Chang and his colleagues conducted a study in which 179 patients were administered GnRHa single dose on FET day. In comparison to the control group, the GnRHa group had considerably higher rates of clinical pregnancy (54.2% vs. 30.6%, *P*=0.004), ongoing pregnancy (47.2% vs. 23.6%, *P*=0.004), and live birth (44.4% vs. 22.2%, *P*=0.005). Chang and his colleagues had downregulated all HRT-FET cycles in this study by long-acting GnRHa (3.75 mg), given subcutaneously. Although the endometrium's locally expressed GnRH-R may have been downregulated by long-acting GnRHa, the scientists found that the embryonic effects of GnRHa supplementation on ET day were responsible for its favorable benefits[20]. Additionally, according to previous research, fresh IVF outcomes can be enhanced with the use of luteal GnRHa when following the GnRHa long protocol[29]. In addition, Xu et al. demonstrated that depot GnRHa treatment, as opposed to long GnRHa or GnRH antagonist protocols, had increased mRNA and protein expression of endometrial HOXA10, MEIS1, and LIF[12]. These studies did not provide any evidence that GnRHa inhibited GnRH-R in the endometrium.

On the contrary, when 287 HRT-FET cycles were included in a recent randomized controlled trial (RCT), the administration of two GnRHa boluses did not lead to a substantial increase in the rates of clinical pregnancy or live birth[31]. In order to confirm that luteal GnRHa treatment improves IVF results, especially FET cycles, more large-scale RCTs are required.

STRENGTH POINTS

First, it was a prospective randomized controlled study design and having no patients who were lost during the study period.

Second, the decision to administrate luteal GnRHa was made based on randomization, rather than on the preferences of the patients after physician consultation, which may have introduced bias.

LIMITATIONS

Being a single-center study instead of a multicentric one, the sample size was small in relation to the outcomes, which was the main drawback of the study. In order to confirm our findings, it is necessary to conduct large-scale RCTs.

Additionally, the selection of embryos was conducted using morphological classification rather than euploidy testing. Consequently, it was impossible to exclude the embryo aneuploidy confounding effects.

CONCLUSIONS

According to our results, the incidence of clinical pregnancy in patients who are undergoing the FET-HRT protocol may be improved by administering a single dose of GnRHa during the luteal phase.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Kushnir VA, Barad DH, Albertini DF, Darmon SK, Gleicher N. Systematic review of worldwide trends in assisted reproductive technology 2004- 2013. Reprod Biol Endocrinol. 2017;15(1):6.

2. Zaat T, Zagers M, Mol F, Goddijn M, van Wely M, Mastenbroek S. Fresh versus frozen embryo transfers in assisted reproduction. Cochrane Database Syst Rev. 2021;2(2): Cd011184.

3. Sha T, Yin X, Cheng W, Massey IY. Pregnancy-related complications and perinatal outcomes resulting from transfer of cryopreserved versus fresh embryos in vitro fertilization: a meta-analysis. Fertil Steril. 2018;109(2):330–42. e9.

4. Wu H, Zhou P, Lin X, Wang S, Zhang S. Endometrial preparation for frozen-thawed embryo transfer cycles: a systematic review and network meta-analysis. J Assist Reprod Genet. 2021;38(8):1913–26.

5. Wu HM, Wang HS, Huang HY, Soong YK, MacCalman CD, Leung PC. GnRH signaling in intrauterine tissues. Reproduction. 2009;137(5):769–77.

6. Cheng CK, Leung PC. Molecular biology of gonadotropin-releasing hormone (GnRH)-I, GnRH-II, and their receptors in humans. Endocr Rev. 2005;26(2):283–306.

7. Raga F, Casañ EM, Kruessel JS, Wen Y, Huang HY, Nezhat C, et al. Quantitative gonadotropin-releasing hormone gene expression and immunohistochemical localization in human endometrium throughout the menstrual cycle. Biol Reprod. 1998;59(3):661–9.

8. Casañ EM, Raga F, Kruessel JS, Wen Y, Nezhat C, Polan ML. Immunoreactive gonadotropin-releasing hormone expression in cycling human endometrium of fertile patients. Fertil Steril. 1998;70(1):102–6.

9. Casañ EM, Raga F, Polan ML. GnRH mRNA and protein expression in human preimplantation embryos. Mol Hum Reprod. 1999;5(3):234–9.

10. Raga F, Casañ EM, Kruessel J, Wen Y, Bonilla-Musoles F, Polan ML. The role of gonadotropin-releasing hormone in murine preimplantation embryonic development. Endocrinology. 1999;140(8):3705–12.

11. Li L, Liu L, Kou Z, Huo M, An J, Zhang X. GnRH agonist treatment regulates IL-6 and IL-11 expression in endometrial stromal cells for patients with HRT regiment in frozen embryo transfer cycles. Reprod Biol. 2022;22(2):100608.

12. Xu B, Geerts D, Hu S, Yue J, Li Z, Zhu G, et al. The depot GnRH agonist protocol improves the live birth rate per fresh embryo transfer cycle, but not the cumulative live birth rate in normal responders: a randomized controlled trial and molecular mechanism study. Hum Reprod. 2020;35(6):1306–18.

13. Nam DH, Lee SH, Kim HS, Lee GS, Jeong YW, Kim S, et al. The role of gonadotropin-releasing hormone (GnRH) and its receptor in development of porcine preimplantation embryos derived from in vitro fertilization. Theriogenology. 2005;63(1):190–201.

14. Kawamura K, Fukuda J, Kumagai J, Shimizu Y, Kodama H, Nakamura A, et al. Gonadotropin-releasing hormone I analog acts as an antiapoptotic factor in mouse blastocysts. Endocrinology. 2005;146(9):4105–16.

15. van der Linden M, Buckingham K, Farquhar C, Kremer JA, Metwally M. Luteal phase support for assisted reproduction cycles. Cochrane Database Syst Rev. 2015;(7):CD009154.

16. Oliveira JB, Baruffi R, Petersen CG, Mauri AL, Cavagna M, Franco JG Jr. Administration of single-dose GnRH agonist in the luteal phase in ICSI cycles: a meta-analysis. Reprod Biol Endocrinol. 2010; 8:107.

17. Kyrou D, Kolibianakis EM, Fatemi HM, Tarlatzi TB, Devroey P, Tarlatzis BC. Increased live birth rates with GnRH agonist addition for luteal support in ICSI/IVF cycles: a systematic review and meta-analysis. Hum Reprod Update. 2011;17(6):734–40.

18. Ma X, Du W, Hu J, Yang Y, Zhang X. Effect of gonadotrophin-releasing hormone agonist addition for luteal support on pregnancy outcome in vitro fertilization/intracytoplasmic sperm injection cycles: a Meta-analysis based on randomized controlled trials. Gynecol Obstet Investig. 2020;85(1):13–25.

19. Chau LTM, Tu DK, Lehert P, Dung DV, Thanh LQ, Tuan VM. Clinical pregnancy following GnRH agonist administration in the luteal phase of fresh or frozen assisted reproductive technology (ART) cycles: systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol X. 2019; 3:100046.

20. Chang WS, Lin PH, Li CJ, Chern CU, Chen YC, Lin LT, Tsui KH. Additional single dose GnRH agonist during luteal phase support may improve live birth rate in GnRHa-HRT frozen–thawed embryo transfer cycle: a retrospective cohort study. BMC Pregnancy and Childbirth. 2023 Mar 14;23(1):174

21. Chou CS, Tai CJ, MacCalman CD, Leung PC. Dose-dependent effects of gonadotropin releasing hormone on matrix metalloproteinase (MMP)-2, and MMP-9 and tissue specific inhibitor of metalloproteinases-1 messenger ribonucleic acid levels in human decidual stromal cells in vitro. J Clin Endocrinol Metab. 2003;88(2):680–8.

22. Vu TH, Werb Z. Matrix metalloproteinases: effectors of development and normal physiology. Genes Dev. 2000;14(17):2123–33.

23. Wu HM, Huang HY, Lee CL, Soong YK, Leung PC, Wang HS. Gonadotropin- releasing hormone type II (GnRH-II) agonist regulates the motility of human decidual endometrial stromal cells: possible effect on embryo implantation and pregnancy. Biol Reprod. 2015;92(4):98.

24. Hardy K, Spanos S. Growth factor expression and function in the human and mouse preimplantation embryo. J Endocrinol. 2002;172(2):221–36.

25. Kane MT, Morgan PM, Coonan C. Peptide growth factors and preimplantation development. Hum Reprod Update. 1997;3(2):137–57.

26. Chou CS, Zhu H, Shalev E, MacCalman CD, Leung PC. The effects of gonadotropin-releasing hormone (GnRH) I and GnRH II on the urokinase- type plasminogen activator/plasminogen activator inhibitor system in human extravillous cytotrophoblasts in vitro. J Clin Endocrinol Metab. 2002;87(12):5594–603.

27. Lin LS, Roberts VJ, Yen SS. Expression of human gonadotropin-releasing hormone receptor gene in the placenta and its functional relationship to human chorionic gonadotropin secretion. J Clin Endocrinol Metab. 1995;80(2):580–5.

28. Iwashita M, Kudo Y, Shinozaki Y, Takeda Y. Gonadotropin-releasing hormone increases serum human chorionic gonadotropin in pregnant women. Endocr J. 1993;40(5):539–44.

29. Wu H, Zhang S, Lin X, Wang S, Zhou P. Luteal phase support for in vitro fertilization/intracytoplasmic sperm injection fresh cycles: a systematic review and network meta-analysis. Reprod Biol Endocrinol. 2021;19(1):103.

30. Liu Y, Wu Y, Pan Z, Jiang F, Lu Y, Meng Y. Single-dose versus multiple-dose GnRH agonist for luteal-phase support in women undergoing IVF/ICSI cycles: a network Meta-analysis of randomized controlled trials. Front Endocrinol (Lausanne). 2022; 13:802688.

31. Alsbjerg B, Kesmodel US, Elbaek HO, Laursen R, Laursen SB, Andreasen D, et al. GnRH agonist supplementation in hormone replacement therapy-frozen embryo transfer cycles: a randomized controlled trial. Reprod BioMed Online. 2022;44(2):261–70.